RTG Tools Operations Manual

Release 3.12

Real Time Genomics

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CONTENTS

CHAPTER

ONE

OVERVIEW

This chapter introduces the features, operational options, and installation requirements of the data analysis software from Real Time Genomics.

1.1 Introduction

RTG software enables the development of fast, efficient software pipelines for deep genomic analysis. RTG is built on innovative search technologies and new algorithms designed for processing high volumes of high-throughput sequencing data from different sequencing technology platforms. The RTG sequence search and alignment functions enable read mapping and protein searches with a unique combination of sensitivity and speed.

The RTG Tools platform provides a subset of the functionality available from the full suite of functions for analyzing and manipulating variant call results. These utilities can be used to perform a variety of tasks such as:

- **Accuracy Evaluation** – Compare called variants to a set of known variants to find specificity and sensitivity, check mendelian consistency for the variants from a family, finding basic variant statistics for a set of calls.
- **Result Filtering** – Find a subset of variants that match a given set of filtering criteria, extracting only the variant information required for a specific task.
- **Variant Set Manipulation** – Merging multiple sets of variant results together, adding additional annotation information to existing variants.

1.2 RTG software description

RTG software is delivered as a single executable with multiple commands executed through a command line interface (CLI). Commands are delivered in product packages, and for commercial users each command can be independently enabled through a license key.

Usage:

```
rtg COMMAND [OPTIONS] <REQUIRED>
```

See also:

For detailed information about RTG command syntax and usage of individual commands, refer to *RTG Command Reference*.

1.3 Installation and deployment

RTG is a self-contained tool that sets minimal expectations on the environment in which it is placed. It comes with the application components it needs to execute completely, yet performance can be enhanced with some simple modifications to the deployment configuration. This section provides guidelines for installing and creating an optimal configuration, starting from a typical recommended system.

RTG software pipeline runs in a wide range of computing environments from dual-core processor laptops to compute clusters with racks of dual processor quad core server nodes. However, internal human genome analysis benchmarks suggest the use of six server nodes of the configuration shown in below.

Table : Recommended system requirements

Processor	Intel Core i7-2600
Memory	48 GB RAM DDR3
Disk	5 TB, 7200 RPM (prefer SAS disk)

RTG Software can be run as a Java JAR file, but platform specific wrapper scripts are supplied to provide improved pipeline ergonomics. Instructions for a quick start installation are provided here.

For further information about setting up per-machine configuration files, please see the `README.txt` contained in the distribution zip file (a copy is also included in this manual's appendix).

1.3.1 Quick start instructions

These instructions are intended for an individual to install and operate the RTG software without the need to establish root / administrator privileges.

RTG software is delivered in a compressed zip file, such as: `rtg-core-3.3.zip`. Unzip this file to begin installation.

Linux and Windows distributions include a Java Virtual Machine (JVM) version 1.8 that has undergone quality assurance testing. RTG may be used on other operating systems for which a JVM version 1.8 or higher is available, such as MacOS X or Solaris, by using the 'no-jre' distribution.

RTG for Java is delivered as a Java application accessed via executable wrapper script (`rtg` on UNIX systems, `rtg.bat` on Windows) that allows a user to customize initial memory allocation and other configuration options. It is recommended that these wrapper scripts be used rather than directly executing the Java JAR.

Here are platform-specific instructions for RTG deployment.

Linux/MacOS X:

- Unzip the RTG distribution to the desired location.
- If your installation requires a license file (`rtg-license.txt`), copy the license file provided by Real Time Genomics into the RTG distribution directory.
- **In a terminal, cd to the installation directory and test for success** by entering `./rtg version`
- On MacOS X, depending on your operating system version and configuration regarding unsigned applications, you may encounter the error message:

```
-bash: rtg: /usr/bin/env: bad interpreter: Operation not permitted
```

  If this occurs, you must clear the OS X quarantine attribute with the command:

```
$ xattr -d com.apple.quarantine rtg
```

- The first time rtg is executed you will be prompted with some questions to customize your installation. Follow the prompts.

- Enter `./rtg help` for a list of rtg commands. Help for any individual command is available using the `--help` flag, e.g.: `./rtg format --help`
- By default, RTG software scripts establish a memory space of 90% of the available RAM - this is automatically calculated. One may override this limit in the `rtg.cfg` settings file or on a per-run basis by supplying `RTG_MEM` as an environment variable or as the first program argument, e.g.: `./rtg RTG_MEM=48g map`
- [OPTIONAL] If you will be running RTG on multiple machines and would like to customize settings on a per-machine basis, copy `rtg.cfg` to `/etc/rtg.cfg`, editing per-machine settings appropriately (requires root privileges). An alternative that does not require root privileges is to copy `rtg.cfg` to `rtg.HOSTNAME.cfg`, editing per-machine settings appropriately, where `HOSTNAME` is the short host name output by the command `hostname -s`

Windows:

- Unzip the RTG distribution to the desired location.
- If your installation requires a license, copy the license file provided by Real Time Genomics (`rtg-license.txt`) into the RTG distribution directory.
- Test for success by entering `rtg version` at the command line. The first time RTG is executed you will be prompted with some questions to customize your installation. Follow the prompts.
- Enter `rtg help` for a list of rtg commands. Help for any individual command is available using the `--help` flag, e.g.: `./rtg format --help`
- By default, RTG software scripts establish a memory space of 90% of the available RAM - this is automatically calculated. One may override this limit by setting the `RTG_MEM` variable in the `rtg.bat` script or as an environment variable.

1.3.2 License Management

Commercial distributions of RTG products require the presence of a valid license key file for operation.

The license key file must be located in the same directory as the RTG executable. The license enables the execution of a particular command set for the purchased product(s) and features.

A license key allows flexible use of the RTG package on any node or CPU core.

To view the current license features at the command prompt, enter:

```
$ rtg license
```

See also:

For more data center deployment and instructions for editing scripts, see *Administration & Capacity Planning*.

1.4 Technical assistance and support

For assistance with any technical or conceptual issue that may arise during use of the RTG product, contact Real Time Genomics Technical Support via email at `support@realtimegenomics.com`

In addition, a discussion group is available at: https://groups.google.com/a/realtimegenomics.com/forum/#!forum/rtg-users

A low-traffic announcements-only group is available at: https://groups.google.com/a/realtimegenomics.com/forum/#!forum/rtg-announce

CHAPTER

TWO

RTG COMMAND REFERENCE

This chapter describes RTG commands with a generic description of parameter options and usage. This section also includes expected operation and output results.

2.1 Command line interface (CLI)

RTG is installed as a single executable in any system subdirectory where permissions authorize a particular community of users to run the application. RTG commands are executed through the RTG command-line interface (CLI). Each command has its own set of parameters and options described in this section. The availability of each command may be determined by the RTG license that has been installed. Contact `support@realtimegenomics.com` to discuss changing the set of commands that are enabled by your license.

Results are organized in results directories defined by command parameters and settings. The command line shell environment should include a set of familiar text post-processing tools, such as `grep`, `awk`, or `perl`. Otherwise, no additional applications such as databases or directory services are required.

2.2 RTG command syntax

Usage:

```
rtg COMMAND [OPTIONS] <REQUIRED>
```

To run an RTG command at the command prompt (either DOS window or Unix terminal), type the product name followed by the command and all required and optional parameters. For example:

```
$ rtg format -o human_REF_SDF human_REF.fasta
```

Typically results are written to output files specified with the `-o` option. There is no default filename or filename extension added to commands requiring specification of an output directory or format.

Many times, unfiltered output files are very large; the built-in compression option generates block compressed output files with the `.gz` extension automatically unless the parameter `-Z` or `--no-gzip` is issued with the command.

Many command parameters require user-supplied information of various types, as shown in the following:

Type	Description
DIR, FILE	File or directory name(s)
SDF	Sequence data that has been formatted to SDF
INT	Integer value
FLOAT	Floating point decimal value
STRING	A sequence of characters for comments, filenames, or labels
REGION	A genomic region specification (see below)

Genomic region parameters take one of the following forms:

- sequence_name (e.g.: `chr21`) corresponds to the entirety of the named sequence.
- sequence_name:start (e.g.: `chr21:100000`) corresponds to a single position on the named sequence.
- sequence_name:start-end (e.g.: `chr21:100000-110000`) corresponds to a range that extends from the specified start position to the specified end position (inclusive). The positions are 1-based.
- sequence_name:position+length (e.g.: `chr21:100000+10000`) corresponds to a range that extends from the specified start position that includes the specified number of nucleotides.
- sequence_name:position~padding (e.g.: `chr21:100000~10000`) corresponds to a range that spans the specified position by the specified amount of padding on either side.

To display all parameters and syntax associated with an RTG command, enter the command and type `--help`. For example: all parameters available for the RTG `format` command are displayed when `rtg format --help` is executed, the output of which is shown below.

```
Usage: rtg format [OPTION]... -o SDF FILE+
                  [OPTION]... -o SDF -I FILE
                  [OPTION]... -o SDF -l FILE -r FILE

Converts the contents of sequence data files (FASTA/FASTQ/SAM/BAM) into the RTG
Sequence Data File (SDF) format.

File Input/Output
  -f, --format=FORMAT            format of input. Allowed values are [fasta,
                                 fastq, sam-se, sam-pe, cg-fastq, cg-sam]
                                 (Default is fasta)
  -I, --input-list-file=FILE     file containing a list of input read files (1
                                 per line)
  -l, --left=FILE                left input file for FASTA/FASTQ paired end
                                 data
  -o, --output=SDF               name of output SDF
  -p, --protein                  input is protein. If this option is not
                                 specified, then the input is assumed to
                                 consist of nucleotides
  -q, --quality-format=FORMAT    format of quality data for fastq files (use
                                 sanger for Illumina 1.8+). Allowed values are
                                 [sanger, solexa, illumina]
  -r, --right=FILE               right input file for FASTA/FASTQ paired end
                                 data
      FILE+                      input sequence files. May be specified 0 or
                                 more times

Filtering
      --duster                   treat lower case residues as unknowns
      --exclude=STRING           exclude input sequences based on their name.
                                 If the input sequence contains the specified
                                 string then that sequence is excluded from the
                                 SDF. May be specified 0 or more times
      --select-read-group=STRING when formatting from SAM/BAM input, only
                                 include reads with this read group ID
      --trim-threshold=INT       trim read ends to maximise base quality above
                                 the given threshold

Utility
      --allow-duplicate-names    disable checking for duplicate sequence names
  -h, --help                     print help on command-line flag usage
      --no-names                 do not include name data in the SDF output
      --no-quality               do not include quality data in the SDF output
      --sam-rg=STRING|FILE       file containing a single valid read group SAM
                                 header line or a string in the form
                                 "@RG\tID:READGROUP1\tSM:BACT_SAMPLE\tPL:ILLUMINA"
```

Required parameters are indicated in the usage display; optional parameters are listed immediately below the usage information in organized categories.

Use the double-dash when typing the full-word command option, as in `--output`:

```
$ rtg format --output human_REF_SDF human_REF.fasta
```

Commonly used command options provide an abbreviated single-character version of a full command parameter, indicated with only a single dash, (Thus `--output` is the same as specifying the command option with the abbreviated character `-o`):

```
$ rtg format -o human_REF human_REF.fasta
```

A set of utility commands are provided through the CLI: `version`, `license`, and `help`. Start with these commands to familiarize yourself with the software.

The `rtg version` command invokes the RTG software and triggers the launch of RTG product commands, options, and utilities:

```
$ rtg version
```

It will display the version of the RTG software installed, RAM requirements, and license expiration, for example:

```
$rtg version
Product: RTG Core 3.5
Core Version: 6236f4e (2014-10-31)
RAM: 40.0GB of 47.0GB RAM can be used by rtg (84%)
License: Expires on 2015-09-30
License location: /home/rtgcustomer/rtg/rtg-license.txt
Contact: support@realtimegenomics.com

Patents / Patents pending:
US: 7,640,256, 13/129,329, 13/681,046, 13/681,215, 13/848,653,
13/925,704, 14/015,295, 13/971,654, 13/971,630, 14/564,810
UK: 1222923.3, 1222921.7, 1304502.6, 1311209.9, 1314888.7, 1314908.3
New Zealand: 626777, 626783, 615491, 614897, 614560
Australia: 2005255348, Singapore: 128254

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M. De La Vega. "Joint Variant and De Novo Mutation Identification on
Pedigrees from High-Throughput Sequencing Data." Journal of
Computational Biology. June 2014, 21(6): 405-419.
doi:10.1089/cmb.2014.0029.
(c) Real Time Genomics Inc, 2014
```

To see what commands you are licensed to use, type `rtg license`:

```
$rtg license
License: Expires on 2015-03-30
Licensed to: John Doe
License location: /home/rtgcustomer/rtg/rtg-license.txt

    Command name    Licensed?  Release Level

Data formatting:
    format          Licensed   GA
    sdf2fasta       Licensed   GA
    sdf2fastq       Licensed   GA

Utility:
```

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```
    bgzip           Licensed   GA
    index           Licensed   GA
    extract         Licensed   GA
    sdfstats        Licensed   GA
    sdfsubset       Licensed   GA
    sdfsubseq       Licensed   GA
    mendelian       Licensed   GA
    vcfstats        Licensed   GA
    vcfmerge        Licensed   GA
    vcffilter       Licensed   GA
    vcfannotate     Licensed   GA
    vcfsubset       Licensed   GA
    vcfeval         Licensed   GA
    pedfilter       Licensed   GA
    pedstats        Licensed   GA
    rocplot         Licensed   GA
    version         Licensed   GA
    license         Licensed   GA
    help            Licensed   GA
```

To display all commands and usage parameters available to use with your license, type `rtg help`:

```
$ rtg help
Usage: rtg COMMAND [OPTION]...
       rtg RTG_MEM=16G COMMAND [OPTION]...  (e.g. to set maximum memory use to 16␣
↪GB)

Type ``rtg help COMMAND`` for help on a specific command. The
following commands are available:

Data formatting:
      format                convert a FASTA file to SDF
      cg2sdf                convert Complete Genomics reads to SDF
      sdf2fasta             convert SDF to FASTA
      sdf2fastq             convert SDF to FASTQ
      sdf2sam               convert SDF to SAM/BAM
Read mapping:
      map                   read mapping
      mapf                  read mapping for filtering purposes
      cgmap                 read mapping for Complete Genomics data
Protein search:
      mapx                  translated protein search
Assembly:
      assemble              assemble reads into long sequences
      addpacbio             add Pacific Biosciences reads to an assembly
Variant detection:
      calibrate             create calibration data from SAM/BAM files
      svprep                prepare SAM/BAM files for sv analysis
      sv                    find structural variants
      discord               detect structural variant breakends using discordant␣
↪reads
      coverage              calculate depth of coverage from SAM/BAM files
      snp                   call variants from SAM/BAM files
      family                call variants for a family following Mendelian␣
↪inheritance
      somatic               call variants for a tumor/normal pair
      population            call variants for multiple potentially-related␣
↪individuals
      lineage               call de novo variants in a cell lineage
      avrbuild              AVR model builder
      avrpredict            run AVR on a VCF file
```

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```
      cnv                    call CNVs from paired SAM/BAM files
Metagenomics:
      species                estimate species frequency in metagenomic samples
      similarity             calculate similarity matrix and nearest neighbor tree
Simulation:
      genomesim              generate simulated genome sequence
      cgsim                  generate simulated reads from a sequence
      readsim                generate simulated reads from a sequence
      readsimeval            evaluate accuracy of mapping simulated reads
      popsim                 generate a VCF containing simulated population␣
↪variants
      samplesim              generate a VCF containing a genotype simulated from a␣
↪population
      childsim               generate a VCF containing a genotype simulated as a␣
↪child of two parents
      denovosim              generate a VCF containing a derived genotype␣
↪containing de novo variants
      samplereplay           generate the genome corresponding to a sample genotype
      cnvsim                 generate a mutated genome by adding CNVs to a template
Utility:
      bgzip                  compress a file using block gzip
      index                  create a tabix index
      extract                extract data from a tabix indexed file
      sdfstats               print statistics about an SDF
      sdfsplit               split an SDF into multiple parts
      sdfsubset              extract a subset of an SDF into a new SDF
      sdfsubseq              extract a subsequence from an SDF as text
      sam2bam                convert SAM file to BAM file and create index
      sammerge               merge sorted SAM/BAM files
      samstats               print statistics about a SAM/BAM file
      samrename              rename read id to read name in SAM/BAM files
      mapxrename             rename read id to read name in mapx output files
      mendelian              check a multi-sample VCF for Mendelian consistency
      vcfstats               print statistics from about variants contained within␣
↪a VCF file
      vcfmerge               merge single-sample VCF files into a single multi-
↪sample VCF
      vcffilter              filter records within a VCF file
      vcfannotate            annotate variants within a VCF file
      vcfsubset              create a VCF file containing a subset of the original␣
↪columns
      vcfeval                evaluate called variants for agreement with a␣
↪baseline variant set
      pedfilter              filter and convert a pedigree file
      pedstats               print information about a pedigree file
      avrstats               print statistics about an AVR model
      rocplot                plot ROC curves from vcfeval ROC data files
      usageserver            run a local server for collecting RTG command usage␣
↪information
      version                print version and license information
      license                print license information for all commands
      help                   print this screen or help for specified command
```

The help command will only list the commands for which you have a license to use.

To display help and syntax information for a specific command from the command line, type the command and then the –help option, as in:

```
$ rtg format --help
```

Note: The following commands are synonymous: `rtg help format` and `rtg format --help`

See also:

Refer to *Installation and deployment* for information about installing the RTG product executable.

2.3 Data Formatting Commands

2.3.1 format

Synopsis:

The `format` command converts the contents of sequence data files (FASTA/FASTQ/SAM/BAM) into the RTG Sequence Data File (SDF) format. This step ensures efficient processing of very large data sets, by organizing the data into multiple binary files within a named directory. The same SDF format is used for storing sequence data, whether it be genomic reference, sequencing reads, protein sequences, etc.

Syntax:

Format one or more files specified from command line into a single SDF:

```
$ rtg format [OPTION] -o SDF FILE+
```

Format one or more files specified in a text file into a single SDF:

```
$ rtg format [OPTION] -o SDF -I FILE
```

Format mate pair reads into a single SDF:

```
$ rtg format [OPTION] -o SDF -l FILE -r FILE
```

Examples:

For FASTA (`.fa`) genome reference data:

```
$ rtg format -o maize_reference maize_chr*.fa
```

For FASTQ (`.fq`) sequence read data:

```
$ rtg format -f fastq -q sanger -o h1_reads -l h1_sample_left.fq -r h1_sample_
↪right.fq
```

Parameters:

File Input/Output		
`-f`	`--format=FORMAT`	The format of the input file(s). Allowed values are [fasta, fastq, fastq-interleaved, sam-se, sam-pe] (Default is fasta).
`-I`	`--input-list-file=FILE`	Specifies a file containing a list of sequence data files (one per line) to be converted into an SDF.
`-l`	`--left=FILE`	The left input file for FASTA/FASTQ paired end data.
`-o`	`--output=SDF`	The name of the output SDF.
`-p`	`--protein`	Set if the input consists of protein. If this option is not specified, then the input is assumed to consist of nucleotides.
`-q`	`--quality-format=FORMAT`	The format of the quality data for fastq format files. (Use sanger for Illumina1.8+). Allowed values are [sanger, solexa, illumina].
`-r`	`--right=FILE`	The right input file for FASTA/FASTQ paired end data.
	`FILE+`	Specifies a sequence data file to be converted into an SDF. May be specified 0 or more times.

Filtering		
	`--duster`	Treat lower case residues as unknowns.
	`--exclude=STRING`	Exclude individual input sequences based on their name. If the input sequence name contains the specified string then that sequence is excluded from the SDF. May be specified 0 or more times.
	`--select-read-group=STRING`	Set to only include only reads with this read group ID when formatting from SAM/BAM files.
	`--trim-threshold=INT`	Set to trim the read ends to maximise the base quality above the given threshold.

Utility		
	`--allow-duplicate-names`	Set to disable duplicate name detection.
`-h`	`--help`	Prints help on command-line flag usage.
	`--no-names`	Do not include sequence names in the resulting SDF.
	`--no-quality`	Do not include sequence quality data in the resulting SDF.
	`--sam-rg=STRING|FILE`	Specifies a file containing a single valid read group SAM header line or a string in the form `@RG\tID:RG1\tSM:G1_SAMP\tPL:ILLUMINA`.

Usage:

Formatting takes one or more input data files and creates a single SDF. Specify the type of file to be converted, or allow default to FASTA format. To aggregate multiple input data files, such as when formatting a reference genome consisting of multiple chromosomes, list all files on the command line or use the `--input-list-file` flag to specify a file containing the list of files to process.

For input FASTA and FASTQ files which are compressed, they must have a filename extension of `.gz` (for gzip compressed data) or `.bz2` (for bzip2 compressed data).

When formatting human reference genome data, it is recommended that the resulting SDF be augmented with chromosome reference metadata, in order to enable automatic sex-aware features during mapping and variant calling. The `format` command will automatically recognize several common human reference genomes and install a reference configuration file. If your reference genome is not recognized, a configuration can be manually adapted from one of the examples provided in the RTG distribution and installed in the SDF directory. The reference configuration is described in *RTG reference file format*.

When using FASTQ input files you must specify the quality format being used as one of `sanger`, `solexa` or `illumina`. As of Illumina pipeline version 1.8 and higher, quality values are encoded in Sanger format and so should be formatted using `--quality-format=sanger`. Output from earlier Illumina pipeline versions should be formatted using `--quality-format=illumina` for Illumina pipeline versions starting with 1.3 and before 1.8, or `--quality-format=solexa` for Illumina pipeline versions less than 1.3.

For FASTQ files that represent paired-end read data, indicate each side respectively using the `--left=FILE` and `--right=FILE` flags. Sometimes paired-end reads are represented in a single FASTQ file by interleaving each side of the read. This type of input can be formatted by specifying `fastq-interleaved` as the format type.

The `mapx` command maps translated DNA sequence data against a protein reference. You must use the `-p`, `--protein` flag to format the protein reference used by `mapx`.

Use the `sam-se` format for single end SAM/BAM input files and the `sam-pe` format for paired end SAM/BAM input files. Note that if the input SAM/BAM files are sorted in coordinate order (for example if they have already been aligned to a reference), it is recommended that they be shuffled before formatting, so that subsequent mapping is not biased by processing reads in chromosome order. For example, a BAM file can be shuffled using `samtools collate` as follows:

```
$ samtools collate -uOn 256 reads.bam tmp-prefix >reads_shuffled.bam
```

And this can be carried out on the fly during formatting using bash process redirection in order to reduce intermediate I/O, for example:

```
$ rtg format --format sam-pe <(samtools collate -uOn 256 reads.bam temp-prefix) ...
```

The SDF for a read set can contain a SAM read group which will be automatically picked up from the input SAM/BAM files if they contain only one read group. If the input SAM/BAM files contain multiple read groups you must select a single read group from the SAM/BAM file to format using the `--select-read-group` flag or specify a custom read group with the `--sam-rg` flag. The `--sam-rg` flag can also be used to add read group information to reads given in other input formats. The SAM read group stored in an SDF will be automatically used during mapping the reads it contains to provide tracking information in the output BAM files.

The `--trim-threshold` flag can be used to trim poor quality read ends from the input reads by inspecting base qualities from FASTQ input. If and only if the quality of the final base of the read is less than the threshold given, a new read length is found which maximizes the overall quality of the retained bases using the following formula.

$$\arg\max x \left(\sum_{i=x+1}^{l} (T - q(i)) \right) \text{ if } q(l) < T$$

Where *l* is the original read length, *x* is the new read length, *T* is the given threshold quality and *q(n)* is the quality of the base at the position n of the read.

Note: Sequencing system read files and reference genome files often have the same extension and it may not always be obvious which file is a read set and which is a genome. Before formatting a sequencing system file, open it to see which type of file it is. For example:

```
$ less pf3.fa
```

In general, a read file typically begins with an `@` or + character; a genome reference file typically begins with the characters `chr`.

Normally when the input data contains multiple sequences with the same name the format command will fail with an error. The `--allow-duplicate-names` flag will disable this check conserving memory, but if the input data has multiple sequences with the same name you will not be warned. Having duplicate sequence names can cause problems with other commands, especially for reference data since the output many commands identifies sequences by their names.

See also:

sdf2fasta, *sdf2fastq*, *sdfstats*

2.3.2 sdf2fasta

Synopsis:

Convert SDF data into a FASTA file.

Syntax:

```
$ rtg sdf2fasta [OPTION]... -i SDF -o FILE
```

Example:

```
$ rtg sdf2fasta -i humanSDF -o humanFASTA_return
```

Parameters:

File Input/Output		
`-i`	`--input=SDF`	SDF containing sequences.
`-o`	`--output=FILE`	Output filename (extension added if not present). Use '-' to w output.

Filtering		
	`--end-id=INT`	Only output sequences with sequence id less than the given number. (ids start at 0).
	`--start-id=INT`	Only output sequences with sequence id greater than or equal to the g number. (Sequence ids start at 0).
`-I`	`--id-file=FILE`	Name of a file containing a list of sequences to extract, one per line.
	`--names`	Interpret any specified sequence as names instead of numeric sequenc
	`--taxons`	Interpret any specified sequence as taxon ids instead of numeric sequ This option only applies to a metagenomic reference species SDF.
	`STRING+`	Specify one or more explicit sequences to extract, as sequence id, or name if –names flag is set.

Utility		
`-h`	`--help`	Prints help on command-line flag usage.
	`--interleave`	Interleave paired data into a single output file. Default is to split to separa output files.
`-l`	`--line-length=INT`	Set the maximum number of nucleotides or amino acids to print on a line of FASTA output. Should be nonnegative, with a value of 0 indicating tha the line length is not capped. (Default is 0).
`-Z`	`--no-gzip`	Set this flag to create the FASTA output file without compression. By default the output file is compressed with blocked gzip.

Usage:

Use the `sdf2fasta` command to convert SDF data into FASTA format. By default, `sdf2fasta` creates a separate line of FASTA output for each sequence. These lines will be as long as the sequences themselves. To make them more readable, use the `-l`, `--line-length` flag and define a reasonable record length like 75.

By default all sequences will be extracted, but flags may be specified to extract reads within a range, or explicitly specified reads (either by numeric sequence id or by sequence name if `--names` is set). Additionally, when the input SDF is a metagenomic species reference SDF, the `--taxons` option, any supplied id is interpreted as a taxon id and all sequences assigned directly to that taxon id will be output. This provides a convenient way to extract all sequence data corresponding to a single (or multiple) species from a metagenomic species reference SDF.

Sequence ids are numbered starting at 0, the `--start-id` flag is an inclusive lower bound on id and the `--end-id` flag is an exclusive upper bound. For example if you have an SDF with five sequences (ids: 0, 1, 2, 3, 4) the following command:

```
$ rtg sdf2fasta --start-id=3 -i mySDF -o output
```

will extract sequences with id 3 and 4. The command:

```
$ rtg sdf2fasta --end-id=3 -i mySDF -o output
```

will extract sequences with id 0, 1, and 2. And the command:

```
$ rtg sdf2fasta --start-id=2 --end-id=4 -i mySDF -o output
```

will extract sequences with id 2 and 3.

See also:

format, *sdf2fastq*, *sdfstats*

2.3.3 sdf2fastq

Synopsis:

Convert SDF data into a FASTQ file.

Syntax:

```
$ rtg sdf2fastq [OPTION]... -i SDF -o FILE
```

Example:

```
$ rtg sdf2fastq -i humanSDF -o humanFASTQ_return
```

Parameters:

File Input/Output		
-i	--input=SDF	Specifies the SDF data to be converted.
-o	--output=FILE	Specifies the file name used to write the resulting FASTQ output.

Filtering		
	--end-id=INT	Only output sequences with sequence id less than the given numbe ids start at 0).
	--start-id=INT	Only output sequences with sequence id greater than or equal to th number. (Sequence ids start at 0).
-I	--id-file=FILE	Name of a file containing a list of sequences to extract, one per lin
	--names	Interpret any specified sequence as names instead of numeric sequ
	STRING+	Specify one or more explicit sequences to extract, as sequence id, name if –names flag is set.

Utility		
-h	--help	Prints help on command-line flag usage.
-q	--default-qualty=INT	Set the default quality to use if the SDF does not contain sequence quality data (0-63).
	--interleave	Interleave paired data into a single output file. Default is to split to separate output files.
-l	--line-length=INT	Set the maximum number of nucleotides or amino acids to print on a line of FASTQ output. Should be nonnegative, with a value of 0 indicating that the line length is not capped. (Default is 0).
-Z	--no-gzip	Set this flag to create the FASTQ output file without compression. By default the output file is compressed with blocked gzip.

Usage:

Use the `sdf2fastq` command to convert SDF data into FASTQ format. If no quality data is available in the SDF, use the `-q`, `--default-quality` flag to set a quality score for the FASTQ output. The quality encoding used during output is sanger quality encoding. By default, `sdf2fastq` creates a separate line of FASTQ output for each sequence. As with `sdf2fasta`, there is an option to use the `-l`, `--line-length` flag to restrict the line lengths to improve readability of long sequences.

By default all sequences will be extracted, but flags may be specified to extract reads within a range, or explicitly specified reads (either by numeric sequence id or by sequence name if `--names` is set).

It may be preferable to extract data to unaligned SAM/BAM format using `sdf2sam`, as this preserves read-group information stored in the SDF and may also be more convenient when dealing with paired-end data.

The `--start-id` and `--end-id` flags behave as in `sdf2fasta`.

See also:

format, *sdf2fasta*, *sdf2sam*, *sdfstats*

2.3.4 sdf2sam

Synopsis:

Convert SDF read data into unaligned SAM or BAM format file.

Syntax:

```
$ rtg sdf2sam [OPTION]... -i SDF -o FILE
```

Example:

```
$ rtg sdf2sam -i samplereadsSDF -o samplereads.bam
```

Parameters:

File Input/Output		
-i	--input=SDF	Specifies the SDF data to be converted.
-o	--output=FILE	Specifies the file name used to write the resulting SAM/BAM to. The outp format is automatically determined based on the filename specified. If '-' i given, the data is written as uncompressed SAM to standard output.

Filtering		
	--end-id=INT	Only output sequences with sequence id less than the given numbe ids start at 0).
	--start-id=INT	Only output sequences with sequence id greater than or equal to th number. (Sequence ids start at 0).
-I	--id-file=FILE	Name of a file containing a list of sequences to extract, one per lin
	--names	Interpret any specified sequence as names instead of numeric sequ
	STRING+	Specify one or more explicit sequences to extract, as sequence id, name if –names flag is set.

Utility		
-h	--help	Prints help on command-line flag usage.
-Z	--no-gzip	Set this flag when creating SAM format output to disable compress SAM is compressed with blocked gzip, and BAM is always compr

Usage:

Use the `sdf2sam` command to convert SDF data into unaligned SAM/BAM format. By default all sequences will be extracted, but flags may be specified to extract reads within a range, or explicitly specified reads (either by numeric sequence id or by sequence name if `--names` is set). This command is a useful way to export paired-end data to a single output file while retaining any read group information that may be stored in the SDF.

The output format is either SAM/BAM depending on the specified output file name. e.g. `output.sam` or `output.sam.gz` will output as SAM, whereas `output.bam` will output as BAM. If neither SAM or BAM format is indicated by the file name then BAM will be used and the output file name adjusted accordingly. e.g `output` will become `output.bam`. However if standard output is selected (`-`) then the output will always be in uncompressed SAM format.

The `--start-id` and `--end-if` behave as in `sdf2fasta`.

See also:

format, *sdf2fasta*, *sdf2fastq*, *sdfstats*, *cg2sdf*, *sdfsplit*

2.3.5 fastqtrim

Synopsis:

Trim reads in FASTQ files.

Syntax:

```
$ rtg fastqtrim [OPTION]... -i FILE -o FILE
```

Example:

Apply hard base removal from the start of the read and quality-based trimming of terminal bases:

```
$ rtg fastqtrim -s 12 -E 18 -i S12_R1.fastq.gz -o S12_trimmed_R1.fastq.gz
```

Parameters:

File Input/Output		
-i	--input=FILE	Input FASTQ file, Use '-' to read from standard input.
-o	--output=FILE	Output filename. Use '-' to write to standard output.
-q	--quality-format=FORMAT	Quality data encoding method used in FASTQ input files (Illumina 1.8+ uses sanger). Allowed values are [sanger, solexa, illumina] (Default is sanger)

Filtering		
	--discard-empty-reads	Discard reads that have zero length after trimming. Should not be used with paired-end data.
-E	--end-quality-threshold=INT	Trim read ends to maximise base quality above the give threshold (Default is 0)
	--min-read-length=INT	If a read ends up shorter than this threshold it will be trimmed to zero length (Default is 0)
-S	--start-quality-threshold=INT	Trim read starts to maximise base quality above the given threshold (Default is 0)
-e	--trim-end-bases=INT	Always trim the specified number of bases from read e (Default is 0)
-s	--trim-start-bases=INT	Always trim the specified number of bases from read start (Default is 0)

Utility		
-h	--help	Print help on command-line flag usage.
-Z	--no-gzip	Do not gzip the output.
-r	--reverse-complement	If set, output in reverse complement.
	--seed=INT	Seed used during subsampling.
	--subsample=FLOAT	If set, subsample the input to retain this fraction of reads.
-T	--threads=INT	Number of threads (Default is the number of available cores)

Usage:

Use `fastqtrim` to apply custom trimming and preprocessing to raw FASTQ files prior to mapping and alignment. The `format` command contains some limited trimming options, which are applied to all input files, however in some cases different or specific trimming operations need to be applied to the various input files. For example, for paired-end data, different trimming may need to be applied for the left read files compared to the right read files. In these cases, `fastqtrim` should be used to process the FASTQ files first.

The `--end-quality-threshold` flag can be used to trim poor quality bases from the ends of the input reads by inspecting base qualities from FASTQ input. If and only if the quality of the final base of the read is less than

the threshold given, a new read length is found which maximizes the overall quality of the retained bases using the following formula:

$$\arg\max x \left(\sum_{i=x+1}^{l} (T - q(i)) \right) \text{ if } q(l) < T$$

where *l* is the original read length, *x* is the new read length, *T* is the given threshold quality and *q(n)* is the quality of the base at the position *n* of the read. Similarly, `--start-quality-threshold` can be used to apply this quality-based thresholding to the start of reads.

Some of the trimming options may result in reads that have no bases remaining. By default, these are output as zero-length FASTQ reads, which RTG commands are able to handle normally. It is also possible to remove zero-length reads altogether from the output with the `--discard-empty-reads` option, however this should not be used when processing FASTQ files corresponding to paired-end data, otherwise the pairs in the two files will no longer be matched.

Similarly, when using the `--subsample` option to down-sample a FASTQ file for paired-end data, you should specify an explicit randomization seed via `--seed` and use the same seed value for the left and right files.

Formatting with filtering on the fly

Running custom filtering with `fastqtrim` need not mean that additional disk space is required or that formatting be slowed down due to additional disk I/O. It is possible when using standard unix shells to perform the filtering on the fly. The following example demonstrates how to apply different trimming options to left and right files while formatting to SDF:

```
$ rtg format -f fastq -o S12_trimmed.sdf \
    -l <(rtg fastqtrim -s 12 -E 18 -i S12_R1.fastq.gz -o -)
    -r <(rtg fastqtrim -E 18 -i S12_R2.fastq.gz -o -)
```

See also:

format

2.3.6 petrim

Synopsis:

Trim paired-end read FASTQ files based on read arm alignment overlap.

Syntax:

```
$ rtg petrim [OPTION]... -l FILE -o FILE -r FILE
```

Parameters:

File Input/Output		
`-l`	`--left=FILE`	Left input FASTQ file (AKA R1)
`-o`	`--output=FILE`	Output filename prefix. Use '-' to write to standard output.
`-q`	`--quality-format=FORMAT`	Quality data encoding method used in FASTQ input files (Illumina 1.8+ uses sanger). Allowed values are [sanger, solexa, illumina] (Default is sanger)
`-r`	`--right=FILE`	Right input FASTQ file (AKA R2)

Sensitivity Tuning		
	--aligner-band-width=FLOAT	Aligner indel band width scaling factor, fraction of read length allowed as an indel (Default is 0.5)
	--gap-extend-penalty=INT	Penalty for a gap extension during alignment (Default is 1)
	--gap-open-penalty=INT	Penalty for a gap open during alignment (Default is 19)
-P	--min-identity=INT	Minimum percent identity in overlap to trigger overlap trimming (Default is 90)
-L	--min-overlap-length=INT	Minimum number of bases in overlap to trigger overlap trimming (Default is 25)
	--mismatch-penalty=INT	Penalty for a mismatch during alignment (Default is 9)
	--soft-clip-distance=INT	Soft clip alignments if indels occur INT bp from either end (Default is 5)
	--unknowns-penalty=INT	Penalty for unknown nucleotides during alignment (Default is 5)

Filtering		
	--discard-empty-pairs	If set, discard pairs where both reads have zero length (after any trimming)
	--discard-empty-reads	If set, discard pairs where either read has zero length (after any trimming)
	--left-probe-length=INT	Assume R1 starts with probes this long, and trim R2 bases that overlap into this (Default is 0)
-M	--midpoint-merge	If set, merge overlapping reads at midpoint of overlap region. Result is in R1 (R2 will be empty)
-m	--midpoint-trim	If set, trim overlapping reads to midpoint of overlap region.
	--min-read-length=INT	If a read ends up shorter than this threshold it will be trimmed to zero length (Default is 0)
	--mismatch-adjustment=STRING	Method used to alter bases/qualities at mismatches within overlap region. Allowed values are [none, zero-phred, pick-best] (Default is none)
	--right-probe-length=INT	Assume R2 starts with probes this long, and trim R1 bases that overlap into this (Default is 0)

Utility		
-h	--help	Print help on command-line flag usage.
	--interleave	Interleave paired data into a single output file. Default is to split t output files.
-Z	--no-gzip	Do not gzip the output.
	--seed=INT	Seed used during subsampling.
	--subsample=FLOAT	If set, subsample the input to retain this fraction of reads.
-T	--threads=INT	Number of threads (Default is the number of available cores)

Usage:

Paired-end read sequencing with read lengths that are long relative to the typical library fragment size can often result in the same bases being sequenced by both arms. This repeated sequencing of bases within the same fragment can skew variant calling, and so it can be advantageous to remove such read overlap.

In some cases, complete read-through can occur, resulting in additional adaptor or non-genomic bases being present at the ends of reads.

In addition, some library preparation methods rely on the ligation of synthetic probe sequence to attract target DNA, which is subsequently sequenced. Since these probe bases do not represent genomic material, they must be removed at some point during the analytic pipeline prior to variant calling, otherwise they could act as a reference

bias when calling variants. Removal from the primary arm where the probe is attached is typically easy enough (e.g. via `fastqtrim`), however in cases of high read overlap, probe sequence can also be present in the other read arm.

`petrim` aligns each read arm against it's mate with high stringency in order to identify cases of read overlap. The sensitivity of read overlap detection is primarily controlled through the use of `--min-identity` and `--min-overlap-length`, although it is also possible to adjust the penalties used during alignment.

The following types of trimming or merging may be applied.

- Removal of non-genomic bases due to complete read-through. This removal is always applied.
- Removal of overlap bases impinging into regions occupied by probe bases. For example, if the left arms contain 11-mer probes, using `--left-probe-length=11` will result in the removal of any right arm bases that overlap into the first 11 bases of the left arm. Similar trimming is available for situations where probes are ligated to the right arm by using `--right-probe-length`.
- Adjustment of mismatching read bases inside areas of overlap. Such mismatches indicate that one or other of the bases has been incorrectly sequenced. Alteration of these bases is selected by supplying the `--mismatch-adjustment` flag with a value of `zero-phred` to alter the phred quality score of both bases to zero, or `pick-best` to choose whichever base had the higher reported quality score.
- Removal of overlap regions by trimming both arms back to a point where no overlap is present. An equal number of bases are removed from each arm. This trimming is enabled by specifying `--midpoint-trim` and takes place after any read-through or probe related trimming.
- Merging non-redundant sequence from both reads to create a single read, enabled via `--midpoint-merge`. This is like `--midpoint-trim` with a subsequent moving of the R2 read onto the end of the the R1 read (thus the R2 read becomes empty).

After trimming or merging it is possible that one or both of the arms of the pair have no bases remaining, and a strategy is needed to handle these pairs. The default is to retain such pairs in the output, even if one or both are zero-length. When both arms are zero-length, the pair can be dropped from output with the use of `--discard-empty-pairs`. If downstream processing cannot handle zero-length reads, `--discard-empty-reads` will drop a read pair if either of the arms is zero-length.

`petrim` also provides the ability to down-sample a read set by using the `--subsample` option. This will produce a different sampling each time, unless an explicit randomization seed is specified via `--seed`.

Formatting with paired-end trimming on the fly

Running custom filtering with `petrim` can be done in standard Unix shells without incurring the use of additional disk space or unduly slowing down the formatting of reads. The following example demonstrates how to apply paired-end trimming while formatting to SDF:

```
$ rtg format -f fastq-interleaved -o S12_trimmed.sdf \
    <(rtg petrim -l S12_R1.fastq.gz -r S12_R2.fastq.gz -m -o - --interleaved)
```

This can even be combined with `fastqtrim` to provide extremely flexible trimming:

```
$ rtg format -f fastq-interleaved -o S12_trimmed.sdf \
    <(rtg petrim -m -o - --interleave \
         -l <(rtg fastqtrim -s 12 -E 18 -i S12_R1.fastq.gz -o -) \
         -r <(rtg fastqtrim -E 18 -i S12_R2.fastq.gz -o -) \
     )
```

Note: `petrim` currently assumes Illumina paired-end sequencing, and aligns the reads in FR orientation. Sequencing methods which produce arms in a different orientation can be processed by first converting the input files using `fastqtrim --reverse-complement`, running `petrim`, followed by another `fastqtrim --reverse-complement` to restore the reads to their original orientation.

See also:

fastqtrim, *format*

2.4 Simulation Commands

RTG includes some simulation commands that may be useful for experimenting with effects of various RTG command parameters or when getting familiar with RTG work flows. A simple simulation series might involve the following commands:

```
$ rtg genomesim --output sim-ref-sdf --min-length 500000 --max-length 5000000 \
  --num-contigs 5
$ rtg popsim --reference sim-ref-sdf --output population.vcf.gz
$ rtg samplesim --input population.vcf.gz --output sample1.vcf.gz \
  --output-sdf sample1-sdf --reference sim-ref-sdf --sample sample1
$ rtg readsim --input sample1-sdf --output reads-sdf --machine illumina_pe \
  -L 75 -R 75 --coverage 10
$ rtg map --template sim-ref-sdf --input reads-sdf --output sim-mapping \
  --sam-rg "@RG\tID:sim-rg\tSM:sample1\tPL:ILLUMINA"
$ rtg snp --template sim-ref-sdf --output sim-name-snp sim-mapping/alignments.bam
```

2.4.1 genomesim

Synopsis:

Use the `genomesim` command to simulate a reference genome, or to create a baseline reference genome for a research project when an actual genome reference sequence is unavailable.

Syntax:

Specify number of sequences, plus minimum and maximum lengths:

```
$ rtg genomesim [OPTION]... -o SDF --max-length INT --min-length INT -n INT
```

Specify explicit sequence lengths (one more sequences):

```
$ rtg genomesim [OPTION]... -o SDF -l INT
```

Example:

```
$ rtg genomesim -o genomeTest -l 500000
```

Parameters:

File Input/Output		
`-o`	`--output=SDF`	The name of the output SDF.

Utility		
	--comment=STRING	Specify a comment to include in the generated SDF.
	--freq=STRING	Set the relative frequencies of A,C,G,T in the generated sequence. (Defa is 1,1,1,1).
-h	--help	Prints help on command-line flag usage.
-l	--length=INT	Specify the length of generated sequence. May be specified 0 or more times, or as a comma separated list.
	--max-length=INT	Specify the maximum sequence length.
	--min-length=INT	Specify the minimum sequence length.
-n	--num-contigs=INT	Specify the number of sequences to generate.
	--prefix=STRING	Specify a sequence name prefix to be used for the generated sequences. The default is to name the output sequences 'simulatedSequenceN', whe N is increasing for each sequence.
-s	--seed=INT	Specify seed for the random number generator.

Usage:

The `genomesim` command allows one to create a simulated genome with one or more contiguous sequences - exact lengths of each contig or number of contigs with minimum and maximum lengths provided. The contents of an SDF directory created by `genomesim` can be exported to a FASTA file using the `sdf2fasta` command.

This command is primarily useful for providing a simple randomly generated base genome for use with subsequent simulation commands.

Each generated contig is named by appending an increasing numeric index to the specified prefix. For example `--prefix=chr --num-contigs=10` would yield contigs named `chr1` through `chr10`.

See also:

cgsim, *readsim*, *popsim*, *samplesim*

2.4.2 cgsim

Synopsis:

Simulate Complete Genomics Inc sequencing reads. Supports the original 35 bp read structure (5-10-10-10), and the newer 29 bp read structure (10-9-10).

Syntax:

Generation by genomic coverage multiplier:

```
$ rtg cgsim [OPTION]... -V INT -t SDF -o SDF -c FLOAT
```

Generation by explicit number of reads:

```
$ rtg cgsim [OPTION]... -V INT -t SDF -o SDF -n INT
```

Example:

```
$ rtg cgsim -V 1 -t HUMAN_reference -o CG_3x_readst -c 3
```

Parameters:

File Input/Output		
-t	--input=SDF	SDF containing input genome.
-o	--output=SDF	Name for reads output SDF.

Fragment Generation		
	`--abundance`	If set, the user-supplied distribution represents desired abundance.
`-N`	`--allow-unknowns`	Allow reads to be drawn from template fragments containing unknown nucleotides.
`-c`	`--coverage=FLOAT`	Coverage, must be positive.
`-D`	`--distribution=FILE`	File containing probability distribution for sequence selection.
	`--dna-fraction`	If set, the user-supplied distribution represents desired DNA fraction.
`-M`	`--max-fragment-size=INT`	Maximum fragment size (Default is 500)
`-m`	`--min-fragment-size=INT`	Minimum fragment size (Default is 350)
	`--n-rate=FLOAT`	Rate that the machine will generate new unknowns in the read (Default is 0.0)
`-n`	`--num-reads=INT`	Number of reads to be generated.
	`--taxonomy-distribution=FILE`	File containing probability distribution for sequence selection expressed by taxonomy id.

Complete Genomics		
`-V`	`--cg-read-version=INT`	Select Complete Genomics read structure version, 1 (35 bp) o (29 bp)

Utility		
	`--comment=STRING`	Comment to include in the generated SDF.
`-h`	`--help`	Print help on command-line flag usage.
	`--no-names`	Do not create read names in the output SDF.
	`--no-qualities`	Do not create read qualities in the output SDF.
`-q`	`--qual-range=STRING`	Set the range of base quality values permitted e.g.: 3-40 (Default is fixed qualities corresponding to overall machine base error rate)
	`--sam-rg=STRING|FILE`	File containing a single valid read group SAM header line or a string in the form `@RG\tID:READGROUP1\tSM:BACT_SAMPLE\tPL:ILLUMIN`
`-s`	`--seed=INT`	Seed for random number generator.

Usage:

Use the `cgsim` command to set either `--coverage` or `--num-reads` in simulated Complete Genomics reads. For more information about Complete Genomics reads, refer to http://www.completegenomics.com

RTG simulation tools allows for deterministic experiment repetition. The `--seed` parameter, for example, allows for regeneration of exact same reads by setting the random number generator to be repeatable (without supplying this flag a different set of reads will be generated each time).

The `--distribution` parameter allows you to specify the probability that a read will come from a particular named sequence for use with metagenomic databases. Probabilities are numbers between zero and one and must sum to one in the file.

See also:

genomesim, *readsim*, *popsim*, *samplesim*

2.4.3 readsim

Synopsis:

Use the `readsim` command to generate single or paired end reads of fixed or variable length from a reference genome, introducing machine errors.

Syntax:

Generation by genomic coverage multiplier:

```
$ rtg readsim [OPTION]... -t SDF --machine STRING -o SDF -c FLOAT
```

Generation by explicit number of reads:

```
$ rtg readsim [OPTION]... -t SDF --machine STRING -o SDF -n INT
```

Example:

```
$ rtg readsim -t genome_ref -o sim_reads -r 75 --machine illumina_se  -c 30
```

Parameters:

File Input/Output		
`-t`	`--input=SDF`	SDF containing input genome.
	`--machine=STRING`	Select the sequencing technology to model. Allowed values are [illumina_se, illumina_pe, complete_genomics, complete_genomics_2, 454_pe, 454_se, iontorrent]
`-o`	`--output=SDF`	Name for reads output SDF.

Fragment Generation		
	`--abundance`	If set, the user-supplied distribution represents desired abundance.
`-N`	`--allow-unknowns`	Allow reads to be drawn from template fragments containing unknown nucleotides.
`-c`	`--coverage=FLOAT`	Coverage, must be positive.
`-D`	`--distribution=FILE`	File containing probability distribution for sequence selection.
	`--dna-fraction`	If set, the user-supplied distribution represents desired DNA fraction.
`-M`	`--max-fragment-size=INT`	Maximum fragment size (Default is 250)
`-m`	`--min-fragment-size=INT`	Minimum fragment size (Default is 200)
	`--n-rate=FLOAT`	Rate that the machine will generate new unknowns in the read (Default is 0.0)
`-n`	`--num-reads=INT`	Number of reads to be generated.
	`--taxonomy-distribution=FILE`	File containing probability distribution for sequence selection expressed by taxonomy id.

Illumina PE		
`-L`	`--left-read-length=INT`	Target read length on the left side.
`-R`	`--right-read-length=INT`	Target read length on the right side.

Illumina SE		
`-r`	`--read-length=INT`	Target read length, must be positive.

454 SE/PE		
	`--454-max-total-size=INT`	Maximum 454 read length (in paired end case the sum of the left and the right read lengths)
	`--454-min-total-size=INT`	Minimum 454 read length (in paired end case the sum of the left and the right read lengths)

IonTorrent SE		
	`--ion-max-total-size=INT`	Maximum IonTorrent read length.
	`--ion-min-total-size=INT`	Minimum IonTorrent read length.

Utility		
	`--comment=STRING`	Comment to include in the generated SDF.
`-h`	`--help`	Print help on command-line flag usage.
	`--no-names`	Do not create read names in the output SDF.
	`--no-qualities`	Do not create read qualities in the output SDF.
`-q`	`--qual-range=STRING`	Set the range of base quality values permitted e.g.: 3-40 (Default is fixed qualities corresponding to overall machine base error rate)
	`--sam-rg=STRING|FILE`	File containing a single valid read group SAM header line or a string in the form `@RG\tID:READGROUP1\tSM:BACT_SAMPLE\tPL:ILLUMIN`
`-s`	`--seed=INT`	Seed for random number generator.

Usage:

Create simulated reads from a reference genome by either specifying coverage depth or a total number of reads.

A typical use case involves creating a mutated genome by introducing SNPs or CNVs with `popsim` and `samplesim` generating reads from the mutated genome with `readsim`, and mapping them back to the original reference to verify the parameters used for mapping or variant detection.

RTG simulation tools allows for deterministic experiment repetition. The `--seed` parameter, for example, allows for regeneration of exact same reads by setting the random number generator to be repeatable (without supplying this flag a different set of reads will be generated each time).

The `--distribution` parameter allows you to specify the sequence composition of the resulting read set, primarily for use with metagenomic databases. The distribution file is a text file containing lines of the form:

```
<probability><space><sequence name>
```

Probabilities must be between zero and one and must sum to one in the file. For reference databases containing taxonomy information, where each species may be comprised of more than one sequence, it is instead possible to use the `--taxonomy-distribution` option to specify the probabilities at a per-species level. The format of each line in this case is:

```
<probability><space><taxon id>
```

When using `--distribution` or `--taxonomy-distribution`, the interpretation must be specified one of `--abundance` or `--dna-fraction`. When using `--abundance` each specified probability reflects the chance of selecting the specified sequence (or taxon id) from the set of sequences, and thus for a given abundance a large sequence will be represented by more reads in the resulting set than a short sequence. In contrast, with `--dna-fraction` each specified probability reflects the chance of a read being derived from the designated sequence, and thus for a given fraction, a large sequence will have a lower depth of coverage than a short sequence.

See also:

cgsim, *genomesim*, *popsim*, *samplesim*

2.4.4 popsim

Synopsis:

Use the `popsim` command to generate a VCF containing simulated population variants. Each variant allele generated has an associated frequency `INFO` field describing how frequent in the population that allele is.

Syntax:

```
$ rtg popsim [OPTION]... -o FILE -t SDF
```

Example:

```
$ rtg popsim -o pop.vcf -t HUMAN_reference
```

Parameters:

File Input/Output		
`-o`	`--output=FILE`	Output VCF file name.
`-t`	`--reference=SDF`	SDF containing the reference genome.

Utility		
`-h`	`--help`	Print help on command-line flag usage.
`-Z`	`--no-gzip`	Do not gzip the output.
	`--seed=INT`	Seed for the random number generator.

Usage:

The `popsim` command is used to create a VCF containing variants with frequency in population information that can be subsequently used to simulate individual samples using the `samplesim` command. The frequency in population is contained in a VCF `INFO` field called `AF`. The types of variants and the allele-frequency distribution has been drawn from observed variants and allele frequency distribution in human studies.

See also:

readsim, *genomesim*, *samplesim*, *childsim*, *samplereplay*

2.4.5 samplesim

Synopsis:

Use the `samplesim` command to generate a VCF containing a genotype simulated from population variants according to allele frequency.

Syntax:

```
$ rtg samplesim [OPTION]... -i FILE -o FILE -t SDF -s STRING
```

Example:

From a population frequency VCF:

```
$ rtg samplesim -i pop.vcf -o 1samples.vcf -t HUMAN_reference -s person1 --sex male
```

From an existing simulated VCF:

```
$ rtg samplesim -i 1samples.vcf -o 2samples.vcf -t HUMAN_reference -s person2 \
  --sex female
```

Parameters:

File Input/Output		
-i	--input=FILE	Input VCF containing population variants.
-o	--output=FILE	Output VCF file name.
	--output-sdf=SDF	If set, output an SDF containing the sample genome.
-t	--reference=SDF	SDF containing the reference genome.
-s	--sample=STRING	Name for sample.

Utility		
	--allow-missing-af	If set, treat variants without allele frequency annotation as unifo likely.
-h	--help	Print help on command-line flag usage.
-Z	--no-gzip	Do not gzip the output.
	--ploidy=STRING	Ploidy to use. Allowed values are [auto, diploid, haploid] (Defa auto)
	--seed=INT	Seed for the random number generator.
	--sex=SEX	Sex of individual. Allowed values are [male, female, either] (De either)

Usage:

The `samplesim` command is used to simulate an individuals genotype information from a population variant frequency VCF generated by the `popsim` command or by previous `samplesim` or `childsim` commands. The new output VCF will contain all the existing variants and samples with a new column for the new sample. The genotype at each record of the VCF will be chosen randomly according to the allele frequency specified in the `AF` field.

If input VCF records do not contain an `AF` annotation, by default any ALT allele in that record will not be selected and so the sample will be genotyped as 0/0. Alternatively for simple simulations the `--allow-missing-af` flag will treat each allele in such records as being equally likely (i.e.: effectively equivalent to `AF=0.5` for a biallelic variant, `AF=0.33,0.33` for a triallelic variant, etc).

The ploidy for each genotype is automatically determined according to the ploidy of that chromosome for the specified sex of the individual, as defined in the reference genome `reference.txt` file. For more information see *RTG reference file format*. If the reference SDF does not contain chromosome configuration information, a default ploidy can be specified using the `--ploidy` flag.

The `--output-sdf` flag can be used to optionally generate an SDF of the individuals genotype which can then be used by the `readsim` command to simulate a read set for the individual.

See also:

readsim, *genomesim*, *popsim*, *childsim*, *samplereplay*

2.4.6 denovosim

Synopsis:

Use the `denovosim` command to generate a VCF containing a derived genotype containing *de novo* variants.

Syntax:

```
$ rtg denovosim [OPTION]... -i FILE --original STRING -o FILE -t SDF -s STRING
```

Example:

```
$ rtg denovosim -i sample.vcf --original personA -o 2samples.vcf \
  -t HUMAN_reference -s personB
```

Parameters:

File Input/Output		
-i	--input=FILE	The input VCF containing parent variants.
	--original=STRING	The name of the existing sample to use as the original genotype.
-o	--output=FILE	The output VCF file name.
	--output-sdf=FILE	Set to output an SDF of the genome generated.
-t	--reference=SDF	The SDF containing the reference genome.
-s	--sample=STRING	The name for the new derived sample.

Utility		
-h	--help	Prints help on command-line flag usage.
-Z	--no-gzip	Set this flag to create the VCF output file without compression.
	--num-mutations=INT	Set the expected number of de novo mutations per genome (Default is 70).
	--ploidy=STRING	The ploidy to use when the reference genome does not contain a reference text file. Allowed values are [auto, diploid, haploid] (Defau is auto)
	--seed=INT	Set the seed for the random number generator.
	--show-mutations	Set this flag to display information regarding de novo mutation points

Usage:

The `denovosim` command is used to simulate a derived genotype containing *de novo* variants from a VCF containing an existing genotype.

The output VCF will contain all the existing variants and samples, along with additional *de novo* variants. If the original and derived sample names are different, the output will contain a new column for the mutated sample. If the original and derived sample names are the same, the sample in the output VCF is updated rather than creating an entirely new sample column. When a sample receives a *de novo* mutation, the sample `DN` field is set to "Y".

If *de novo* variants were introduced without regard to neighboring variants, a situation could arise where it is not possible to unambiguously determine the haplotype of the simulated sample. To prevent this, `denovosim` will not output a *de novo* variant that overlaps existing variants. Since `denovosim` chooses candidate *de novo* locations before reading the input VCF, this occasionally mandates skipping a candidate *de novo* so the target number of mutations may not always be reached.

The `--output-sdf` flag can be used to optionally generate an SDF of the derived genome which can then be used by the `readsim` command to simulate a read set for the new genome.

See also:

readsim, *genomesim*, *popsim*, *samplesim*, *samplereplay*

2.4.7 childsim

Synopsis:

Use the `childsim` command to generate a VCF containing a genotype simulated as a child of two parents.

Syntax:

```
$ rtg childsim [OPTION]... --father STRING -i FILE --mother STRING -o FILE -t SDF \
  -s STRING
```

Example:

```
$ rtg childsim --father person1 --mother person2 -i 2samples.vcf -o 3samples.vcf \
  -t HUMAN_reference -s person3
```

Parameters:

File Input/Output		
	`--father=STRING`	Name of the existing sample to use as the father.
`-i`	`--input=FILE`	Input VCF containing parent variants.
	`--mother=STRING`	Name of the existing sample to use as the mother.
`-o`	`--output=FILE`	Output VCF file name.
	`--output-sdf=SDF`	If set, output an SDF containing the sample genome.
`-t`	`--reference=SDF`	SDF containing the reference genome.
`-s`	`--sample=STRING`	Name for new child sample.

Utility		
	`--extra-crossovers=FLOAT`	Probability of extra crossovers per chromosome (Default is
	`--genetic-map-dir=DIR`	If set, load genetic maps from this directory for recombinat point selection.
`-h`	`--help`	Print help on command-line flag usage.
`-Z`	`--no-gzip`	Do not gzip the output.
	`--ploidy=STRING`	Ploidy to use. Allowed values are [auto, diploid, haploid] (Default is auto)
	`--seed=INT`	Seed for the random number generator.
	`--sex=SEX`	Sex of individual. Allowed values are [male, female, either (Default is either)
	`--show-crossovers`	If set, display information regarding haplotype selection an crossover points.

Usage:

The `childsim` command is used to simulate an individuals genotype information from a VCF containing the two parent genotypes generated by previous `samplesim` or `childsim` commands. The new output VCF will contain all the existing variants and samples with a new column for the new sample.

The ploidy for each genotype is generated according to the ploidy of that chromosome for the specified sex of the individual, as defined in the reference genome `reference.txt` file. For more information see *RTG reference file format*. The generated genotypes are all consistent with Mendelian inheritance (*de novo* variants can be simulated with the `denovosim` command).

The `--output-sdf` flag can be used to optionally generate an SDF of the child's genotype which can then be used by the `readsim` command to simulate a read set for the child.

By default positions for crossover events are chosen according to a uniform distribution. However, if linkage information is available, then this can be used to inform the crossover selection procedure. The expected format for this information is described in *Genetic map directory*, and the directory containing the relevant files can be specified by using the `--genetic-map-dir` flag.

See also:

readsim, *genomesim*, *popsim*, *samplesim*, *samplereplay*

2.4.8 pedsamplesim

Synopsis:

Generates simulated genotypes for all members of a pedigree. `pedsamplesim` automatically simulates founder individuals, inheritance by children, and de novo mutations.

Syntax:

```
$ rtg pedsamplesim [OPTION]... -i FILE -o DIR -p FILE -t SDF
```

Example:

```
$ rtg pedsamplesim -t reference.sdf -p family.ped -i popvars.vcf \
  -o family_sim --remove-unused
```

Parameters:

File Input/Output		
`-i`	`--input=FILE`	Input VCF containing parent variants.
`-o`	`--output=DIR`	Directory for output.
	`--output-sdf`	If set, output an SDF for the genome of each simulated sample.
`-p`	`--pedigree=FILE`	Genome relationships PED file.
`-t`	`--reference=SDF`	SDF containing the reference genome.

Utility		
	`--extra-crossovers=FLOAT`	Probability of extra crossovers per chromosome (Default is
	`--genetic-map-dir=DIR`	If set, load genetic maps from this directory for recombinat point selection.
`-h`	`--help`	Print help on command-line flag usage.
`-Z`	`--no-gzip`	Do not gzip the output.
	`--num-mutations=INT`	Expected number of mutations per genome (Default is 70)
	`--ploidy=STRING`	Ploidy to use. Allowed values are [auto, diploid, haploid] (Default is auto)
	`--remove-unused`	If set, output only variants used by at least one sample.
	`--seed=INT`	Seed for the random number generator.

Usage:

The `pedsamplesim` uses the methods of `samplesim`, `denovosim`, and `childsim` to greatly ease the simulation of multiple samples. The input VCF should contain standard allele frequency INFO annotations that will be used to simulate genotypes for any sample identified as a founder. Any samples present in the pedigree that are already present in the input VCF will not be regenerated. To simulate genotypes for a subset of the members of the pedigree, use `pedfilter` to create a filtered pedigree file that includes only the subset required.

The supplied pedigree file is first examined to identify any individuals that cannot be simulated according to inheritance from other samples in the pedigree. Note that simulation according to inheritance requires both parents to be present in the pedigree. These samples in the pedigree are treated as founder individuals.

Founder individuals are simulated using `samplesim`, where the genotypes are chosen according to the allele frequency annotation in the input VCF.

All newly generated samples may have *de novo* mutations introduced according to the `--num-mutations` setting. As with the `denovosim` command, any *de novo* mutations introduced in a sample will be genotyped as homozygous reference in other pre-existing samples, and introduced variants will not overlap any pre-existing variant loci.

Samples that can be simulated according to Mendelian inheritance are then generated, using `childsim`. As expected, as well as inheriting *de novo* variants from parents, each child may obtain new *de novo* mutations of their own.

If the simulated samples will be used for subsequent simulated sequencing, such as via `readsim`, it is possible to automatically output an SDF containing the simulated genome for each sample by specifying the `--output-sdf` option, obviating the need to separately use `samplereplay`.

By default positions for crossover events are chosen according to a uniform distribution. However, if linkage information is available, then this can be used to inform the crossover selection procedure. The expected format for this information is described in *Genetic map directory*, and the directory containing the relevant files can be specified by using the `--genetic-map-dir` flag.

See also:

pedfilter, *popsim*, *samplesim*, *childsim*, *denovosim*, *samplereplay*, *readsim*

2.4.9 samplereplay

Synopsis:

Use the `samplereplay` command to generate the genome SDF corresponding to a sample genotype in a VCF file.

Syntax:

```
$ rtg samplereplay [OPTION]... -i FILE -o SDF -t SDF -s STRING
```

Example:

```
$ rtg samplereplay -i 3samples.vcf -o child.sdf -t HUMAN_reference -s person3
```

Parameters:

File Input/Output		
`-i`	`--input=FILE`	Input VCF containing the sample genotype.
`-o`	`--output=SDF`	Name for output SDF.
`-t`	`--reference=SDF`	SDF containing the reference genome.
`-s`	`--sample=STRING`	Name of the sample to select from the VCF.

Utility		
`-h`	`--help`	Print help on command-line flag usage.

Usage:

The `samplereplay` command can be used to generate an SDF of a genotype for a given sample from an existing VCF file. This can be used to generate a genome from the outputs of the `samplesim` and `childsim` commands. The output genome can then be used in simulating a read set for the sample using the `readsim` command.

Every chromosome for which the individual is diploid will have two sequences in the resulting SDF.

See also:

readsim, *genomesim*, *popsim*, *samplesim*, *childsim*

2.5 Utility Commands

2.5.1 bgzip

Synopsis:

Block compress a file or decompress a block compressed file. Block compressed outputs from the mapping and variant detection commands can be indexed with the `index` command. They can also be processed with standard gzip tools such as `gunzip` and `zcat`.

Syntax:

```
$ rtg bgzip [OPTION]... FILE+
```

Example:

```
$ rtg bgzip alignments.sam
```

Parameters:

File Input/Output		
`-l`	`--compression-level=INT`	The compression level to use, between 1 (least but fast) and 9 (highest but slow) (Default is 5)
`-d`	`--decompress`	Decompress.
`-f`	`--force`	Force overwrite of output file.
	`--no-terminate`	If set, do not add the block gzip termination block.
`-c`	`--stdout`	Write on standard output, keep original files unchanged. Implied when using standard input.
	`FILE+`	File to (de)compress, use '-' for standard input. Must be specified 1 or more times.

Utility		
`-h`	`--help`	Print help on command-line flag usage.

Usage:

Use the `bgzip` command to block compress files. Files such as VCF, BED, SAM, TSV must be block-compressed before they can be indexed for fast retrieval of records corresponding to specific genomic regions.

See also:

index

2.5.2 index

Synopsis:

Create tabix index files for block compressed TAB-delimited genome position data files or BAM index files for BAM files.

Syntax:

Multi-file input specified from command line:

```
$ rtg index [OPTION]... FILE+
```

Multi-file input specified in a text file:

```
$ rtg index [OPTION]... -I FILE
```

Example:

```
$ rtg index -f sam alignments.sam.gz
```

Parameters:

File Input/Output		
-f	--format=FORMAT	Format of input to index. Allowed values are [sam, bam, cram, sv, coveragetsv, bed, vcf, auto] (Default is auto)
-I	--input-list-file=FILE	File containing a list of block compressed files (1 per line) containing genome position data.
	FILE+	Block compressed files containing data to be indexed. May be specified 0 or more times.

Utility		
-h	--help	Print help on command-line flag usage.

Usage:

Use the `index` command to produce tabix indexes for block compressed genome position data files like SAM files, VCF files, BED files, and the TSV output from RTG commands such as `coverage`. The `index` command can also be used to produce BAM indexes for BAM files with no index.

See also:

map, *coverage*, *snp*, *extract*, *bgzip*

2.5.3 extract

Synopsis:

Extract specified parts of an indexed block compressed genome position data file.

Syntax:

Extract whole file:

```
$ rtg extract [OPTION]... FILE
```

Extract specific regions:

```
$ rtg extract [OPTION]... FILE STRING+
```

Example:

```
$ rtg extract alignments.bam 'chr1:2500000~1000'
```

Parameters:

File Input/Output		
	FILE	The indexed block compressed genome position data file to extract.

Filtering		
	REGION+	The range to display. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<len <sequence_name>:<pos>~<padding>. May be specified 0 or more

Reporting		
	`--header`	Set to also display the file header.
	`--header-only`	Set to only display the file header.

Utility		
`-h`	`--help`	Prints help on command-line flag usage.

Usage:

Use the `extract` command to view specific parts of indexed block compressed genome position data files such as those in SAM/BAM/BED/VCF format.

See also:

map, *coverage*, *snp*, *index*, *bgzip*

2.5.4 aview

Synopsis:

View read mapping and variants corresponding to a region of the genome, with output as ASCII to the terminal, or HTML.

Syntax:

```
$ rtg aview [OPTION]... --region STRING -t SDF FILE+
```

Example:

```
$ rtg aview -t hg19 -b omni.vcf -c calls.vcf map/alignments.bam \
  --region Chr10:100000+3 -padding 30
```

Parameters:

File Input/Output		
`-b`	`--baseline=FILE`	VCF file containing baseline variants.
`-B`	`--bed=FILE`	BED file containing regions to overlay. May be specified 0 or more times.
`-c`	`--calls=FILE`	VCF file containing called variants. May be specified 0 or more times.
`-I`	`--input-list-file=FILE`	File containing a list of SAM/BAM format files (1 per line)
`-r`	`--reads=SDF`	Read SDF (only needed to indicate correctness of simulated read mappings). May be specified 0 or more times.
`-t`	`--template=SDF`	SDF containing the reference genome.
	`FILE+`	Alignment SAM/BAM files. May be specified 0 or more times.

Filtering		
`-p`	`--padding=INT`	Padding around region of interest (Default is to automatically determine padding to avoid read truncation)
	`--region=REGION`	The region of interest to display. The format is one of <sequence_name> <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> <sequence_name>:<pos>~<padding>
	`--sample=STRING`	Specify name of sample to select. May be specified 0 or more times, or comma separated list.

Reporting		
	`--html`	Output as HTML.
	`--no-base-colors`	Do not use base-colors.
	`--no-color`	Do not use colors.
	`--no-dots`	Display nucleotide instead of dots.
	`--print-cigars`	Print alignment cigars.
	`--print-mapq`	Print alignment MAPQ values.
	`--print-mate-position`	Print mate position.
	`--print-names`	Print read names.
	`--print-readgroup`	Print read group id for each alignment.
	`--print-reference-line=INT`	Print reference line every N lines (Default is 0)
	`--print-sample`	Print sample id for each alignment.
	`--print-soft-clipped-bases`	Print soft clipped bases.
	`--project-track=INT`	If set, project highlighting for the specified track down through reads (Default projects the union of tracks)
	`--sort-readgroup`	Sort reads first on read group and then on start position.
	`--sort-reads`	Sort reads on start position.
	`--sort-sample`	Sort reads first on sample id and then on start position.
	`--unflatten`	Display unflattened CGI reads when present.

Utility		
`-h`	`--help`	Print help on command-line flag usage.

Usage:

Use the `aview` command to display a textual view of mappings and variants corresponding to a small region of the reference genome. This is useful when examining evidence for variant calls in a server environment where a graphical display application such as IGV is not available. The `aview` command is easy to script in order to output displays for multiple regions for later viewing (either as text or HTML).

See also:

map, *snp*

2.5.5 sdfstats

Synopsis:

Print statistics that describe a directory of SDF formatted data.

Syntax:

```
$ rtg sdfstats [OPTION]... SDF+
```

Example:

```
$ rtg sdfstats human_READS_SDF

Location           : C:\human_READS_SDF
Parameters         : format -f solexa -o human_READS_SDF
                              c:\users\Elle\human\SRR005490.fastq.gz
SDF Version        : 6
Type               : DNA
Source             : SOLEXA
Paired arm         : UNKNOWN
Number of sequences: 4193903
Maximum length     : 48
Minimum length     : 48
```

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```
N                   : 931268
A                   : 61100096
C                   : 41452181
G                   : 45262380
T                   : 52561419
Total residues      : 201307344
Quality scores available on this SDF
```

Parameters:

File Input/Output		
	SDF+	Specifies an SDF on which statistics are to be reported. May b

Reporting		
	--lengths	Set to print out the name and length of each sequence. (Not recomm sets).
-p	--position	Set to include information about unknown bases (Ns) by read positi
-q	--quality	Set to display mean of quality.
	--sex=SEX	Set to display the reference sequence list for the given sex. Allowed female, either]. May be specified 0 or more times, or as a comma se
	--taxonomy	Set to display information about the taxonomy.
-n	--unknowns	Set to include information about unknown bases (Ns).

Utility		
-h	--help	Prints help on command-line flag usage.

Usage:

Use the `sdfstats` command to get information about the contents of SDFs.

See also:

format, *sdf2fasta*, *sdf2fastq*, *sdfstats*

2.5.6 sdfsubset

Synopsis:

Extracts a specified subset of sequences from one SDF and outputs them to another SDF.

Syntax:

Individual specification of sequence ids:

```
$ rtg sdfsubset [OPTION]... -i SDF -o SDF STRING+
```

File list specification of sequence ids:

```
$ rtg sdfsubset [OPTION]... -i SDF -o SDF -I FILE
```

Example:

```
$ rtg sdfsubset -i reads -o subset_reads 10 20 30 40 50
```

Parameters:

File Input/Output		
-i	--input=SDF	Specifies the input SDF.
-o	--output=SDF	The name of the output SDF.

Filtering		
	--end-id=INT	Only output sequences with sequence id less than the given number. ids start at 0).
	--start-id=INT	Only output sequences with sequence id greater than or equal to the number. (Sequence ids start at 0).
-I	--id-file=FILE	Name of a file containing a list of sequences to extract, one per line.
	--names	Interpret any specified sequence as names instead of numeric sequen
	STRING+	Specifies the sequence id, or sequence name if the names flag is set t from the input SDF. May be specified 0 or more times.

Utility		
-h	--help	Prints help on command-line flag usage.

Usage:

Use this command to obtain a subset of sequences from an SDF. Either specify the subset on the command line as a list of space-separated sequence ids or using the --id-file parameter to specify a file containing a list of sequence ids, one per line. Sequence ids start from zero and are the same as the ids that map uses by default in the QNAME field of its BAM files.

For example:

```
$ rtg sdfsubset -i reads -o subset_reads 10 20 30 40 50
```

This will produce an SDF called subset_reads with sequences 10, 20, 30, 40 and 50 from the original SDF contained in it.

See also:

sdfsubseq, *sdfstats*

2.5.7 sdfsubseq

Synopsis:

Prints a subsequence of a given sequence in an SDF.

Syntax:

Print sequences from sequence names:

```
$ rtg sdfsubseq [OPTION]... -i FILE STRING+
```

Print sequences from sequence ids:

```
$ rtg sdfsubseq [OPTION]... -i FILE -I STRING+
```

Example:

```
$ rtg sdfsubseq -i reads -I 0:1+100
```

Parameters:

File Input/Output		
-i	--input=FILE	Specifies the input SDF.

Filtering		
-I	--sequence-id	If set, use sequence id instead of sequence name in region (0-based)
	REGION+	The range to display. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> <sequence_name>:<pos>~<padding>. Must be specified 1 or more tim

Utility		
-f	--fasta	Set to output in FASTA format.
-q	--fastq	Set to output in FASTQ format.
-h	--help	Prints help on command-line flag usage.
-r	--reverse-complement	Set to output in reverse complement.

Usage:

Prints out the nucleotides or amino acids of specified regions in a set of sequences.

For example:

```
$ rtg sdfsubseq --input reads --sequence-id 0:1+20
AGGCGTCTGCAGCCGACGCG
```

See also:

sdfsubset, *sdfstats*

2.5.8 mendelian

Synopsis:

The mendelian command checks a multi-sample VCF file for variant calls which do not follow Mendelian inheritance, and compute aggregate sample concordance.

Syntax:

```
$ rtg mendelian [OPTION]... -i FILE -t SDF
```

Example:

```
$ rtg mendelian -i family.vcf.gz -t genome_ref
```

Parameters:

File Input/Output		
-i	--input=FILE	VCF file containing multi-sample variant calls. Use '-' to read from standard input.
-o	--output=FILE	If set, output annotated calls to this VCF file. Use '-' to write to standard output.
	--output-consistent=FILE	If set, output only consistent calls to this VCF file.
	--output-inconsistent=FILE	If set, output only non-Mendelian calls to this VCF file.
-t	--template=SDF	SDF containing the reference genome.

Sensitivity Tuning		
	`--all-records`	Use all records, regardless of filters (Default is to only process records where FILTER is `.` or `PASS`)
`-l`	`--lenient`	Allow homozygous diploid calls in place of haploid calls and assume missing values are equal to the reference.
	`--min-concordance=FLOAT`	Percentage concordance required for consistent parentage (Default is 99.0)
	`--pedigree=FILE`	Genome relationships PED file (Default is to extract pedigree information from VCF header fields)

Utility		
`-h`	`--help`	Print help on command-line flag usage.
`-Z`	`--no-gzip`	Do not gzip the output.

Usage:

Given a multi-sample VCF file for a nuclear family with a defined pedigree, the `mendelian` command examines the variant calls and outputs the number of violations of Mendelian inheritance. If the `--output-inconsistent` parameter is set, all detected violations are written into an output VCF file. As such, this command may be regarded as a VCF filter, outputting those variant calls needing a non-Mendelian explanation. Such calls may be the consequence of sequencing error, calling on low-coverage, or genuine novel variants in one or more individuals.

Pedigree information regarding the relationships between samples and the sex of each sample is extracted from the VCF headers automatically created by the RTG pedigree-aware variant calling commands. If this pedigree information is absent from the VCF header or is incorrect, a pedigree file can be explicitly supplied with the `--pedigree` flag.

To ensure correct behavior when dealing with sex chromosomes it is necessary to specify a sex-aware reference and ensure the sex of each sample is supplied as part of the pedigree information. While it is best to give the reference SDF used in the creation of the VCF, for checking third-party outputs any reference SDF containing the same chromosome names and an appropriate `reference.txt` file will work. For more information, see *RTG reference file format*. Variants calls where the call ploidy does not match what is expected are annotated in the output VCF with an `MCP` FORMAT annotation.

Particularly when evaluating VCF files that have been produced by third party tools or when the VCF is the result of combining independent per-sample calling, it is common to end up with situations where calls are not available for every member of the family. Under normal circumstances `mendelian` will attempt to determine Mendelian consistency on the basis of the values that have been provided. Records where the presence of missing values makes the Mendelian consistency undecidable contain `MCU` INFO annotations in the annotated output VCF. The following examples illustrate some consistent, undecidable, and inconsistent calls in the presence of missing values:

```
CHROM  FATHER_GT  MOTHER_GT  SON_GT  STATUS
chrX   .          0/1        1       OK
chr1   ./.        1/1        1/2     MCU
chr1   ./.        1/1        2/2     MCV
```

Since the number of calls where one sample is missing can be quite high, an alternative option is to treat missing values as equal to the reference by using the `--lenient` parameter. Note that while this approach will be correct in most cases, it will give inaccurate results where the calling between different samples has reported the variant in an equivalent but slightly different position or representation (e.g. positioning of indels within homopolymer regions, differences of representation such as splitting MNPs into multiple SNPs etc).

The `mendelian` command computes overall concordance between related samples to assist detecting cases where pedigree has been incorrectly recorded or samples have been mislabeled. For each child in the pedigree, pairwise concordance is computed with respect to each parent by identifying diploid calls where the parent does not contain either allele called in the child. Low pairwise concordance with a single parent may indicate that the

parent is the source of the problem, whereas low pairwise concordance with both parents may indicate that the child is the source of the problem. A stricter three-way concordance is also recorded.

By default, only VCF records with the `FILTER` field set to PASS or missing are processed. All variant records can be examined by specifying the `--all-records` parameter.

See also:

family, *population*, *vcfstats*

2.5.9 vcfannotate

Synopsis:

Used to add annotations to a VCF file, either to the VCF `ID` field, as a VCF `INFO` sub-field, or as a VCF `FORMAT` sub-field.

Syntax:

```
$ rtg vcfannotate [OPTION]... -b FILE -i FILE -o FILE
```

Example:

```
$ rtg vcfannotate -b dbsnp.bed -i snps.vcf.gz -o snps-dbsnp.vcf.gz
```

Parameters:

File Input/Output		
	`--bed-regions=FILE`	If set, only read VCF records that overlap the ranges contained in the specified BED file.
`-i`	`--input=FILE`	VCF file containing variants to annotate. Use '-' to read from standard input.
`-o`	`--output=FILE`	Output VCF file name. Use '-' to write to standard output.
	`--region=REGION`	If set, only read VCF records within the specified range. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> or <sequence_name>:<pos>~<padding>

Reporting		
`-A`	`--annotation=STRING`	Add computed annotation to VCF records. Allowed values are [AC, AN, EP, GQD, IC, LAL, MEANQAD, NAA, PD, QA, QD, RA, SCONT, VAF, VAF1, ZY]. May be specified 0 or more times, or as a comma separated list.
	`--bed-ids=FILE`	Add variant IDs from BED file. May be specified 0 or more times.
	`--bed-info=FILE`	Add INFO annotations from BED file. May be specified 0 or more times.
	`--fill-an-ac`	Add or update the AN and AC INFO fields.
	`--info-description=STRING`	If the BED INFO field is not already declared, use this description in the header (Default is Annotation)
	`--info-id=STRING`	The INFO ID for BED INFO annotations (Default is ANN)
	`--relabel=FILE`	Relabel samples according to `old-name new-name` pairs in specified file.
	`--vcf-ids=FILE`	Add variant IDs from VCF file. May be specified 0 or more times.

Utility		
-a	--add-header=STRING|FILE	File containing VCF header lines to add, or a literal header li May be specified 0 or more times.
-h	--help	Print help on command-line flag usage.
-Z	--no-gzip	Do not gzip the output.
	--no-header	Prevent VCF header from being written.

Usage:

Use `vcfannotate` to add text annotations to variants.

A common use case is to add annotations to only those variants that fall within ranges specified in a BED or VCF file, supplied via `--bed-ids` or `--vcf-ids` respectively. The annotations from the BED file are added as an `INFO` field in the output VCF file. It can also be used to compute or fill in certain additional annotations from the existing content. Note that this annotation method is solely based on the position and span of the variant, ignoring actual alleles and genotypes.

If the `--bed-ids` flag is used, instead of adding the annotation to the `INFO` fields, it is added to the `ID` column of the VCF file instead. If the `--vcf-ids` flag is used, the `ID` column of the input VCF file is used to update the `ID` column of the output VCF file instead.

If the `--fill-an-ac` flag is set, the output VCF will have the `AN` and `AC` info fields (as defined in the VCF 4.2 specification) created or updated.

It is also possible to use `vcfannotate` to insert additional VCF header lines into the VCF header. These are supplied using the `--add-header` flag which may either be a literal VCF header line (useful for adding one or two header lines), or from a file.

```
$ rtg vcfannotate -i in.vcf.gz -o out.vcf.gz \
--add-header "##SAMPLE=<ID=NA24385,Sex=MALE>" \
--add-header "##SAMPLE=<ID=NA24143,Sex=FEMALE>" \
--add-header "##SAMPLE=<ID=NA24149,Sex=MALE>" \
--add-header "##PEDIGREE=<Child=NA24385,Mother=NA24143,Father=NA24149>"
```

or alternatively:

```
$ rtg vcfannotate -i in.vcf.gz -o out.vcf.gz --add-header ped_vcf_headers.txt
```

Care should be taken that the lines being inserted are valid VCF header lines.

If the `--annotation` flag is set, `vcfannotate` attempts to compute the specified annotation(s) and add them as `FORMAT` fields in the corresponding records. Records for which particular annotations cannot be computed, due to a lack of pre-requisite fields, will not be modified.

For a description of the meaning of fields available for annotation, see *Small-variant VCF output file description*. The `SCONT` annotation is a convenience to annotate with all of the contrary evidence annotations: `DCOC`, `DCOF`, `OCOC`, `OCOF`.

See also:

snp, *family*, *somatic*, *population*, *vcffilter*, *vcfsubset*

2.5.10 vcfdecompose

Synopsis:

Decomposes complex variants within a VCF file into smaller components.

Syntax:

```
$ rtg vcfdecompose [OPTION]... -i FILE -o FILE
```

Parameters:

File Input/Output		
-i	--input=FILE	VCF file containing variants to decompose. Use '-' to read fr input.
-o	--output=FILE	Output VCF file name. Use '-' to write to standard output.
-t	--template=SDF	SDF of the reference genome the variants are called against.

Sensitivity Tuning		
	--break-indels	If set, peel as many SNPs off an indel as possible.
	--break-mnps	If set, break MNPs into individual SNPs.

Utility		
-h	--help	Print help on command-line flag usage.
-Z	--no-gzip	Do not gzip the output.
	--no-header	Prevent VCF header from being written.

Usage:

The `vcfdecompose` command decomposes and trims variants based on a multiple sequence alignment between the alleles in each VCF record. Only records where every ALT allele is an ordinary allele (i.e. consisting of nucleotides) will undergo decomposition. In addition, if there are redundant same-as-reference bases in the alleles, these will be trimmed off.

The default behavior is to break the variant at positions where there is at least one base aligned to the reference across all ALT alleles, so the output may contain MNPs or impure indels. If desired, MNPs can be split into individual SNPs via `--break-mnps`. Similarly, impure indels can be split into a combination of SNPs and pure indels via `--break-indels`.

Although decomposed variants carry through the original `INFO` and `FORMAT` annotations, the decomposition may mean that some annotations are no longer semantically correct. In particular, any VCF `FORMAT` fields declared to be of type `A`, `G`, or `R` will no longer be valid if the set of alleles has changed.

Note that the reference genome is an optional parameter. When variants are decomposed and trimmed, the resulting variant may require a padding base to be added, as required by the VCF specification. The VCF specification suggests that the padding base should be the base before the variant (i.e. padding on the left), but sometimes this requires knowledge of reference bases not present in the original record. When the reference genome is supplied, `vcfdecompose` will ensure that any padding bases are added on the left of the variant. If the reference genome is not supplied, padding bases may sometimes be on the right hand side of the variant. For example:

```
1  20  .  GCGCGCGCGCG  TTTGCGCGCTTGCGCGTTT  .  PASS  .              GT  1/0
```

will decompose without a reference genome as:

```
1  20  .  G            TTTG                 .  PASS  ORP=20;ORL=11  GT  1/0
1  25  .  C            CTT                  .  PASS  ORP=20;ORL=11  GT  1/0
```

and with a reference genome (where the reference base at position 19 can be determined to be a `T`) as:

```
1  19  .  T     TTTT    .  PASS  ORP=20;ORL=11  GT  1/0
1  25  .  C     CTT     .  PASS  ORP=20;ORL=11  GT  1/0
```

The variants that are left vs right-padded are equivalent and identified as such by haplotype-aware comparison tools such as `vcfeval`.

See also:

vcffilter, *vcfeval*

2.5.11 vcfeval

Synopsis:

Evaluates called variants for agreement with a baseline variant set irrespective of representational differences. Outputs a weighted ROC file which can be viewed with `rtg rocplot` and VCF files containing false positives (called variants not matched in the baseline), false negatives (baseline variants not matched in the call set), and true positives (variants that match between the baseline and calls).

The baseline variants might be the variants that were used to generate a synthetic simulated sample (such as via `popsim`, `samplesim`, etc), a gold-standard VCF corresponding to a reference sample such as NA12878, or simply an alternative call-set being used as a basis for comparison.

Syntax:

```
$ rtg vcfeval [OPTION]... -b FILE -c FILE -o DIR -t SDF
```

Example:

```
$ rtg vcfeval -b goldstandard.vcf.gz -c snps.vcf.gz -t HUMAN_reference \
  --sample daughter -f AVR -o eval
```

Parameters:

File Input/Output		
`-b`	`--baseline=FILE`	VCF file containing baseline variants.
	`--bed-regions=FILE`	If set, only read VCF records that overlap the ranges contained in the specified BED file.
`-c`	`--calls=FILE`	VCF file containing called variants.
`-e`	`--evaluation-regions=FILE`	If set, evaluate within regions contained in the supplied BED file, allowing transborder matches. To be used for truth-set high-confidence regions or other regions of interest where region boundary effects should be minimized.
`-o`	`--output=DIR`	Directory for output.
	`--region=REGION`	If set, only read VCF records within the specified range. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> or <sequence_name>:<pos>~<padding>
`-t`	`--template=SDF`	SDF of the reference genome the variants are called against.

Filtering		
	--all-records	Use all records regardless of FILTER status (Default is to only process records where FILTER is . or PASS)
	--decompose	Decompose complex variants into smaller constituents to allow partial credit.
	--ref-overlap	Allow alleles to overlap where bases of either allele are same-as-ref (Default is to only allow VCF anchor base overlap)
	--sample=STRING	The name of the sample to select. Use <baseline_sample>,<calls_sample> to select different sample names f baseline and calls. (Required when using multi-sample VCF files)
	--sample-ploidy=INT	Expected ploidy of samples (Default is 2)
	--squash-ploidy	Treat heterozygous genotypes as homozygous ALT in both baseline ar calls, to allow matches that ignore zygosity differences.

Reporting		
	--at-precision=FLOAT	Output summary statistics where precision >= supplied value (Default is to summarize at maximum F-measure)
	--at-sensitivity=FLOAT	Output summary statistics where sensitivity >= supplied value (Default is to summarize at maximum F-measure)
	--no-roc	Do not produce ROCs.
-m	--output-mode=STRING	Output reporting mode. Allowed values are [split, annotate, combine, ga4gh, roc-only] (Default is split)
	--roc-expr=STRING	Output ROC file for variants matching custom JavaScript expression. Use the form <LABEL>=<EXPRESSION>. May be specified 0 or more times.
	--roc-regions=STRING	Output ROC file for variants overlapping custom regions supplied in BED file. Use the form <LABEL>=<FILENAME>. May be specified 0 or more times.
	--roc-subset=STRING	Output ROC file for preset variant subset. Allowed values are [hom, het, snp, non-snp, mnp, indel]. May be specified 0 or more times, or as a comma separated list.
-O	--sort-order=STRING	The order in which to sort the ROC scores so that good scores come before bad scores. Allowed values are [ascending, descending] (Default is descending)
-f	--vcf-score-field=STRING	The name of the VCF FORMAT field to use as the ROC score. Also valid are QUAL, INFO.<name> or FORMAT.<name> to select the named VCF FORMAT or INFO field (Default is GQ)

Utility		
-h	--help	Print help on command-line flag usage.
-Z	--no-gzip	Do not gzip the output.
-T	--threads=INT	Number of threads (Default is the number of available cores)

Usage:

The vcfeval command can be used to generate VCF files containing called variants that were in the baseline VCF, called variants that were not in the baseline VCF and baseline variants that were not in the called variants. It also produces ROC curve data files based on a score contained in a VCF field which show the predictive power of that field for the quality of the variant calls.

When developing and validating sequencing pipelines and variant calling algorithms, the comparison of variant call sets is a common problem. The naïve way of computing these numbers is to look at the same reference locations in the baseline (ground truth) and called variant set, and see if genotype calls match at the same position. However, a complication arises due to possible differences in representation for indels between the baseline and the call sets within repeats or homopolymers, and in multiple-nucleotide polymorphisms (MNPs), which encompass several nearby nucleotides and are locally phased. The vcfeval command includes a novel dynamic-

programming algorithm for comparing variant call sets that deals with complex call representation discrepancies, and minimizes false positives and negatives across the entire call sets for accurate performance evaluation. A primary advantage of `vcfeval` (compared to other tools) is that the evaluation does not depend on normalization or decomposition, and so the results of analysis can easily be used to relate to the original variant calls and their annotations.

Note that `vcfeval` operates at the level of local haplotypes for a sample, so for a diploid genotype, both alleles must match in order to be considered correct. Some of the vcfeval output modes (described below) automatically perform an additional haploid analysis phase to identify variants which may not have a diploid match but which share a common allele (for example, zygosity errors made during calling). If desired, this more lenient haploid comparison can be used at the outset by setting the `--squash-ploidy` flag (see below).

Note that variants selected for inclusion in a haplotype cannot be permitted to overlap each other (otherwise the question arises of which variant should have priority when determining the resulting haplotype), and any well-formed call-set should not contain these situations in order to avoid such ambiguity. When such cases are encountered by `vcfeval`, the best non-overlapping result is determined. A special case of overlapping variants is where calls are denoted as partially the same as the reference (for example, a typical heterozygous call). Strictly speaking such variants are an assertion that the relevant haplotype bases must not be altered from the reference and overlap should not be permitted (this is the interpretation that `vcfeval` employs by default). However, sometimes as a result of using non-haplotype-aware variant calling tools or when using naïve merging of multiple call sets, a more lenient comparison is desired. The `--ref-overlap` flag will permit such overlapping variants to both match, as long as any overlap only occurs where one variant or other has asserted haplotype bases as being the same as reference.

Common allele matching with `--squash-ploidy`

When `--squash-ploidy` is specified, a haploid match is attempted using *each* of the non-reference alleles used in the sample genotype. For example if the baseline and call VCFs each had a record with the same REF and ALT alleles declared, the following GT fields would be considered a match:

```
0/1, 1/1, 1/2   (genotypes match due to the 1 allele)
0/2, 1/2, 2/2   (genotypes match due to the 2 allele)
```

Thus `--squash-ploidy` matches any case where the baseline and calls share a common allele. This is most often used to run matching that does not penalize for genotyping errors. For example, it is recommended to use this option when matching somatic variant calls, as since somatic variation is usually associated with variable allelic fractions and heterogeneity that mean strict diploid genotype comparisons are not appropriate.

Comparing non-diploid genomes

By default, `vcfeval` assumes diploid organisms (that is, the expected ploidy of any GT call is 2). As a special case to ease the comparison of male calls on sex chromosomes (where callers often continue to use diploid representation), haploid calls are treated as homozygous diploid. Any calls made with unexpected ploidy are ignored and reported in the `vcfeval` log file.

To compare genomes with non-diploid ploidy, the expected sample ploidy can be overridden via `--sample-ploidy` – for example `--sample-ploidy=4` would be used to compare tetraploid organisms.

Comparing with a VCF that has no sample column

A common scenario is to match a call set against a baseline which contains no sample column, where the objective is to identify which baseline alleles which have been called. One example of this is to identify whether calls match a database of known high-priority somatic variants such as COSMIC, or to find calls which have been previously seen in a population allele database such as ExAC. Ordinarily `vcfeval` requires the input VCFs to contain a sample column containing a genotype in the GT field, however, it is possible to specify a special sample name of 'ALT' in order to indicate that the the genotypes for comparison should be derived from the ALT alleles of the record. This can be specified independently for baseline and calls, for example:

```
$ rtg vcfeval -t build37.sdf -b cosmic.vcf.gz -c tumor-calls.vcf.gz \
--squash-ploidy --sample ALT,tumor -o tumor-vs-cosmic
```

Which would perform a haploid matching of the GT of the called sample 'tumor' against all possible haploid genotypes in the COSMIC VCF. The resulting true positives file contains all the calls containing an allele present in the COSMIC VCF.

Note: It is also possible to run a diploid comparison by omitting `--squash-ploidy`, but this is not usually required, and is computationally more intensive since there may be many more possible diploid genotypes to explore, particularly if the ALT VCF contains many multiallelic records.)

Evaluation with respect to regions

When evaluating exome variant calls, it may be useful to restrict analysis only to exome target regions. In this case, supply a BED file containing the list of regions to restrict analysis to via the `--bed-regions` flag. For a quick way to restrict analysis only to a single region, the `--region` flag is also accepted. Note that when restricting analysis to regions, there may be variants which can not be correctly evaluated near the borders of each analysis region, if determination of equivalence would require inclusion of variants outside of the region. For this reason, it is recommended that such regions be relatively inclusive.

When matching against gold standard truth sets which have an accompanying high-confidence regions BED file, the flag `--evaluation-regions` should be used instead of `--bed-regions`, as it has special matching semantics that aims to reduce comparison region boundary effects. When this comparison method is used, call variants which match a baseline variant are only considered a true positive if the baseline variant is inside the high confidence regions, and call variants are only considered false positive if they fall inside the high confidence regions.

vcfeval outputs

The primary outputs of `vcfeval` are VCF files indicating which variants matched between the baseline and the calls VCF, and data files containing information used to generate ROC curves with the `rocplot` command (or via spreadsheet). `vcfeval` supports different VCF output modes which can be selected with the `--output-mode` flag according to the type of analysis workflow desired. The following modes are available:

Split (`--output-mode=split`)

This output mode is the default, and produces separate VCF files for each of the match categories. The individual VCF records in these files are not altered in any way, preserving all annotations present in the input files.

- `tp.vcf` – contains those variants from the *calls* VCF which agree with variants in the baseline VCF
- `tp-baseline.vcf` – contains those variants from the *baseline* VCF which agree with variants in the calls VCF. Thus, the variants in `tp.vcf` and `tp-baseline.vcf` are equivalent. This file can be used to successively refine a highly sensitive baseline variant set to produce a consensus from several call sets.
- `fp.vcf` – contains variants from the *calls* VCF which do not agree with baseline variants.

- `fn.vcf` – contains variants from the *baseline* VCF which were not correctly called.

This mode performs a single pass comparison, either in diploid mode (the default), or haploid mode (if `--squash-ploidy` has been set). The separate output files produced by this mode allow the use of `vcfeval` as an advanced haplotype-aware VCF intersection tool.

Annotate (`--output-mode=annotate`)

This output mode does not split the input VCFs by match status, but instead adds `INFO` annotations containing the match status of each record:

- `calls.vcf` – contains variants from the *calls* VCF, augmented with match status annotations.
- `baseline.vcf` – contains variants from the *baseline* VCF, augmented with match status annotations.

This output mode automatically performs two comparison passes, the first finds diploid matches (assigned a match status of `TP`), and a second pass that applies a haploid mode to the false positives and false negatives in order to find calls (such as zygosity errors) that contain a common allele. This second category of match are annotated with status `FN_CA` or `FP_CA` in the output VCFs, and those calls which do not have any match are assigned status `FN` or `FP`. A status value of `IGN` indicates a VCF record which was ignored (for example, due to having a non-PASS filter status, representing a structural variant, or otherwise containing a non-variant genotype). A status of `OUT` indicates a VCF record which does not contain a match status due to falling outside the evaluation regions when `--evaluation-regions` is being used. The annotated VCF files produced in this mode may also be used with `vcf2rocplot` to produce additional post-evaluation ROC data files.

Combine (`--output-mode=combine`)

This output mode provides an easy way to view the baseline and call variants in a single two-sample VCF.

- `output.vcf` – contains variants from both the *baseline* and *calls* VCFs, augmented with match status annotations. The sample under comparison from each of the input VCFs is extracted as a column in the output. As the VCF records from the baseline and calls typically have very different input annotations which can be difficult to merge, and to keep the output format simple, there is no attempt to preserve any of the original variant annotations.

As with the annotation output mode, this output mode automatically performs two comparison passes to find both diploid matches and haploid (lenient) matches.

ROC-only (`--output-mode=roc-only`)

This output mode provides a lightweight way to run performance benchmarking, as VCF file output is omitted, and only ROC data files are produced.

Note: In addition, `vcfeval` has an output mode (`--output-mode=ga4gh`) which produces the intermediate evaluation format defined by the GA4GH Benchmarking Team, without additional statistics files. This mode is not generally intended for end users, rather it is used when `vcfeval` is selected as the comparison engine inside the `hap.py` benchmarking tool see: https://github.com/ga4gh/benchmarking-tools and https://github.com/Illumina/hap.py

---

Additional ROC stratifications

All of the output modes produce the following ROC data files (unless disabled by `--no-roc`):

- `weighted_roc.tsv` – contains ROC data derived from all analyzed call variants, regardless of their representation. Columns include the score field, and standard accuracy metrics such as true positives, false positives, false negatives, precision, sensitivity, and f-measure corresponding to each score threshold.
- `snp_roc.tsv` – contains ROC data derived from only those variants which were represented as SNPs. Since the representation conventions can differ between the baseline and calls, there are some subtleties to be aware of when interpreting metrics such as precision, sensitivity, etc, described below.
- `non_snp_roc.tsv` – contains ROC data derived from those variants which were not represented as SNPs. As above, not all metrics are computed for this file.

`vcfeval` also provides the ability to produce additional ROC data files corresponding to preset and customized variant stratifications with the following flags:

Preset stratifications

The `--roc-subset` flag allows selection from preset stratifications based on variant type (according to their representation in the relevant input VCF):

- `hom` – homozygous variants only
- `het` – heterozygous variants only
- `snp` – SNP variants (enabled by default)
- `non-snp` – non-SNP variants (enabled by default)
- `mnp` – multi-nucleotide polymorphisms only
- `indel` – length-changing variants only

Multiple presets can be enabled in a single run, e.g. `--roc-subset hom,het,indel`

Region-based stratifications

The `--roc-regions` flag produces a stratified ROC data file using only variants that overlap regions specified in a user-supplied BED file. The special syntax for this flag is: `--roc-regions LABEL=FILE`, where `LABEL` is a short tag used to determine ROC output file names and `FILE` is the path to the relevant BED file. For example, to produce additional stratifications based on BED files partitioning the genome based on GC content:

```
$ rtg vcfeval -t build37.sdf -b baseline.vcf.gz -c calls.vcf.gz \
  --roc-regions GC55TO60=/path/to/GCcontent/GRCh37_gc55to60_slop50.bed.gz \
  --roc-regions GC60TO65=/path/to/GCcontent/GRCh37_gc60to65_slop50.bed.gz
```

Custom JavaScript based stratifications

The above stratification flags will satisfy most common usages, but `vcfeval` also includes the ability to write custom stratifications using JavaScript expressions (similar to `vcffilter --keep-expr`). The special syntax for this flag is: `--roc-expr LABEL=EXPRESSION`, where `LABEL` is a short tag used to determine ROC output file names and `EXPRESSION` is the JavaScript expression that accepts a variant for inclusion in the stratification. This is most useful when the input VCFs contain annotations useful for the stratification. For example, to produce stratifications based on depth of coverage during variant calling:

```
$ rtg vcfeval -t build37.sdf -b baseline.vcf.gz -c calls.vcf.gz \
  --roc-expr "DP10TO20=has(INFO.DP) && INFO.DP>=10 && INFO.DP<20" \
  --roc-expr "DP20TO30=has(INFO.DP) && INFO.DP>=20 && INFO.DP<30" \
  --roc-expr "DP30TO40=has(INFO.DP) && INFO.DP>=30 && INFO.DP<40"
```

Tips:

- Ensure the expression is valid to evaluate on all variants (for example, take care when referring to sample fields names if the sample names are different between baseline and calls files).
- It may be useful to test or debug the expression (without the label) via `vcffilter --keep-expr`.

For more information on JavaScript expressions, see *RTG JavaScript filtering API*

Benchmarking comparisons using ROC and precision/sensitivity curves

Multiple ROC data files (from a single or several `vcfeval` runs) can be plotted with the `rocplot` command, which allows output to a PNG or SVG image or analysis in an interactive GUI that provides zooming and visualization of the effects of threshold adjustment. As these files are simple tab-separated-value format, they can also be loaded into a spreadsheet tool or processed with shell scripts.

While ROC curve analysis provides a much more thorough method for examining the performance of a call set with respect to a baseline truth set, for convenience, `vcfeval` also produces a `summary.txt` file which indicates match summary statistics that correspond to two key points on the ROC curve. The first point is where all called variants are included (i.e., no thresholding on a score value); and second point corresponding to a score threshold that maximises the F-measure of the curve. While this latter point is somewhat arbitrary, it represents a balanced tradeoff between precision and sensitivity which is likely to provide a fairer comparison when comparing call sets from different callers.

Sometimes it is useful to perform the summary statistic evaluation at some point other than maximized F-measure (for example when comparing a large number of results at a particular precision level). This can be accomplished by specifying a different point using either the `--at-precision` or `--at-sensitivity` flag with a value in the range `[0, 1]`.

Note that `vcfeval` reports true positives both counted using the baseline variant representation as well as counted using the call variant representation. When these numbers differ greatly, it indicates a general difference in representational conventions used between the two call sets. Since false negatives can only be measured in terms of the baseline representation, sensitivity is defined as:

$$\text{Sensitivity} = \text{TP}_{\text{baseline}} / (\text{TP}_{\text{baseline}} + \text{FN}).$$

Conversely since false positives can only be measured in terms of the call representation, precision is defined as:

$$\text{Precision} = \text{TP}_{\text{call}} / (\text{TP}_{\text{call}} + \text{FP}).$$

Note: For definitions of the terminology used when evaluating caller accuracy, see: https://en.wikipedia.org/wiki/Receiver_operating_characteristic and https://en.wikipedia.org/wiki/Sensitivity_and_specificity

Benchmarking performance for SNPs versus indels

A common desire is to perform analysis separately for SNPs versus indels. However, it is important to note that due the representation ambiguity problem, it is not always trivial to decide in a global sense whether a variant is a SNP or an indel or other complex variant. A group of variants that may be represented as single SNPs in one call-set may be represented as a single complex variant in another call-set. Consider the following example reference and alternate haplotypes:

```
     12345678901234567
REF: ATCGTAAATAAAATGCA
ALT: ATCGTAAAATAAATGCA
```

One variant caller might represent the haplotypes as the following VCF records:

```
chr1 5 . T TA . . . GT 1/1
chr1 9 . TA T . . . GT 1/1
```

While another variant caller could represent the same haplotypes as:

```
chr1 9 . T A . . . GT 1/1
chr1 10 . A T . . . GT 1/1
```

The decision as to which representation to use is essentially arbitrary, yet one caller has used indels (and no SNPs), and the other has used SNPs (and no indels). For this reason it is certainly a poor idea to attempt to divide baseline and called variants into separate SNP and indel datasets up front and perform evaluation on each set separately, as any variants that use different representation categories will not be matched across the independent comparisons. Any variant-type specific metrics should be computed after matching is carried out on the full variant sets.

Note that when there are different representational conventions between the baseline and calls (or between calls from one variant caller and another), then at some level there is really a semantic difference between a "baseline indel" and a "call-set indel" (or "variant-caller-A indel" and "variant-caller-B indel"), so caution should be applied when making conclusions related to SNP versus indel accuracy.

In the `snp_roc.tsv` and `non_snp_roc.tsv` output files (and other preset stratifications available via `--roc-subset`), `vcfeval` notes the number of baseline and call variants of each variant type. When considering benchmarking metrics in the absence of any thresholding with respect to a score field, it is straight-forward to use the previous formulae (i.e. sensitivity is computed using the counts from baseline variants, and precision is computed using the counts from called variants). When computing threshold-specific metrics for ROC data points, the computation is more involved. Since only the call variants contain the score field used to rank variants, the number of (say) TP baseline indels that exceed threshold x is not defined. `vcfeval` computes a scaled count as:

$$\mathrm{TP}_{\mathrm{baseline\_indel}}(x) = \mathrm{TP}_{\mathrm{call\_indel}}(x) \times \mathrm{TP}_{\mathrm{baseline\_indel}} / \mathrm{TP}_{\mathrm{call\_indel}}$$

and thus threshold-specific sensitivity is computed as

$$\mathrm{Sensitivity}_{\mathrm{indel}}(x) = \mathrm{TP}_{\mathrm{baseline\_indel}}(x) / (\mathrm{TP}_{\mathrm{baseline\_indel}} + \mathrm{FN}_{\mathrm{indel}})$$

This scaling ensures that the end point of the variant type specific ROC or precision/sensitivity curve ends at the same point that is obtained when computing metrics without any threshold.

The scaling described above is applied to all of the preset stratifications available via `--roc-subset`, but is not applied to any custom stratifications produced via `--roc-regions` or `--roc-expr`.

Variant decomposition and benchmarking

In general, it is not necessary to run any variant decomposition and/or normalization on variant call sets prior to evaluation with `vcfeval`, as the haplotype aware matching process can account for representation differences. However, since matching is at the granularity of entire variants, a single long complex call will be categorized as either correct or incorrect, even if part of the call may match. If partial credit in the case of long calls is of interest, `vcfeval` includes an option to internally decompose variants prior to matching, using the `--decompose` flag. This decomposition is applied to both baseline and call variants, and any output VCFs will contain the decomposed representation. External VCF decomposition (with more control over decomposition options) is also available via `rtg vcfdecompose`.

See also:

snp, *popsim*, *samplesim*, *childsim*, *rocplot*, *vcf2rocplot*, *vcfdecompose*

2.5.12 vcffilter

Synopsis:

Filters VCF records based on various criteria. When filtering on multiple samples, if any of the specified samples fail the criteria, the record will be filtered. By default filtered records are removed, but see the –fail, –clear-failed-samples, and –fail-samples options for alternatives.

Syntax:

```
$ rtg vcffilter [OPTION]... -i FILE -o FILE
```

Examples:

Keep only records where the sample has depth of coverage at least 5:

```
$ rtg vcffilter -i snps.vcf.gz -o snps_cov5.vcf.gz -d 5
```

Keep only biallelic records:

```
$ rtg vcffilter -i snps.vcf.gz -o snps_biallelic.vcf.gz --max-alleles 2
```

Parameters:

File Input/Output		
	`--all-samples`	Apply sample-specific criteria to all samples contained in the input VCF.
	`--bed-regions=FILE`	If set, only read VCF records that overlap the ranges contained in the specified BED file.
`-i`	`--input=FILE`	VCF file containing variants to be filtered. Use '-' to read from standard input.
`-o`	`--output=FILE`	Output VCF file. Use '-' to write to standard output. This option is required, unless `--javascript` is being used.
	`--region=REGION`	If set, only read VCF records within the specified range. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> or <sequence_name>:<pos>~<padding>
	`--sample=STRING`	Apply sample-specific criteria to the named sample contained in the input VCF. May be specified 0 or more times.

Filtering (Record based)		
-w	--density-window=INT	Window within which multiple variants are discarded.
	--exclude-bed=FILE	Discard all variants within the regions in this BED file.
	--exclude-vcf=FILE	Discard all variants that overlap with the ones in this file.
	--include-bed=FILE	Only keep variants within the regions in this BED file.
	--include-vcf=FILE	Only keep variants that overlap with the ones in this file.
-j	--javascript=STRING	Javascript filtering functions for determining whether to keep record. May be either an expression or a file name. May be specified 0 or more times. *See Examples*
-e	--keep-expr=STRING	Records for which this expression evaluates to true will be retained. *See Examples*
-k	--keep-filter=STRING	Only keep variants with this FILTER tag. May be specified 0 or more times, or as a comma separated list.
-K	--keep-info=STRING	Only keep variants with this INFO tag. May be specified 0 or more times, or as a comma separated list.
	--max-alleles=INT	Maximum number of alleles (REF included)
-C	--max-combined-read-depth=INT	Maximum allowed combined read depth.
-Q	--max-quality=FLOAT	Maximum allowed quality.
	--min-alleles=INT	Minimum number of alleles (REF included)
-c	--min-combined-read-depth=INT	Minimum allowed combined read depth.
-q	--min-quality=FLOAT	Minimum allowed quality.
-r	--remove-filter=STRING	Remove variants with this FILTER tag. May be specified 0 or more times, or as a comma separated list.
-R	--remove-info=STRING	Remove variants with this INFO tag. May be specified 0 or more times, or as a comma separated list.
	--remove-overlapping	Remove records that overlap with previous records.

Filtering (Sample based)		
-A	--max-ambiguity-ratio=FLOAT	Maximum allowed ambiguity ratio.
	--max-avr-score=FLOAT	Maximum allowed AVR score.
	--max-denovo-score=FLOAT	Maximum de novo score threshold.
-G	--max-genotype-quality=FLOAT	Maximum allowed genotype quality.
-D	--max-read-depth=INT	Maximum allowed sample read depth.
	--min-avr-score=FLOAT	Minimum allowed AVR score.
	--min-denovo-score=FLOAT	Minimum de novo score threshold.
-g	--min-genotype-quality=FLOAT	Minimum allowed genotype quality.
-d	--min-read-depth=INT	Minimum allowed sample read depth.
	--non-snps-only	Only keep where sample variant is MNP or INDEL
	--remove-all-same-as-ref	Remove where all samples are same as reference.
	--remove-hom	Remove where sample is homozygous.
	--remove-same-as-ref	Remove where sample is same as reference.
	--snps-only	Only keep where sample variant is a simple SNP.

Reporting		
	`--clear-failed-samples`	Retain failed records, set the sample GT field to missing.
`-f`	`--fail=STRING`	Retain failed records, add the provided label to the FILTER
`-F`	`--fail-samples=STRING`	Retain failed records, add the provided label to the sample F

Utility		
`-a`	`--add-header=STRING|FILE`	File containing VCF header lines to add, or a literal header li May be specified 0 or more times.
`-h`	`--help`	Print help on command-line flag usage.
`-Z`	`--no-gzip`	Do not gzip the output.
	`--no-header`	Prevent VCF header from being written.

Usage:

Use `vcffilter` to get a subset of the results from variant calling based on the filtering criteria supplied by the filter flags. Multiple criteria can be specified at once, and advanced processing can be specified via JavaScript scripting.

When filtering on multiple samples, if any of the specified samples fail the criteria, the record will be filtered. The default behavior is for filtered records to be excluded from output altogether, but alternatively the records can be retained but with an additional user-specified VCF `FILTER` status set via `--fail` option, or if sample-specific filtering criteria is being applied, only those samples can be filtered either by setting their `GT` field to missing by using the `--clear-failed-samples` option, or by setting the `FORMAT FT` field with a user-specified status via the `--fail-samples` option.

The `--bed-regions` option makes use of tabix indexes to avoid loading VCF records outside the supplied regions, which can give faster filtering performance. If the input VCF is not indexed or being read from standard input, or if records failing filters are to be annotated via the `--fail` option, use the `--include-bed` option instead.

The flags `--min-denovo-score` and `--max-denovo-score` can only be used on a single sample. Records will only be kept if the specified sample is flagged as a *de novo* variant and the score is within the range specified by the flags. It will also only be kept if none of the other samples for the record are also flagged as a *de novo* variant within the specified score range.

The `--add-header` option allows inserting arbitrary VCF header lines into the output VCF. For more information, see *vcfannotate*.

A powerful general-purpose filtering capability has been included that permits the specification of filter criteria as simple JavaScript expressions (`--keep-expr`) or more comprehensive JavaScript processing functions (`--javascript`). Both `--keep-expr` and `--javascript` can take JavaScript on the command line or if a filename is supplied then the script/expression will be read from that file. `--keep-expr` will be applied before `--javascript`, so the `--javascript` record function will not be called for records filtered out by `--keep-expr`.

See also:

For full details of functions available in `--keep-expr` and `--javascript` see *RTG JavaScript filtering API*

Simple filtering by JavaScript expression with `--keep-expr`

The `--keep-expr` flag aims to provide a convenient way to apply some simple (typically one line) filtering expressions which are evaluated in the context of each record. The final expression of the fragment must evaluate to a boolean value. Records which evaluate to `true` will be retained, while `false` will be removed. The value must be of type boolean, simply being truthy/falsy (in the JavaScript sense) will raise an error.

`--keep-expr` examples:

The following expression keeps records where the `NA12878` sample has `GQ` > 30 and the total depth is > 20. JavaScript will auto convert numerical strings when comparing a string with a number, so calls to `parseInt` can be omitted.

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz \
--keep-expr "'NA12878'.GQ > 30 && INFO.DP > 20"
```

If the field of interest may contain the missing value ('.') or may be entirely missing on a per-record basis, the `has()` function can be used to control whether such records are kept vs filtered. For example, to keep records with depth greater than 20, and remove any without a `DP` annotation:

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz \
--keep-expr "has(INFO.DP) && INFO.DP > 20"
```

Alternatively, to keep records with depth greater than 20, as well as those without a DP annotation:

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz \
--keep-expr "!has(INFO.DP) || INFO.DP > 20"
```

The next example keeps records where all samples have a depth > 10. The standard JavaScript array methods `every` and `some` can be used to apply a condition on every sample column.

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz \
  --keep-expr "SAMPLES.every(function(s) {return s.DP > 10})"
```

Similarly, the following example retains records where the `FILTER` field is unset, or if set must be either `PASS` or `MED_QUAL`:

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz \
  --keep-expr "FILTER.every(function(f) {return f == 'PASS' || f == 'MED_QUAL'})"
```

Note that multi-valued `INFO` and `FORMAT` fields are not split into sub-values, so in some cases correct filtering may require splitting the values first. For example, to select bi-allelic records with `AF` greater than 0.1, the following simple selection will work:

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz \
  --keep-expr "INFO.AF>=0.1"
```

However, in the presence of multi-allelic records, something like the following is required:

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz \
  --keep-expr "INFO.AF.split(',').some(function(af) {return af >= 0.1})"
```

Advanced JavaScript filtering with `--javascript`

The `--javascript` option aims to support more complicated processing than `--keep-expr`, permitting modification of the output VCF, or supporting use cases where the script is tasked to compute and output alternative information in addition to (or instead of) the output VCF. The scripts specified by the user are evaluated once at the start of processing. Two special functions may be defined in a `--javascript` script, which will then be executed in different contexts:

- A function with the name `record` will be executed once for each VCF record. If the `record` function has a return value it must have type boolean. Records which evaluate to `true` will be retained, while `false` will be removed. If the record function has no return value then the record will be retained. The `record` function is applied after any `--keep-expr` expression.
- A function with the name `end` will be called once at the end of processing. This allows reporting of summary statistics collected during the filter process.

This `--javascript` flag may be specified multiple times, they will be evaluated in order, in a shared JavaScript namespace, before VCF processing commences. This permits a use case where an initial JavaScript expression supplies parameter values which will be required by a subsequent JavaScript file.

Example `--javascript` scripts:

To find indels with length greater than 5, save the following to a file named `find-indels.js`:

```
// Finds indels with length > 5
function record() {
  var deltas = ALT.map(function (alt) {
    return Math.abs(alt.length - REF.length);
  });
  return deltas.some(function (delta) {return delta > 5});
}
```

Then perform the filtering via:

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz --javascript find-indels.js
```

The following example derives a new `FORMAT` column containing variant allelic fraction to two decimal places based on the values in the `AD` and `DP` `FORMAT` annotations, for every sample contained in the VCF. Save the following to a file named `add-vaf.js`:

```
// Derive new VAF FORMAT field for each sample
ensureFormatHeader('##FORMAT=<ID=VAF,Number=1,Type=Float,' +
  'Description="Variant Allelic Fraction">');

function record() {
  SAMPLES.forEach(function(sample) {
    // Take all but the first AD value as numerics
    var altDepths = sample.AD.split(",").slice(1);
    // Find the max
    var maxAltDepth = Math.max.apply(null, altDepths);
    if (maxAltDepth > 0) {
      sample.VAF = (maxAltDepth / sample.DP).toFixed(2);
    }
  });
}
```

Then run the filtering via:

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz --javascript add-vaf.js
```

The next example produces a table of binned indel lengths, save the following to a file named `indel-lengths.js`:

```
// bin breakpoints can be customised by defining your own bins[] in a
// previous -j flag
if (typeof bins == "undefined") {
  var bins = [-10, -5, -3, 0, 4, 6, 11];
}

var counts = [0];
bins.forEach(function () {counts.push(0)});
function record() {
  if (ALT.length == 0) {
    return false;
  }
  var deltas = ALT.map(function (alt) { return alt.length - REF.length; });
  var maxDel = Math.min.apply(null, deltas);
  var maxIns = Math.max.apply(null, deltas);
  var delta = Math.abs(maxDel) > maxIns ? maxDel : maxIns;

  if (delta == 0) {
    return false;
  }
  for (var i = 0; i < bins.length; i++) {
    if (delta < bins[i]) {
      counts[i]++;
      break;
    }
  }
  if (delta > bins[bins.length - 1]) {
    counts[counts.length - 1]++;
  }
  return false;
}

function end() {
  print("Delta\\tCount");
  for (var i = 0; i < bins.length; i++) {
    print("<" + bins[i] + "\\t" + counts[i]);
  }
  print(">" + bins[bins.length - 1] + "\\t" + counts[counts.length - 1]);
}
```

Then run the filtering via:

```
$ rtg vcffilter -i in.vcf.gz -o out.vcf.gz --javascript indel-lengths.js
```

We could use this same script with adjusted bins and omitting the output of the VCF via:

```
$ rtg vcffilter -i in.vcf.gz -j "var bins = [-20, -10, 0, 20, 20];" \
  -j indel-lengths.js
```

See also:

For full details of functions available in `--keep-expr` and `--javascript` see *RTG JavaScript filtering API*

See also:

snp, *family*, *somatic*, *population*, *vcfannotate*, *vcfmerge*, *vcfsubset*

2.5.13 vcfmerge

Synopsis:

Combines the contents of two or more VCF files. The `vcfmerge` command can concatenate the outputs of per-chromosome variant detection runs to create a complete genome VCF file, and also merge VCF outputs from multiple samples to form a multi-sample VCF file.

Syntax:

```
$ rtg vcfmerge [OPTION]... -o FILE FILE+
```

Example:

```
$ rtg vcfmerge -o merged.vcf.gz snp1.vcf.gz snp2.vcf.gz
```

Parameters:

File Input/Output		
	`--bed-regions=FILE`	If set, only read VCF records that overlap the ranges contained in the specified BED file.
`-I`	`--input-list-file=FILE`	File containing a list of VCF format files (1 per line) to be merged.
`-o`	`--output=FILE`	Output VCF file. Use '-' to write to standard output.
	`--region=REGION`	If set, only read VCF records within the specified range. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> or <sequence_name>:<pos>~<padding>
	`FILE+`	Input VCF files to merge. May be specified 0 or more times.

Utility		
`-a`	`--add-header=STRING|FILE`	File containing VCF header lines to add, or a literal header line. May be specified 0 or more times.
`-f`	`--force-merge=STRING`	Allow merging of specified header ID even when descriptions do not match. May be specified 0 or more times, or as a comma separated list.
`-F`	`--force-merge-all`	Attempt merging of all non-matching header declarations.
`-h`	`--help`	Print help on command-line flag usage.
`-Z`	`--no-gzip`	Do not gzip the output.
	`--no-header`	Prevent VCF header from being written.
	`--no-merge-alts`	Do not merge multiple records if the ALTs are different.
	`--no-merge-records`	Do not merge multiple records at the same position into one.
	`--preserve-formats`	Do not merge multiple records containing unmergeable FORMAT fields (Default is to remove those FORMAT fields so the variants can be combined)
	`--stats`	Output statistics for the merged VCF file.

Usage:

The `vcfmerge` command takes a list of VCF files and outputs to a single VCF file. Each VCF file must be block compressed and have a corresponding tabix index file, which is the default for outputs from RTG variant detection tools, but may also be created from an existing VCF file using the RTG `bgzip` and `index` commands.

There are two primary usage scenarios for the `vcfmerge` command. The first is to combine input VCFs corresponding to different genomic regions (for example, if variant calling was carried out for each chromosome independently on different nodes of a compute cluster). The second scenario is when combining VCFs containing variant calls for different samples (e.g. combining calls made for separate cohorts into a single VCF).

The input files must have consistent header lines, although specific similar header lines can be forced to merge using the `--force-merge` parameter, or inconsistent header checking can be entirely disabled with `--force-merge-all`.

The `--add-header` option allows inserting arbitrary VCF header lines into the output VCF. For more information, see *vcfannotate*.

Merging records at the same position

When multiple records occur with the same position and length on the reference, `vcfmerge` will attempt to combine the records into a single record. Combining multiple fully annotated records is non-trivial, and can lead to loss of information depending on the annotations present. The default behavior takes a pragmatic approach to merging, with options to adjust the merging behavior as required. Multi-record merging can be disabled entirely by setting `--no-merge-records`.

The first point to note is that the `QUAL`, `FILTER`, `INFO` fields are taken from the first record only (those values from other records at the position are ignored).

If the combined record would result in a change in the set of ALT alleles, any VCF `INFO` or `FORMAT` fields declared to be of type `A`, `G`, or `R` cannot meaningfully be retained. By default such fields will be removed or set to the missing value (`.`). (Other annotations may also become semantically meaningless, but it isn't possible to tell in general.)

Similarly, if multiple input records with the same position and length on the reference contain information for the same sample, only the information from the first record will be retained.

These behaviors can be altered with additional flags: `--no-merge-alts` will simply prevent record merging if the ALTs are not the same across the records; `--preserve-formats` will attempt merging as long as the records do not contain problematic `A`, `G`, or `R` annotations, or contain the same sample.

See also:

snp, *family*, *population*, *somatic*, *vcffilter*, *vcfannotate*, *vcfsubset*, *bgzip*, *index*

2.5.14 vcfsplit

Synopsis:

Splits samples contained within a VCF into separate files, one per sample.

Syntax:

```
$ rtg vcfsplit [OPTION]... -i FILE -o DIR
```

Example:

```
$ rtg vcfsplit --keep-sample NA12878,NA12891,NA12892 -i population-ceph-calls.vcf.
↪gz -o trio-vcfs
```

Parameters:

File Input/Output		
	`--bed-regions=FILE`	If set, only read VCF records that overlap the ranges contained in the specified BED file.
`-i`	`--input=FILE`	The input VCF, or '-' to read from standard input.
`-o`	`--output=DIR`	Directory for output.
	`--region=REGION`	If set, only read VCF records within the specified range. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> or <sequence_name>:<pos>~<padding>

Filtering		
	`--keep-ref`	Keep records where the sample is reference.
	`--keep-sample=STRING|FILE`	File containing sample IDs to select, or a literal sample name. May be specified 0 or more times, or as a comma separated list.
	`--remove-sample=STRING|FILE`	File containing sample IDs to ignore, or a literal sample name. May be specified 0 or more times, or as a comma separated list.

Utility		
`-h`	`--help`	Print help on command-line flag usage.
`-Z`	`--no-gzip`	Do not gzip the output.

Usage:

The `vcfsplit` command allows producing separate single-sample VCF files from a single multi-sample VCF, and is more efficient than running `vcfsubset` separately for each required sample, particularly when the input VCF contains many samples, as the input VCF only needs to be read once.

The output VCFs are all written into the specified output directory, as `sample_name.vcf.gz`. For any particular output VCF, only records where the sample has a non-reference genotype will be output, unless `--keep-ref` is used.

See also:

vcfsubset

2.5.15 vcfstats

Synopsis:

Display simple statistics about the contents of a set of VCF files.

Syntax:

```
$ rtg vcfstats [OPTION]... FILE+
```

Example:

```
$ rtg vcfstats /data/human/wgs/NA19240/snp_chr5.vcf.gz

Location                      : /data/human/wgs/NA19240/snp_chr5.vcf.gz
Passed Filters                : 283144
Failed Filters                : 83568
SNPs                          : 241595
MNPs                          : 5654
Insertions                    : 15424
Deletions                     : 14667
Indels                        : 1477
Unchanged                     : 4327
SNP Transitions/Transversions : 1.93 (210572/108835)
Total Het/Hom ratio           : 2.13 (189645/89172)
SNP Het/Hom ratio             : 2.12 (164111/77484)
MNP Het/Hom ratio             : 3.72 (4457/1197)
Insertion Het/Hom ratio       : 1.69 (9695/5729)
Deletion Het/Hom ratio        : 2.33 (10263/4404)
Indel Het/Hom ratio           : 3.13 (1119/358)
Insertion/Deletion ratio      : 1.05 (15424/14667)
Indel/SNP+MNP ratio           : 0.13 (31568/247249)
```

Parameters:

File Input/Output		
	`--known`	Set to only calculate statistics for known variants.
	`--novel`	Set to only calculate statistics for novel variants.
	`--sample=FILE`	Set to only calculate statistics for the specified sample. (Default is t all samples). May be specified 0 or more times.
	FILE+	VCF file from which to derive statistics. Use '-' to read from stand Must be specified 1 or more times.

Reporting		
	`--allele-lengths`	Set to output variant length histogram.

Utility		
`-h`	`--help`	Prints help on command-line flag usage.

Usage:

Use the `vcfstats` command to display summary statistics for a set of VCF files. If a VCF file contains multiple sample columns, the statistics for each sample are shown individually.

When determining the categorization of a REF to ALT transformation, some normalization is carried out to ignore same as reference bases at the start and end of the alleles. Thus the following REF to ALT transformations are categorized as `SNPs`:

```
A    -> G      (simple case)
ATGC -> ATGG   (leading bases match)
ATGC -> ACGC   (leading and trailing bases match)
```

Cases where multiple bases change, but the lengths of the two alleles do not are considered to be `MNPs`:

```
ATGC -> TTGG   (two bases change)
ATGC -> GTCT   (three bases change)
```

Cases where there is pure addition or removal of bases are classified as `Insertions` or `Deletions` respectively:

```
A     -> AT       (one base insertion)
ATT   -> ATTTT    (two base insertion)
AT    -> A        (one base deletion)
ATTTT -> ATT      (two base deletion)
```

The remaining case is there there is a length change between the REF and ALT, but it is not pure. These are called `Indels`:

```
ATT   -> CTTT     (one base changed, one base inserted)
CTTT  -> ATT      (one base changed, one base deleted)
```

In the per-sample summary output of `vcfstats`, each genotype is classified as a whole into one of the above categories, preferring the more complex of the transformations when ploidy is greater than one.

When computing the per-sample variant length histograms, note that the histograms are incremented for each called allele (thus a diploid homozygous call will increment the appropriate cell by two), and the length of an indel is taken as the change in length rather than the overall length.

See also:

snp, *family*, *somatic*, *vcffilter*, *vcfmerge*, *vcfsubset*

2.5.16 vcfsubset

Synopsis:

Create a VCF file containing a subset of the original columns.

Syntax:

```
$ rtg vcfsubset [OPTION]... -i FILE -o FILE
```

Example:

```
$ rtg vcfsubset -i snps.vcf.gz -o frequency.vcf.gz --keep-info AF --remove-samples
```

Parameters:

File Input/Output		
	--bed-regions=FILE	If set, only read VCF records that overlap the ranges contained in the specified BED file.
-i	--input=FILE	VCF file containing variants to manipulate. Use '-' to read from standard input.
-o	--output=FILE	Output VCF file. Use '-' to write to standard output.
	--region=REGION	If set, only read VCF records within the specified range. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> or <sequence_name>:<pos>~<padding>

Filtering		
	--keep-filter=STRING	Keep the specified FILTER tag. May be specified 0 or more times, or as a comma separated list.
	--keep-format=STRING	Keep the specified FORMAT field. May be specified 0 or more times, or as a comma separated list.
	--keep-info=STRING	Keep the specified INFO tag. May be specified 0 or more times, or as a comma separated list.
	--keep-sample=STRING|FILE	File containing sample IDs to keep, or a literal sample name. May be specified 0 or more times, or as a comma separated list.
	--remove-filter=STRING	Remove the specified FILTER tag. May be specified 0 or more times, or as a comma separated list.
	--remove-filters	Remove all FILTER tags.
	--remove-format=STRING	Remove the specified FORMAT field. May be specified 0 or more times, or as a comma separated list.
	--remove-ids	Remove the contents of the ID field.
	--remove-info=STRING	Remove the specified INFO tag. May be specified 0 or more times, or as a comma separated list.
	--remove-infos	Remove all INFO tags.
	--remove-qual	Remove the QUAL field.
	--remove-sample=STRING|FILE	File containing sample IDs to remove, or a literal sample name. May be specified 0 or more times, or as a comma separated list.
	--remove-samples	Remove all samples.

Utility		
-h	--help	Print help on command-line flag usage.
-Z	--no-gzip	Do not gzip the output.
	--no-header	Prevent VCF header from being written.

Usage:

Use the `vcfsubset` command to produce a smaller copy of an original VCF file containing only the columns and information desired. For example, to produce a VCF containing only the information for one sample from a multiple sample VCF file use the `--keep-sample` flag to specify the sample to keep. The various `--keep` and `--remove` options can either be specified multiple times or with comma separated lists, for example, `--keep-format GT --keep-format DP` is equivalent to `-keep-format GT,DP`.

See also:

snp, *family*, *somatic*, *population*, *vcffilter*, *vcfannotate*

2.5.17 vcf2rocplot

Synopsis:

Produce rocplot compatible ROC data files from vcfeval annotated VCFs. The primary use cases for this command are to produce aggregate ROCs from several independent `vcfeval` evaluation runs, and to generate ROCs with respect to different criteria than were used during the initial `vcfeval` evaluation.

Syntax:

```
$ rtg vcf2rocplot [OPTION]... -o DIR FILE+
```

Create an aggregate ROC from evaluations of several independent samples:

```
$ rtg vcfeval -b goldstandard.vcf.gz -c snps.vcf.gz -t HUMAN_reference \
  --sample daughter1 -f AVR -o eval -m annotate
$ rtg vcfeval -b goldstandard.vcf.gz -c snps.vcf.gz -t HUMAN_reference \
  --sample daughter2 -f AVR -o eval -m annotate
$ rtg vcfeval -b goldstandard.vcf.gz -c snps.vcf.gz -t HUMAN_reference \
  --sample son2 -f AVR -o eval -m annotate
$ rtg vcf2rocplot -o eval_family -f AVR \
  eval_{daughter1,daughter2,son1}/{baseline,calls}.vcf.gz
$ rtg rocplot eval_family/weighted_roc.tsv.gz \
  --png eval_family_roc.png
```

Parameters:

File Input/Output		
	`--bed-regions=FILE`	If set, only read VCF records that overlap the ranges contained in the specified BED file.
`-o`	`--output=DIR`	Directory for output.
	`--region=REGION`	If set, only read VCF records within the specified range. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> or <sequence_name>:<pos>~<padding>
	`FILE+`	Input VCF files containing vcfeval annotations. Must be specified 1 or more times.

Reporting		
	`--at-precision=FLOAT`	Output summary statistics where precision >= supplied value (Default is to summarize at maximum F-measure)
	`--at-sensitivity=FLOAT`	Output summary statistics where sensitivity >= supplied value (Default is to summarize at maximum F-measure)
	`--roc-expr=STRING`	Output ROC file for variants matching custom JavaScript expression. Use the form <LABEL>=<EXPRESSION>. May be specified 0 or more times.
	`--roc-regions=STRING`	Output ROC file for variants overlapping custom regions supplied in BED file. Use the form <LABEL>=<FILENAME>. May be specified 0 or more times.
	`--roc-subset=STRING`	Output ROC file for preset variant subset. Allowed values are [hom, het, snp, non-snp, mnp, indel]. May be specified 0 or more times, or as a comma separated list.
`-O`	`--sort-order=STRING`	The order in which to sort the ROC scores so that `good` scores come before `bad` scores. Allowed values are [ascending, descending] (Default is descending)
`-f`	`--vcf-score-field=STRING`	The name of the VCF FORMAT field to use as the ROC score. Also valid are `QUAL`, `INFO.<name>` or `FORMAT.<name>` to select the named VCF FORMAT or INFO field (Default is GQ)

Utility		
`-h`	`--help`	Print help on command-line flag usage.
`-Z`	`--no-gzip`	Do not gzip the output.
`-T`	`--threads=INT`	Number of threads (Default is the number of available cores)

Usage:

While the ROC outputs generated by `vcfeval` are sufficient for many scenarios, there are some situations where it is useful to regenerate ROC files from one or more existing vcfeval outputs.

One such situation is when evaluating caller accuracy across a cohort of samples where the number of variants per individual sample is low. Individual sample ROC curves are fairly uninformative with regard to overall accuracy or where to place suitable filter thresholds. An ROC curve from the combined evaluations provides a much better indication of overall caller accuracy.

Another use case for generating ROC files from an existing `vcfeval` run is to look at alternative scoring methods or stratifications without having to execute the time-consuming variant matching stage implied by a new `vcfeval` run.

The prerequisite for using `vcf2rocplot` is that `vcfeval` must have been run in "annotation" mode (i.e. via `--output-mode=annotate`) so that the output VCF files contain sufficient annotations regarding variant classification status. It is important that `vcf2rocplot` be provided with both annotated baseline and call VCFs in order to produce correct outputs.

The operation of the various score field selection and ROC stratification flags works the same as when running `vcfeval`.

See also:

rocplot, *vcfeval*

2.5.18 svdecompose

Synopsis:

Split composite structural variants into a breakend representation.

Syntax:

```
$ rtg svdecompose [OPTION]... -i FILE -o FILE
```

Parameters:

File Input/Output		
-i	--input=FILE	VCF file containing variants to filter. Use '-' to read from standa
-o	--output=FILE	Output VCF file name. Use '-' to write to standard output.

Utility		
-h	--help	Print help on command-line flag usage.
-Z	--no-gzip	Do not gzip the output.
	--no-header	Prevent VCF header from being written.

Usage:

The `svdecompose` command is applied to a VCF containing structural variants and converts deletion, insertion, inversion, and tandem duplications with `SVTYPE` of `DEL`, `INS`, `INV`, and `DUP`, respectively, into corresponding breakend events with `SVTYPE=BND`. `svdecompose` will also decompose sequence-resolved insertions and deletions greater than `--min-indel-length` into breakend representation. Records of others types are passed through without modification.

This operation can be useful for the purposes of reducing output from various structural variant callers to a common representation to better facilitate comparison with the `bndeval` command.

For insertions, `svdecompose` will represent the insertion as breakends between the reference and a "virtual haplotype", where for example, contig "<INS_A>" represents the destination of all insertions made on chromosome A. So if another caller produced a similar insertion event (in position and/or length), the break end versions will also be nearby on the virtual contig. For the following insertions:

```
1  54712   .     T  TTTTTTTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTC  .  .  .
1  934144  I_7   C  CGGAGGGGAGGGCGCGGAGCGGAGG                            .  .  .
1  934144  I_22  C  CGGAGGGGAGGGCGCGGAGCGGAGGGGAGGGCGCGGAGCGGAGG        .  .  .
```

each insertion gets two breakends like this:

```
1  54712   .     T  T[<INS_1>:54712[   .  .  SVTYPE=BND;CIPOS=0,0
1  54712   .     T  ]<INS_1>:54765]C   .  .  SVTYPE=BND;CIPOS=0,0
1  934144  I_7   C  C[<INS_1>:934144[  .  .  SVTYPE=BND;CIPOS=0,0
1  934144  I_22  C  C[<INS_1>:934144[  .  .  SVTYPE=BND;CIPOS=0,0
1  934144  I_7   C  ]<INS_1>:934168]G  .  .  SVTYPE=BND;CIPOS=0,0
1  934144  I_22  C  ]<INS_1>:934187]G  .  .  SVTYPE=BND;CIPOS=0,0
```

See also:

bndeval

2.5.19 bndeval

Synopsis:

Evaluate called breakends for agreement with a baseline breakend set. Outputs a weighted ROC file which can be viewed with `rtg rocplot` and VCF files containing false positives (called breakends not matched in the baseline), false negatives (baseline breakends not matched in the call set), and true positives (breakends that match between the baseline and calls).

Syntax:

```
$ rtg bndeval [OPTION]... -b FILE -c FILE -o DIR
```

Parameters:

File Input/Output		
`-b`	`--baseline=FILE`	VCF file containing baseline variants.
	`--bed-regions=FILE`	If set, only read VCF records that overlap the ranges contained in the specified BED file.
`-c`	`--calls=FILE`	VCF file containing called variants.
`-o`	`--output=DIR`	Directory for output.
	`--region=REGION`	If set, only read VCF records within the specified range. The format is one of <sequence_name>, <sequence_name>:<start>-<end>, <sequence_name>:<pos>+<length> or <sequence_name>:<pos>~<padding>

Filtering		
	`--all-records`	Use all records regardless of FILTER status (Default is to only proc where FILTER is "." or "PASS")
	`--bidirectional`	If set, allow matches between flipped breakends.
	`--tolerance=INT`	Positional tolerance for breakend matching (Default is 100)

Reporting		
	`--no-roc`	Do not produce ROCs.
`-m`	`--output-mode=STRING`	Output reporting mode. Allowed values are [split, annotate] (Default is split)
`-O`	`--sort-order=STRING`	The order in which to sort the ROC scores so that `good` scores come before `bad` scores. Allowed values are [ascending, descending] (Default is descending)
`-f`	`--vcf-score-field=STRING`	The name of the VCF FORMAT field to use as the ROC score. Also valid are `QUAL`, `INFO.<name>` or `FORMAT.<name>` to select the named VCF FORMAT or INFO field (Default is INFO.DP)

Utility		
`-h`	`--help`	Print help on command-line flag usage.
`-Z`	`--no-gzip`	Do not gzip the output.

Usage:

The `bndeval` command operates on VCF files containing breakends such as those produced by the `discord` command. In particular, it considers records having the breakend structural variant type (`SVTYPE=BND`) as defined in the VCF specification. Other types of record are ignored, but the `svdecompose` command can be applied beforehand to split certain other structural variants (e.g., `INV` and `DEL`) or sequence-resolved insertions and deletions into constituent breakend events.

The input and output requirements of `bndeval` are broadly similar to the `vcfeval` command. The primary inputs to `bndeval` are a truth/baseline VCF containing expected breakends, and a query/call VCF containing the called breakends. Evaluation can be restricted to particular regions by specifying a BED file.

The regions contained in the evaluation regions BED file are intersected with the breakend records contained in the truth VCF in order to obtain a list of *truth breakend regions*. An evaluation region is included if there is any overlapping truth VCF record (no attempt is made to look at the degree of overlap). Thus by supplying either evaluation regions corresponding to targeted regions or larger gene-level regions `bndeval` can be used to evaluate at different levels of granularity.

Similarly, the evaluation regions are intersected with the breakend records records contained in the calls VCF to obtain *called breakend regions*.

The *truth breakend regions* are then intersected with the *called breakend regions* to obtain TP/FP/FN metrics. The intersection supports a user-selectable tolerance in position. Further, be default, a breakend must occur in the same orientation to be considered a match, but this constraint can be relaxed by supplying the `--bidirectional` command line option.

bndeval outputs

Once complete, `bndeval` command produces summary statistics and the following primary result files in the output directory:

- `weighted_roc.tsv.gz` - contains ROC data that can be plotted with `rocplot`
- `baseline.bed.gz` contains the *truth breakend regions*, where each BED record contains the region status as `TP` or `FN`, the `SVTYPE`, and the span of the original truth VCF record.
- `calls.bed.gz` contains the *called breakend regions*, where each BED record contains the region status as `TP` or `FP`, the `SVTYPE`, the span of the original calls VCF record, and the score value used for ranking in the ROC plot.
- `summary.txt` contains the same summary statistics printed to standard output.

See also:

discord, *svdecompose*, *vcfeval*, *rocplot*

2.5.20 pedfilter

Synopsis:

Filter and convert a pedigree file.

Syntax:

```
$ rtg pedfilter [OPTION]... FILE
```

Example:

```
$ rtg pedfilter --remove-parentage mypedigree.ped
```

Parameters:

File Input/Output		
	`FILE`	The pedigree file to process, may be PED or VCF, use '-' to read from

Filtering		
	`--keep-family=STRING`	Keep only individuals with the specified family ID. May be specifi or more times, or as a comma separated list.
	`--keep-ids=STRING`	Keep only individuals with the specified ID. May be specified 0 or more times, or as a comma separated list.
	`--keep-primary`	Keep only primary individuals (those with a PED individual line / VCF sample column)
	`--remove-parentage`	Remove all parent-child relationship information.

Reporting		
	`--vcf`	Output pedigree in in the form of a VCF header rather than PED.

Utility		
`-h`	`--help`	Print help on command-line flag usage.

Usage:

The `pedfilter` command can be used to perform manipulations on pedigree information and convert pedigree information between PED and VCF header format. For more information about the PED file format see *Pedigree PED input file format*.

The VCF files output by the `family` and `population` commands contain full pedigree information represented as VCF header lines, and the `pedfilter` command allows this information to be extracted in PED format.

This command produces the pedigree output on standard output, which can be redirected to a file or another pipeline command as required.

See also:

family, *population*, *mendelian*, *pedstats*

2.5.21 pedstats

Synopsis:

Output information from pedigree files of various formats.

Syntax:

```
$ rtg pedstats [OPTION]... FILE
```

Example:

For a summary of pedigree information:

```
$ rtg pedstats ceph_pedigree.ped

Pedigree file: /data/ceph/ceph_pedigree.ped

Total samples:               17
Primary samples:             17
Male samples:                 9
Female samples:               8
Afflicted samples:            0
Founder samples:              4
Parent-child relationships:  26
Other relationships:          0
Families:                     3
```

To output a list of all founders:

```
$ rtg pedstats --founder-ids ceph_pedigree.ped
NA12889
NA12890
NA12891
NA12892
```

For quick pedigree visualization using Graphviz and ImageMagick, use a command-line such as:

```
$ dot -Tpng <(rtg pedstats --dot "A Title" mypedigree.ped) | display -
```

Parameters:

File Input/Output		
	FILE	The pedigree file to process, may be PED or VCF, use '-' to read from

Reporting		
-d	--delimiter=STRING	Output id lists using this separator (Default is \n)
	--dot=STRING	Output pedigree in Graphviz format, using the supplied text as a title.
	--families	Output information about family structures.
	--female-ids	Output ids of all females.
	--founder-ids	Output ids of all founders.
	--male-ids	Output ids of all males.
	--maternal-ids	Output ids of maternal individuals.
	--paternal-ids	Output ids of paternal individuals.
	--primary-ids	Output ids of all primary individuals.
	--simple-dot	When outputting Graphviz format, use a layout that looks less like a traditional pedigree diagram but works better with large complex pedigrees.

Utility		
-h	--help	Print help on command-line flag usage.

Usage:

This command is used to show pedigree summary statistics or select groups of individual IDs.

When using `pedstats` to output a list of sample IDs, the default is to print one ID per line. Depending on subsequent use, it may be convenient to use a different separator between output IDs. For example, with comma separated output it is possible to directly use the results as an argument to `vcfsubset`:

```
$ rtg vcfsubset -i pedigree-calls.vcf.gz -o family1.vcf.gz \
    --keep-samples <(rtg pedstats -d , --founder-ids ceph_pedigree.ped)
```

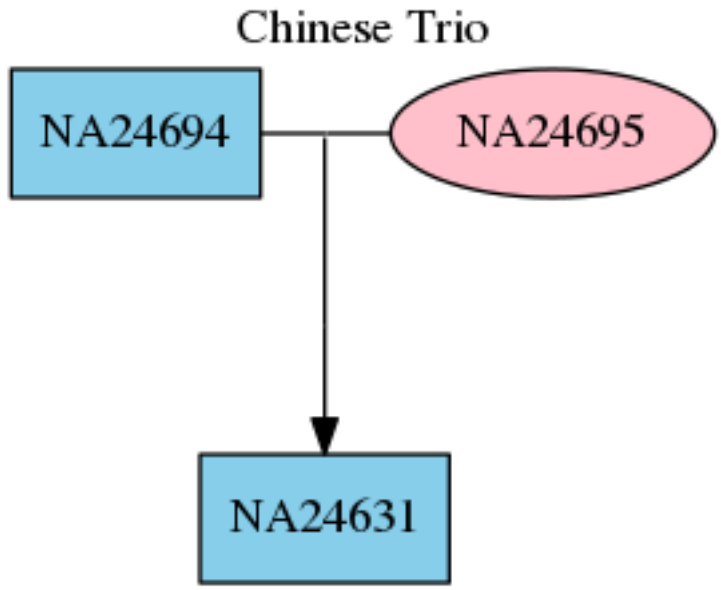
In addition, `pedstats` can be used to generate a simple pedigree visualization, using the well-known Graphviz graphics drawing package, which can be saved to PNG or PDF. For example, with the following `chinese-trio.ped`:

```
#PED format pedigree
#
#fam-id/ind-id/pat-id/mat-id: 0=unknown
#sex: 1=male; 2=female; 0=unknown
#phenotype: -9=missing, 0=missing; 1=unaffected; 2=affected
#
#fam-id ind-id  pat-id  mat-id  sex     phen
0       NA24631 NA24694 NA24695 1       0
0       NA24694 0       0       1       0
0       NA24695 0       0       2       0
```

We can visualize the pedigree with:

```
$ dot -Tpng <(rtg pedstats --dot "Chinese Trio" chinese-trio.ped) -o chinese-trio.
↪png
```

This will create a PNG image that can be displayed in any image viewing tool and contains the pedigree structure as shown below.

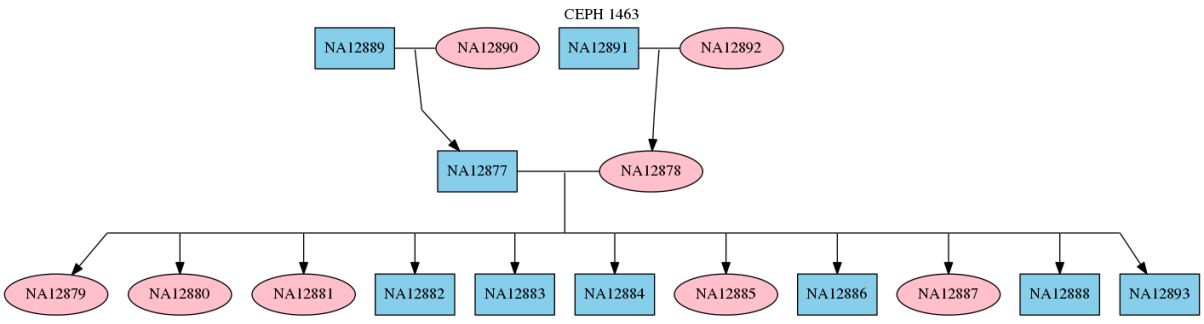


For more information about the PED file format see *Pedigree PED input file format*.

The VCF files output by the RTG pedigree-aware variant calling commands contain full pedigree information represented as VCF header lines, and the `pedstats` command can also take these VCFs as input. For example, given a VCF produced by the `population` command after calling the CEPH-1463 pedigree:

```
$ dot -Tpng <(rtg pedstats --dot "CEPH 1463" population-ceph-calls.vcf.gz) -o ceph-
↪1463.png
```

Would produce the following pedigree directly from the VCF:



Note: Graphviz is provided directly via many operating system package managers, and can also be downloaded from their web site: https://www.graphviz.org/

See also:

family, *population*, *pedfilter*, *vcfsubset*

2.5.22 rocplot

Synopsis:

Plot ROC curves from `readsimeval` and `vcfeval` ROC data files, either to an image, or using an interactive GUI.

Syntax:

```
$ rtg rocplot [OPTION]... FILE+
```

```
$ rtg rocplot [OPTION]... --curve STRING
```

Example:

```
$ rtg rocplot eval/weighted_roc.tsv.gz
```

Parameters:

File Input/Output		
	`--curve=STRING`	ROC data file with title optionally specified (path[=title]). May be s 0 or more times.
	`--png=FILE`	If set, output a PNG image to the given file.
	`--svg=FILE`	If set, output a SVG image to the given file.
	`--zoom=STRING`	Show a zoomed view with the given coordinates, supplied in the for <xmax>,<ymax> or <xmin>,<ymin>,<xmax>,<ymax>
	`FILE+`	ROC data file. May be specified 0 or more times.

Reporting		
	`--cmd=FILE`	If set, print rocplot command used in previously saved image.
	`--hide-sidepane`	If set, hide the side pane from the GUI on startup.
	`--interpolate`	If set, interpolate curves at regular intervals.
	`--line-width=INT`	Sets the plot line width (Default is 2)
	`--palette=STRING`	Name of color palette to use. Allowed values are [blind_13, blind_15, blind_8, brewer_accent, brewer_dark2, brewer_paired, brewer_pastel1, brewer_pastel2, brewer_set1, brewer_set2, brewer_set3, classic] (Default is classic)
	`--plain`	If set, use a plain plot style.
`-P`	`--precision-sensitivity`	If set, plot precision vs sensitivity rather than ROC.
	`--scores`	If set, show scores on the plot.
`-t`	`--title=STRING`	Title for the plot.

Utility		
`-h`	`--help`	Print help on command-line flag usage.

Usage:

Used to produce ROC plots from the ROC files produced by `readsimeval`, `bndeval` and `vcfeval`. By default this opens the ROC plots in an interactive viewer. On a system with only console access the plot can be saved directly to an image file using the either the `--png` or `--svg` parameter.

ROC data files may be specified either as direct file arguments to the command, or via the `--curve` flag. The former method is useful when selecting files using shell wild card globbing, and the latter method allows specifying a custom title for each curve, so use whichever method is most convenient.

Strictly speaking, a true ROC curve should use *rates* rather than absolute numbers on the X and Y axes (e.g. True Positive / Total Positives rather than True Positives on the Y, and False Positive / Total Negatives on the X axis). However, there are a couple of difficulties involved with computing these rates with variant calling datasets.

Firstly, the truth sets do not include any indication of the set of negatives (the closest we may get is in the cases of truth sets which contain a set of confidence regions, where it can be assumed that no other variants may be present inside the specified regions); secondly even with knowledge of negative regions, how do you count the set of possible negative calls, when a call could occupy multiple reference bases, or even (in the case of insertions) zero reference bases. It is conceptually even possible to have a call-set contain more false positives than there are reference bases. For this reason the ROC curves are plotted using the absolute counts.

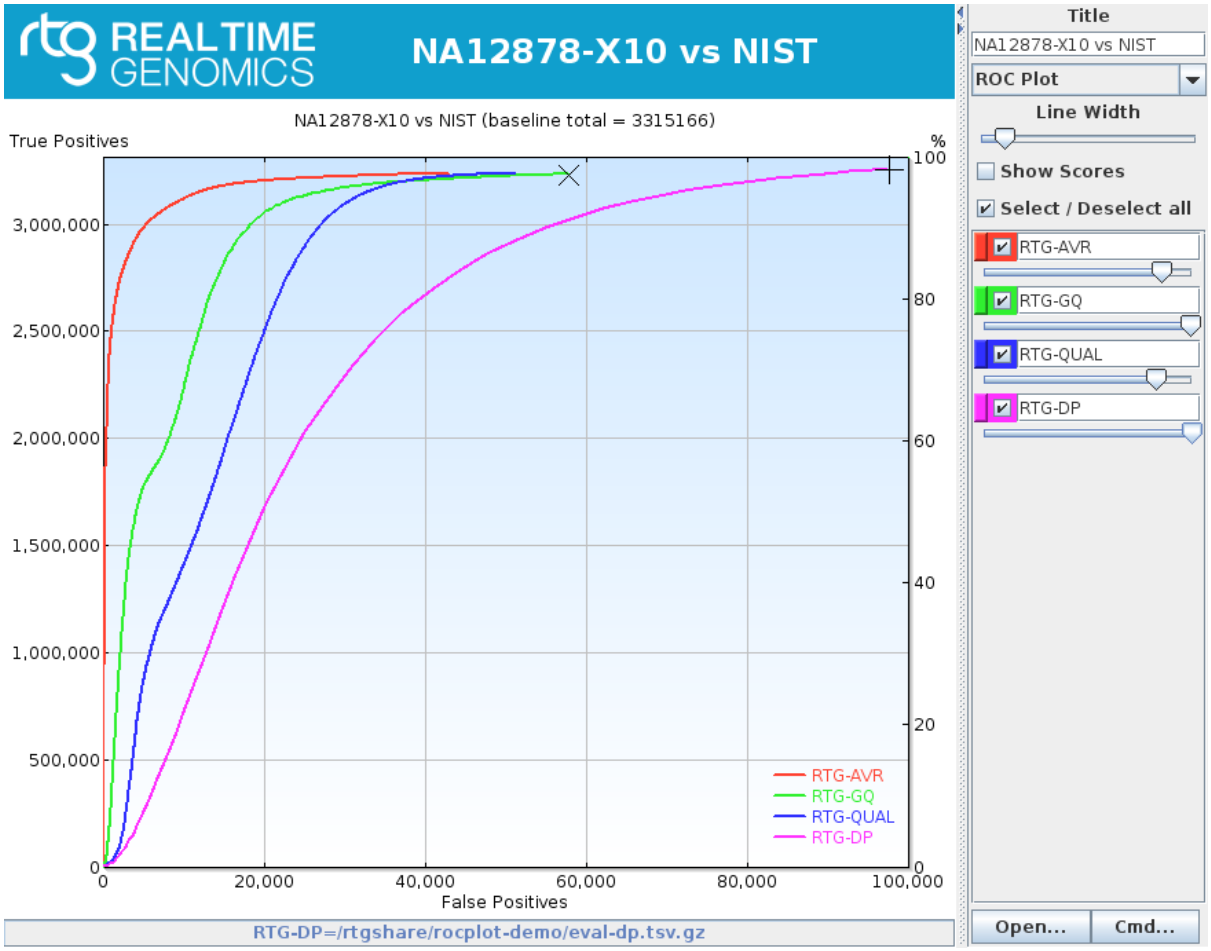
Precision/sensitivity (also known as precision/recall) curves are another popular means of visualizing call-set accuracy, and these metrics also do not require a count of Total Negatives and so cause no particular difficulty to plot. Precision/sensitivity graphs can be selected from the command line via the `--precision-sensitivity` flag, or may be interactively selected in the GUI.

An interesting result of ROC analysis is that although there may be few data points on an ROC graph, it is possible to construct a filtered dataset corresponding to any point that lies on a straight line between two points on the graph. (For example, using threshold A for 25% of the variants and threshold B for 75% of the variants would result in accuracy that is 75% of the way between the points corresponding to thresholds A and B on the ROC plot). So in a sense it is meaningful to connect points on an ROC graph with straight lines. However, for precision/sensitivity graphs, it's incorrect to connect adjacent points with a straight line, as this does not correspond to achievable accuracy on the ROC convex hull and can over-estimate the accuracy. Instead, we can plot appropriately interpolated values with the `--interpolate` option.

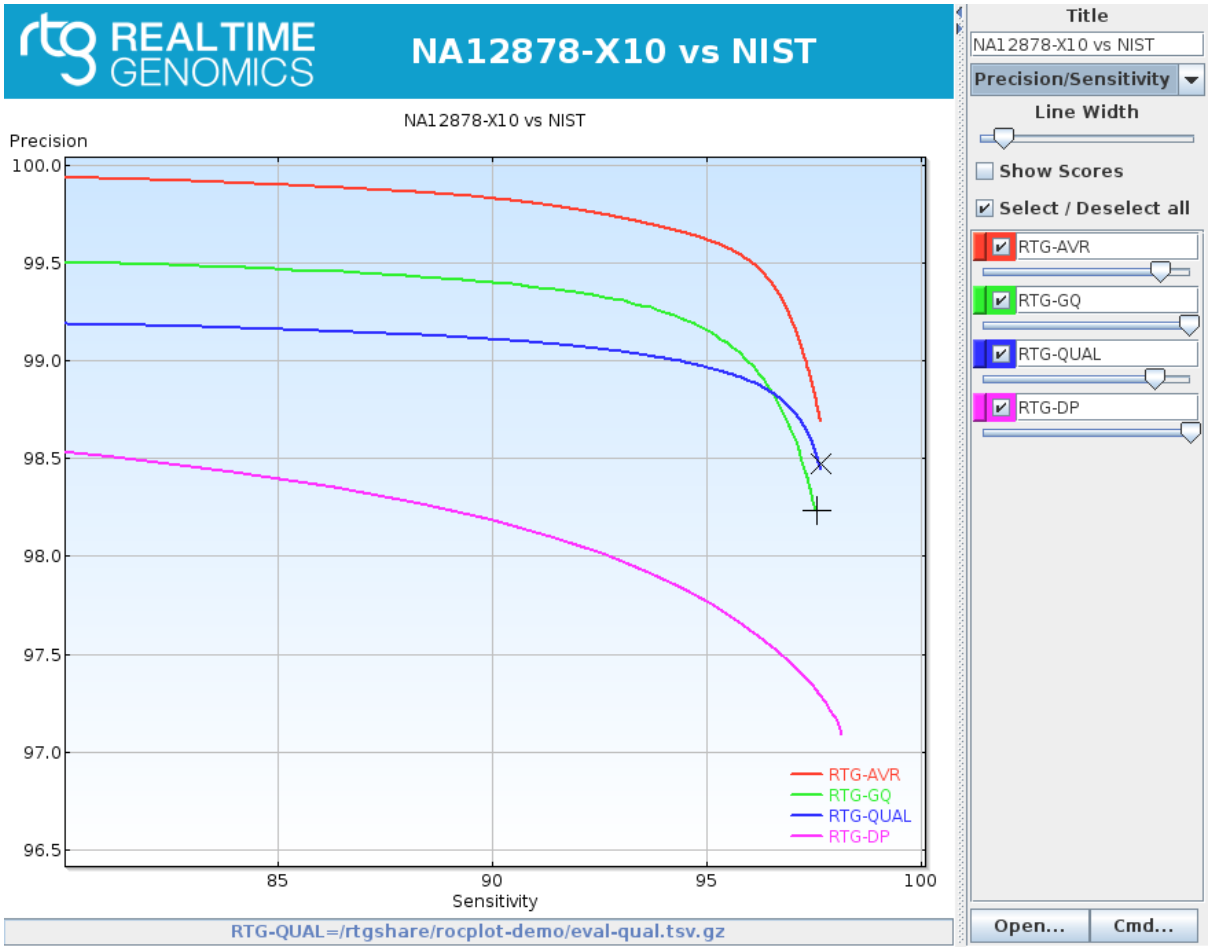
The default ROC graphs include some flourishes such as background gradients and axes drop shadows, these can be disabled via the `--plain` parameter. Alternative preset color palettes for the curve colors may be selected with the `--palette` parameter, and in particular some palettes are more color-blind friendly than the default palette. In addition, PNG images saved by `rocplot` include metadata indicating the graph configuration that may be useful when recreating graphs. This metadata can be displayed (when present) via the `--cmd` parameter.

Interactive GUI

The following image shows the `rocplot` GUI with an example ROC plot :



Similarly, here is an example precision/sensitivity plot:



Some quick tips for the interactive GUI:

- Select regions within the graph to zoom in. Right click within the graph area to bring up a context menu that allows undoing the zoom one level at a time, or resetting the zoom to the default.
- The graph right click menu also allows exporting the image as PNG or SVG. (The saved image does not include the RTG banner).
- Click on a spot in the graph to show the equivalent accuracy metrics for that location in the status bar. Clicking to the left or below the axes will remove the cross-hair. Note that sensitivity depends on the baseline total number of variants being correct. If for example the ROC curve corresponds to evaluating an exome call-set against a whole-genome baseline, this number will be inaccurate.
- A secondary cross-hair is also available by holding down shift when placing (or removing) the cross-hair. When two cross-hairs are placed or moved, metrics in the status bar indicate the difference between the two positions.
- Additional ROC data files can be loaded by clicking on the "Open..." button, and multiple ROC data files within a directory can be loaded at once using multi-select. Alternatively, you may use Drag and Drop from your file browser to drop ROC data files into either the graph area or the right hand ROC curve widget area.
- The "Cmd" button will open a message window that contains a command-line equivalent to the currently displayed set of curves. This command-line may be copy-pasted, providing an easy way to replicate the current set of curves in another session, generate a curve in a script, or share with a colleague.
- There is a drop down that allows for switching between ROC and precision/sensitivity graph types.

Each curve in the GUI has a customization widget on the right hand side of the window that allows several operations:

- Rename the title used for the curve via the editable text.
- Temporarily hide/show the curve via selection checkbox.

- Reorder curves via drag and drop using the colored handle on the left.
- Right click within the ROC widget area to bring up a context menu that allows permanently removing that curve, or customizing the color used for the curve
- Each curve has a slider to simulate the effect of applying a threshold on the scoring attribute. If the "show scores" option is set, this provides an easy way to select appropriate filter threshold values, which you might apply to variant sets using `rtg vcffilter` or similar VCF filtering tools.

Note: For definitions of the terminology used when evaluating caller accuracy, see: https://en.wikipedia.org/wiki/Receiver_operating_characteristic and https://en.wikipedia.org/wiki/Sensitivity_and_specificity

Note: For a description of the precision/sensitivity interpolation, see: "The relationship between Precision-Recall and ROC curves", Davis, J., (2006), https://dx.doi.org/10.1145/1143844.1143874

See also:

readsimeval, *bndeval*, *vcfeval*

2.5.23 version

Synopsis:

The RTG version display utility.

Syntax:

```
$ rtg version
```

Example:

```
$ rtg version

Product: RTG Core 3.9
Core Version: 718f8317b7 (2018-05-29)
RAM: 25.0GB of 31.3GB RAM can be used by rtg (79%)
CPU: Defaulting to 4 of 4 available processors (100%)
JVM: Java HotSpot(TM) 64-Bit Server VM 1.8.0_161
License: Expires on 2019-05-20
Contact: support@realtimegenomics.com

Patents / Patents pending:
US: 7,640,256, 9,165,253, 13/129,329, 13/681,046, 13/681,215, 13/848,653, 13/925,
↪704, 14/015,295, 13/971,654, 13/971,630, 14/564,810
UK: 1222923.3, 1222921.7, 1304502.6, 1311209.9, 1314888.7, 1314908.3
New Zealand: 626777, 626783, 615491, 614897, 614560
Australia: 2005255348, Singapore: 128254

Citation (variant calling):
John G. Cleary, Ross Braithwaite, Kurt Gaastra, Brian S. Hilbush, Stuart Inglis,␣
↪Sean A. Irvine, Alan Jackson, Richard Littin, Sahar Nohzadeh-Malakshah, Mehul␣
↪Rathod, David Ware, Len Trigg, and Francisco M. De La Vega. "Joint Variant and␣
↪De Novo Mutation Identification on Pedigrees from High-Throughput Sequencing␣
↪Data." Journal of Computational Biology. June 2014, 21(6): 405-419. doi:10.1089/
↪cmb.2014.0029.

Citation (vcfeval):
John G. Cleary, Ross Braithwaite, Kurt Gaastra, Brian S. Hilbush, Stuart Inglis,␣
↪Sean A. Irvine, Alan Jackson, Richard Littin, Mehul Rathod, David Ware, Justin M.
↪ Zook, Len Trigg, and Francisco M. De La Vega. "Comparing Variant Call Files for␣
↪Performance Benchmarking of Next-Generation Sequencing Variant Calling Pipelines.
↪" bioRxiv, 2015. doi:10.1101/023754.
```

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```
(c) Real Time Genomics, 2017
```

Parameters:

There are no options associated with the `version` command.

Usage:

Use the `version` command to display release and version information.

See also:

help, *license*

2.5.24 license

Synopsis:

The RTG license display utility.

Syntax:

```
$ rtg license
```

Example:

```
$ rtg license
```

Parameters:

There are no options associated with the license command.

Usage:

Use the `license` command to display license information and expiration date. Output at the command line (standard output) shows command name, licensed status, and command release level.

See also:

help, *version*

2.5.25 help

Synopsis:

The RTG help command provides online help for all RTG commands.

Syntax:

List all commands:

```
$ rtg help
```

Show usage syntax and flags for one command:

```
$ rtg help COMMAND
```

Example:

```
$ rtg help format
```

Parameters:

There are no options associated with the `help` command.

Usage:

Use the `help` command to view syntax and usage information for the main `rtg` command as well as individual RTG commands.

See also:

license, *version*

CHAPTER

THREE

ADMINISTRATION & CAPACITY PLANNING

3.1 Advanced installation configuration

RTG software can be shared by a group of users by installing on a centrally available file directory or shared drive. Assignment of execution privileges can be determined by the administrator, independent of the software license file. For commercial users, the software license prepared by Real Time Genomics (`rtg-license.txt`) need only be included in the same directory as the executable (`RTG.jar`) and the run-time scripts (`rtg` or `rtg.bat`).

During installation on Unix systems, a configuration file named `rtg.cfg` is created in the installation directory. By editing this configuration file, one may alter further configuration variables appropriate to the specific deployment requirements of the organization. On Windows systems, these variables are set in the `rtg.bat` file in the installation directory. These configuration variables include:

Variable	Description
`RTG_MEM`	Specify the maximum memory for Java run-time execution. Use a G suffix for gigabytes, e.g.: `RTG_MEM=48G`. The default memory allocation is 90% of system memory.
`RTG_JAVA`	Specify the path to Java (default assumes current path).
`RTG_JAR`	Indicate the path to the `RTG.jar` executable (default assumes current path).
`RTG_JAVA_OPTS`	Provide any additional Java JVM options.
`RTG_DEFAULT_THREADS`	By default any RTG module with a `--threads` parameter will automatically use the number of cores as the number of threads. This setting makes the specified number the default for the `--threads` parameter instead.
`RTG_PROXY`	Specify the http proxy server for TalkBack exception management (default is no http proxy).
`RTG_TALKBACK`	Send log files for crash-severity exception conditions (default is true, set to false to disable).
`RTG_USAGE`	If set to true, enable simple usage logging.
`RTG_USAGE_DIR`	Destination directory when performing single-user file-based usage logging.
`RTG_USAGE_HOST`	Server URL when performing server-based logging.
`RTG_USAGE_OPTIONAL`	May contain a comma-separated list of the names of optional fields to include in usage logging (when enabled). Any of `username`, `hostname` and `commandline` may be set here.
`RTG_REFERENCES_DIR`	Specifies an alternate directory containing metagenomic pipeline reference datasets.
`RTG_MODELS_DIR`	Specifies an alternate directory containing AVR models.

3.2 Run-time performance optimization

CPU — Multi-core operation finishes jobs faster by processing multiple application threads in parallel. By default RTG uses all available cores of a multi-processor server node. With a command line parameter setting, RTG operation can be limited to a specified number of cores if desired.

Memory — Adding more memory can improve performance where very high read coverage is desired. RTG creates and uses indexes to speed up genomic data processing. The more RAM you have, the more reads you can process in memory in a run. We use 48 GB as a rule of thumb for processing human data. However, a smaller number of reads can be processed in as little as 2 GB.

Disk Capacity — Disk requirements are highly dependent on the size of the underlying data sets, the amount of information needed to hold quality scores, and the number of runs needed to investigate the impact of varying levels of sensitivity. Though all data is handled and stored in compressed form by default, a realistic minimum disk size for handling human data is 1 TB. As a rule of thumb, for every 2 GB of input read data expect to add 1 GB of index data and 1 GB of output files per run. Additionally, leave another 2 GB free for temporary storage during processing.

3.3 Alternate configurations

Demonstration system — For training, testing, demonstrating, processing and otherwise working with smaller genomes, RTG works just fine on a newer laptop system with an Intel processor. For example, product testing in support of this documentation was executed on a MacBook PC (Intel Core 2 Duo processor, 2.1 GHz clock speed, 1 processor, 2 cores, 3 MB L2 Cache, 4 GB RAM, 290 GB 5400 RPM Serial-ATA disk)

Clustered system — The comparison of genomic variation on a large scale demands extensive processing capability. Assuming standard CPU hardware as described above, scale up to meet your institutional or major product needs by adding more rack-mounted boards and blades into rack servers in your data center. To estimate the number of cores required, first estimate the number of jobs to be run, noting size and sensitivity requirements. Then apply the appropriate benchmark figures for different size jobs run with varying sensitivity, dividing the number of reads to be processed by the reads/second/core.

3.4 Exception management - TalkBack and log file

Many RTG commands generate a log file with each run that is saved to the results output directory. The contents of the file contain lists of job parameters, system configuration, and run-time information.

In the case of internal exceptions, additional information is recorded in the log file specific to the problem encountered. Fatal exceptions are trapped and notification is sent to Real Time Genomics with a copy of the log file. This mechanism is called TalkBack and uses an embedded URL to which RTG sends the report.

The following sample log displays the software version information, parameter list, and run-time progress.

```
2009-09-05 21:38:10 RTG version = v2.0b build 20013 (2009-10-03)
2009-09-05 21:38:10 java.runtime.name = Java(TM) SE Runtime Environment
2009-09-05 21:38:10 java.runtime.version = 1.6.0_07-b06-153
2009-09-05 21:38:10 os.arch = x86_64
2009-09-05 21:38:10 os.freememory = 1792544768
2009-09-05 21:38:10 os.name = Mac OS X
2009-09-05 21:38:10 os.totalmemory = 4294967296
2009-09-05 21:38:10 os.version = 10.5.8
2009-09-05 21:38:10 Command line arguments: [-a, 1, -b, 0, -w, 16, -f, topn, -n, 5,
↪ -P, -o, pflow, -i, pfreads, -t, pftemplate]
2009-09-05 21:38:10 NgsParams threshold=20 threads=2
2009-09-05 21:39:59  Index[0] memory performance
```

TalkBack may be disabled by adding `RTG_TALK_BACK=false` to the `rtg.cfg` configuration file (Unix) or the `rtg.bat` file (Window) as described in *Advanced installation configuration*.

3.5 Usage logging

RTG has the ability to record simple command usage information for submission to Real Time Genomics. The first time RTG is run (typically during installation), the user will be asked whether to enable usage logging. This information may be required for customers with a pay-per-use license. Other customers may choose to send this information to give Real Time Genomics feedback on which commands and features are commonly used or to locally log RTG command use for their own analysis.

A usage record contains the following fields:

- Time and date
- License serial number
- Unique ID for the run
- Version of RTG software
- RTG command name, without parameters (e.g. map)
- Status (Started / Failed / Succeeded)
- A command-specific field (e.g. number of reads)

For example:

```
2013-02-11 11:38:38007   4f6c2eca-0bfc-4267-be70-b7baa85ebf66    RTG Core v2.7␣
↪build d74f45d (2013-02-04)   format  Start   N/A
```

No confidential information is included in these records. It is possible to add extra fields, such as the user name running the command, host name of the machine running the command, and full command-line parameters, however as these fields may contain confidential information, they must be explicitly enabled as described in *Advanced installation configuration*.

When RTG is first installed, you will be asked whether to enable user logging. Usage logging can also be manually enabled by editing the `rtg.cfg` file (or `rtg.bat` file on Windows) and setting `RTG_USAGE=true`. If the `RTG_USAGE_DIR` and `RTG_USAGE_HOST` settings are empty, the default behavior is to directly submit usage records to an RTG hosted server via HTTPS. This feature requires the machine running RTG to have access to the Internet.

For cases where the machines running RTG do not have access to the Internet, there are two alternatives for collecting usage information.

3.5.1 Single-user, single machine

Usage information can be recorded directly to a text file. To enable this option, edit the `rtg.cfg` file (or `rtg.bat` file on Windows), and set the `RTG_USAGE_DIR` to the name of a directory where the user has write permissions. For example:

```
RTG_USAGE=true
RTG_USAGE_DIR=/opt/rtg-usage
```

Within this directory, the RTG usage information will be written to a text file named after the date of the current month, in the form `YYYY-MM.txt`. A new file will be created each month. This text file can be manually sent to Real Time Genomics when requested.

3.5.2 Multi-user or multiple machines

In this case, a local server can be started to collect usage information from compute nodes and recorded to local files for later manual submission. To configure this method of collecting usage information, edit the `rtg.cfg` file (or `rtg.bat` file on Windows), and set the `RTG_USAGE_DIR` to the name of a directory where the local server will store usage logs, and `RTG_USAGE_HOST` to a URL consisting of the name of the local machine that will run the server and the network port on which the server will listen. For example if the server will be run on a machine named `gridhost.mylan.net`, listening on port `9090`, writing usage information into the directory `/opt/rtg-usage/`, set:

```
RTG_USAGE=true
RTG_USAGE_DIR=/opt/rtg-usage
RTG_USAGE_HOST=http://gridhost.mylan.net:9090/
```

On the machine `gridhost`, run the command:

$ rtg usageserver

Which will start the local usage server listening. Now when RTG commands are run on other nodes or as other users, they will submit usage records to this sever for collation.

Within the usage directory, the RTG usage information will be written to a text file named after the date of the current month, in the form `YYYY-MM.txt`. A new file will be created each month. This text file can be manually sent to Real Time Genomics when requested.

3.5.3 Advanced usage configuration

If you wish to augment usage information with any of the optional fields, edit the `rtg.cfg` file (or `rtg.bat` file on Windows) and set the `RTG_USAGE_OPTIONAL` to a comma separated list containing any of the following:

- `username` - adds the username of the user running the RTG command.
- `hostname` - adds the machine name running the RTG command.
- `commandline` - adds the command line, including parameters, of the RTG command (this field will be truncated if the length exceeds 1000 characters).

For example:

```
RTG_USAGE_OPTIONAL=username,hostname,commandline
```

3.6 Command-line color highlighting

Some RTG commands make use of ANSI colors to visually enhance terminal output, and the decision as to whether to colorize the output is automatically determined, although some commands also contain additional flags to control colorization.

The default behavior of output colorization can be configured by defining a Java system property named `rtg.default-markup` with an appropriate value and supplying it via RTG_JAVA_OPTS. For example, to disable output colorization, use:

```
RTG_JAVA_OPTS="-Drtg.default-markup=none"
```

The possible values for `rtg.default-markup` are:

- `auto` - automatically enable ANSI markup when running on non-Windows OS and when I/O is detected to be a console.
- `none` - disable ANSI markup.
- `ansi` - enable ANSI markup. This may be useful if you are using Windows OS and have installed an ANSI-capable terminal such as ANSICON, ConEmu or Console 2.

CHAPTER

FOUR

APPENDIX

4.1 RTG reference file format

Many RTG commands can make use of additional information about the structure of a reference genome, such as expected ploidy, sex chromosomes, location of PAR regions, etc. When appropriate, this information may be stored inside a reference genome's SDF directory in a file called `reference.txt`.

The `format` command will automatically identify several common reference genomes during formatting and will create a `reference.txt` in the resulting SDF. However, for non-human reference genomes, or less common human reference genomes, a pre-built reference configuration file may not be available, and will need to be manually provided in order to make use of RTG sex-aware pipeline features.

Several example `reference.txt` files for different human reference versions are included as part of the RTG distribution in the `scripts` subdirectory, so for common reference versions it will suffice to copy the appropriate example file into the formatted reference SDF with the name `reference.txt`, or use one of these example files as the basis for your specific reference genome.

To see how a reference text file will be interpreted by the chromosomes in an SDF for a given sex you can use the `sdfstats` command with the `--sex` flag. For example:

```
$ rtg sdfstats --sex male /data/human/ref/hg19

Location            : /data/human/ref/hg19
Parameters          : format -o /data/human/ref/hg19 -I chromosomes.txt
SDF Version         : 11
Type                : DNA
Source              : UNKNOWN
Paired arm          : UNKNOWN
SDF-ID              : b6318de1-8107-4b11-bdd9-fb8b6b34c5d0
Number of sequences : 25
Maximum length      : 249250621
Minimum length      : 16571
Sequence names      : yes
N                   : 234350281
A                   : 844868045
C                   : 585017944
G                   : 585360436
T                   : 846097277
Total residues      : 3095693983
Residue qualities   : no

Sequences for sex=MALE:
chrM POLYPLOID circular 16571
chr1 DIPLOID linear 249250621
chr2 DIPLOID linear 243199373
chr3 DIPLOID linear 198022430
chr4 DIPLOID linear 191154276
chr5 DIPLOID linear 180915260
chr6 DIPLOID linear 171115067
```

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```
chr7 DIPLOID linear 159138663
chr8 DIPLOID linear 146364022
chr9 DIPLOID linear 141213431
chr10 DIPLOID linear 135534747
chr11 DIPLOID linear 135006516
chr12 DIPLOID linear 133851895
chr13 DIPLOID linear 115169878
chr14 DIPLOID linear 107349540
chr15 DIPLOID linear 102531392
chr16 DIPLOID linear 90354753
chr17 DIPLOID linear 81195210
chr18 DIPLOID linear 78077248
chr19 DIPLOID linear 59128983
chr20 DIPLOID linear 63025520
chr21 DIPLOID linear 48129895
chr22 DIPLOID linear 51304566
chrX HAPLOID linear 155270560 ~=chrY
    chrX:60001-2699520 chrY:10001-2649520
    chrX:154931044-155260560 chrY:59034050-59363566
chrY HAPLOID linear 59373566 ~=chrX
    chrX:60001-2699520 chrY:10001-2649520
    chrX:154931044-155260560 chrY:59034050-59363566
```

The reference file is primarily intended for XY sex determination but should be able to handle ZW and X0 sex determination also.

The following describes the reference file text format in more detail. The file contains lines with TAB separated fields describing the properties of the chromosomes. Comments within the `reference.txt` file are preceded by the character `#`. The first line of the file that is not a comment or blank must be the version line.

```
version1
```

The remaining lines have the following common structure:

```
<sex> <line-type>     <line-setting>...
```

The sex field is one of `male`, `female` or `either`. The line-type field is one of `def` for default sequence settings, `seq` for specific chromosomal sequence settings and `dup` for defining pseudo-autosomal regions. The *line-setting* fields are a variable number of fields based on the line type given.

The default sequence settings line can only be specified with `either` for the sex field, can only be specified once and must be specified if there are not individual chromosome settings for all chromosomes and other contigs. It is specified with the following structure:

```
either        def     <ploidy>        <shape>
```

The *ploidy* field is one of `haploid`, `diploid`, `triploid`, `tetraploid`, `pentaploid`, `hexaploid`, `polyploid` or `none`. The *shape* field is one of `circular` or `linear`.

The specific chromosome settings lines are similar to the default chromosome settings lines. All the sex field options can be used, however for any one chromosome you can only specify a single line for `either` or two lines for `male` and `female`. They are specified with the following structure:

```
<sex> seq     <chromosome-name>       <ploidy>        <shape> [allosome]
```

The *ploidy* and *shape* fields are the same as for the default chromosome settings line. The *chromosome-name* field is the name of the chromosome to which the line applies. The *allosome* field is optional and is used to specify the allosome pair of a haploid chromosome.

The pseudo-autosomal region settings line can be set with any of the *sex* field options and any number of the lines can be defined as necessary. It has the following format:

```
<sex> dup     <region>        <region>
```

The regions must be taken from two haploid chromosomes for a given sex, have the same length and not go past the end of the chromosome. The regions are given in the format `<chromosome-name>:<start>-<end>` where start and end are positions counting from one and the end is non-inclusive.

An example for the HG19 human reference:

```
# Reference specification for hg19, see
# http://genome.ucsc.edu/cgi-bin/hgTracks?hgsid=184117983&chromInfoPage=
version 1
# Unless otherwise specified, assume diploid linear. Well-formed
# chromosomes should be explicitly listed separately so this
# applies primarily to unplaced contigs and decoy sequences
either        def     diploid linear
# List the autosomal chromosomes explicitly. These are used to help
# determine "normal" coverage levels during mapping and variant calling
either        seq     chr1    diploid linear
either        seq     chr2    diploid linear
either        seq     chr3    diploid linear
either        seq     chr4    diploid linear
either        seq     chr5    diploid linear
either        seq     chr6    diploid linear
either        seq     chr7    diploid linear
either        seq     chr8    diploid linear
either        seq     chr9    diploid linear
either        seq     chr10   diploid linear
either        seq     chr11   diploid linear
either        seq     chr12   diploid linear
either        seq     chr13   diploid linear
either        seq     chr14   diploid linear
either        seq     chr15   diploid linear
either        seq     chr16   diploid linear
either        seq     chr17   diploid linear
either        seq     chr18   diploid linear
either        seq     chr19   diploid linear
either        seq     chr20   diploid linear
either        seq     chr21   diploid linear
either        seq     chr22   diploid linear
# Define how the male and female get the X and Y chromosomes
male  seq     chrX    haploid linear  chrY
male  seq     chrY    haploid linear  chrX
female        seq     chrX    diploid linear
female        seq     chrY    none    linear
#PAR1 pseudoautosomal region
male  dup     chrX:60001-2699520      chrY:10001-2649520
#PAR2 pseudoautosomal region
male  dup     chrX:154931044-155260560        chrY:59034050-59363566
# And the mitochondria
either        seq     chrM    polyploid       circular
```

As of the current version of the RTG software the following are the effects of various settings in the `reference.txt` file when processing a sample with the matching sex.

A ploidy setting of `none` will prevent reads from mapping to that chromosome and any variant calling from being done in that chromosome.

A ploidy setting of `diploid`, `haploid` or `polyploid` does not currently affect the output of mapping.

A ploidy setting of `diploid` will treat the chromosome as having two distinct copies during variant calling, meaning that both homozygous and heterozygous diploid genotypes may be called for the chromosome.

A ploidy setting of `haploid` will treat the chromosome as having one copy during variant calling, meaning that only haploid genotypes will be called for the chromosome.

A ploidy setting of `polyploid` will treat the chromosome as having one copy during variant calling, meaning that only haploid genotypes will be called for the chromosome. For variant calling with a pedigree, maternal inheritance is assumed for polyploid sequences.

The shape of the chromosome does not currently affect the output of mapping or variant calling.

The allosome pairs do not currently affect the output of mapping or variant calling (but are used by simulated data generation commands).

The pseudo-autosomal regions will cause the second half of the region pair to be skipped during mapping. During variant calling the first half of the region pair will be called as diploid and the second half will not have calls made for it. For the example `reference.txt` provided earlier this means that when mapping a male the X chromosome sections of the pseudo-autosomal regions will be mapped to exclusively and for variant calling the X chromosome sections will be called as diploid while the Y chromosome sections will be skipped. There may be some edge effects up to a read length either side of a pseudo-autosomal region boundary.

4.2 Pedigree PED input file format

The PED file format is a white space (tab or space) delimited ASCII file. Lines starting with # are ignored. It has exactly six required columns in the following order.

Column	Definition
Family ID	Alphanumeric ID of a family group. This field is ignored by RTG commands.
Individual ID	Alphanumeric ID of an individual. This corresponds to the Sample ID specified in the read group of the individual (`SM` field).
Paternal ID	Alphanumeric ID of the paternal parent for the individual. This corresponds to the Sample ID specified in the read group of the paternal parent (`SM` field).
Maternal ID	Alphanumeric ID of the maternal parent for the individual. This corresponds to the Sample ID specified in the read group of the maternal parent (`SM` field).
Sex	The sex of the individual specified as using 1 for male, 2 for female and any other number as unknown.
Phenotype	The phenotype of the individual specified using -9 or 0 for unknown, 1 for unaffected and 2 for affected.

Note: The PED format is based on the PED format defined by the PLINK project: http://pngu.mgh.harvard.edu/~purcell/plink/data.shtml#ped

The value '0' can be used as a missing value for Family ID, Paternal ID and Maternal ID.

The following is an example of what a PED file may look like.

```
# PED format pedigree
# fam-id ind-id pat-id mat-id sex phen
FAM01 NA19238 0 0 2 0
FAM01 NA19239 0 0 1 0
FAM01 NA19240 NA19239 NA19238 2 0
0 NA12878 0 0 2 0
```

When specifying a pedigree for the `lineage` command, use either the pat-id or mat-id as appropriate to the gender of the sample cell lineage. The following is an example of what a cell lineage PED file may look like.

```
# PED format pedigree
# fam-id ind-id pat-id mat-id sex phen
LIN BASE 0 0 2 0
```

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```
LIN GENA 0 BASE 2 0
LIN GENB 0 BASE 2 0
LIN GENA-A 0 GENA 2 0
```

RTG includes commands such as `pedfilter` and `pedstats` for simple viewing, filtering and conversion of pedigree files.

4.3 Genetic map directory

Certain commands such as `pedsamplesim` are able to make use of genetic linkage information provided in the form of per sequence TSV files in a directory.

Such files must be tab separated with a single header line. The header line must include the columns `pos` and `cM` (for centimorgans). A `chr` column is also recommended, but not essential. Other columns may be present, but are ignored.

For example, the start of such a file for `chr1` is:

```
chr     pos     rate            cM
chr1    6079392 0.0453026       0
chr1    6085392 0.0696987       0.0002718156
chr1    6088671 0.719604        0.0005003576373
chr1    6095199 0.203332        0.0051979325493
chr1    6098266 0.250261        0.0058215517933
```

The columns in the example are:

1. Chromosome name
2. Position in chromosome
3. Rate of crossovers in region ending at this position (ignored)
4. Total number of centimorgans from start of sequence

The corresponding map directory should contain a file or files for each sequence of interest, using the naming convention `[`*sex*`.]`*sequence*`.map` where *sex* is an optional sex specification (either `male` or `female`) and *sequence* is the name of the sequence, for example `chr1`.

When loading these files, an attempt is first made to load the file corresponding to the sex of the sample being processed. If that file does not exist, then an attempt is made to load a nonspecific version of the file. If that also does not exist, then a uniform distribution will be assumed. For example, if processing a female sample for `chr1`, first `female.chr1.map` will be tested. If that does not exist, then `chr1.map` will be tested. If that also does not exist, then the uniform distribution will be used. In this way as many or as few sequences as desired may be covered within a particular directory.

For the human genome, several sources of appropriate linkage information are available. Please note that it will generally be necessary to add headers and possibly make other adjustments to these files before they can be used with RTG.

Specific sources include:

- https://github.com/cbherer/Bherer_etal_SexualDimorphismRecombination
- https://genome.sph.umich.edu/wiki/Polymutt2
- http://bochet.gcc.biostat.washington.edu/beagle/genetic_maps/

A zip file containing the Bherer genetic map files for human build 37, with the required header manipulations already applied is available for download from: https://realtimegenomics.com/news/pre-formatted-reference-datasets

See *pedsamplesim*, *childsim*.

4.4 RTG commands using indexed input files

Several RTG commands require coordinate indexed input files to operate and several more require them when the `--region` or `--bed-regions` parameter is used. The index files used are standard tabix or BAM index files.

The RTG commands which produce the inputs used by these commands will by default produce them with appropriate index files. To produce indexes for files from third party sources or RTG command output where the `--no-index` or `--no-gzip` parameters were set, use the RTG `bgzip` and `index` commands.

4.5 RTG JavaScript filtering API

The `vcffilter` command permits filtering VCF records via user-supplied JavaScript expressions or scripts containing JavaScript functions that operate on VCF records. The JavaScript environment has an API provided that enables convenient access to components of a VCF record in order to satisfy common use cases.

4.5.1 VCF record field access

This section describes the supported methods to access components of an individual VCF record. In the following descriptions, assume the input VCF contains the following excerpt (the full header has been omitted):

```
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NA12877 NA12878
1 11259340 . G C,T . PASS DP=795;DPR=0.581;ABC=4.5 GT:DP 1/2:65 1/0:15
```

CHROM, POS, ID, REF, QUAL

Within the context of a `--keep-expr` or `record` function these variables will provide access to the String representation of the VCF column of the same name.

```
CHROM; // "1"
POS; // "11259340"
ID; // "."
REF; // "G"
QUAL; // "."
```

ALT, FILTER

Will retrieve an array of the values in the column.

```
ALT; // ["C", "T"]
FILTER; // ["PASS"]
```

INFO.{INFO_FIELD}

The values in the `INFO` field are accessible through properties on the `INFO` object indexed by `INFO ID`. These properties will be the string representation of info values with multiple values delimited with ",". Missing fields will be represented by ".". Assigning to these properties will update the VCF record. This will be undefined for fields not declared in the header.

```
INFO.DP; // "795"
INFO.ABC; // "4,5"
```

{SAMPLE_NAME}.{FORMAT_FIELD}

The JavaScript String prototype has been extended to allow access to the format fields for each sample. The string representation of values in the sample column are accessible as properties on the string matching the sample name named after the FORMAT field ID.

```
'NA12877'.GT; // "1/2"
'NA12878'.GT; // "1/0"
```

Note that these properties are only defined for fields that are declared in the VCF header (any that are not declared in the header will be undefined). See below for how to add new INFO or FORMAT field declarations.

4.5.2 VCF record modification

Most components of VCF records can be written or updated in a fairly natural manner by direct assignment in order to make modifications. For example:

```
CHROM = "chr1";       // Will change the CHROM value
POS = 42;             // Will change the POS value
ID = "rs23987382";    // Will change the ID value
QUAL = "50";          // Will change the QUAL value
FILTER = "FAIL";      // Will set the FILTER value
INFO.DPR = "0.01";    // Will change the value of the DPR info field
'NA12877'.DP = "10"; // Will change the DP field of the NA12877 sample
```

Other components of the VCF record (such as REF, and ALT) are considered immutable and can not currently be altered.

Note: Modification of CHROM and/or POS can lead to a VCF file which is incorrectly sorted and this will not necessarily be detected or reported until the resulting VCF file is used with another module or tool. Depending on the new value assigned to CHROM it may also be necessary to modify the sequence dictionary in the VCF header to reflect the change (see *VCF header modification*).

Direct assignment to ID and FILTER fields accept either a string containing semicolon separated values, or a list of values. For example:

```
ID = 'rs23987382;COSM3805';
ID = ['rs23987382', 'COSM3805'];
FILTER = 'BAZ;BANG';
FILTER = ['BAZ', 'BANG'];
```

Note that although the FILTER field returns an array when read, any changes made to this array directly are not reflected back into the VCF record.

Adding a filter to existing filters is a common operation and can be accomplished by the above assignment methods, for example by adding a value to the existing list and then setting the result:

```
var f = FILTER;
f.push('BOING');
FILTER = f;
```

However, since this is a little unwieldy, a convenience function called *add()* can be used (and may be chained):

```
FILTER.add('BOING');
FILTER.add(['BOING', 'DOING');
FILTER.add('BOING').add('DOING');
```

4.5.3 VCF header modification

Functions are provided that allow the addition of new `FILTER`, `INFO` and `FORMAT` fields to the header and records. It is recommended that the following functions only be used within the run-once portion of `--javascript`.

ensureFormatHeader({FORMAT_HEADER_STRING})

Add a new `FORMAT` field to the VCF if it is not already present. This will add a `FORMAT` declaration line to the header and define the corresponding accessor methods for use in record processing.

```
ensureFormatHeader('##FORMAT=<ID=GL,Number=G,Type=Float,' +
  'Description="Log_10 scaled genotype likelihoods.">');
```

ensureInfoHeader({INFO_HEADER_STRING})

Add a new `INFO` field to the VCF if it is not already present. This will add an `INFO` declaration line to the header and define the corresponding accessor methods for use in record processing.

```
ensureInfoHeader('##INFO=<ID=CT,Number=1,Type=Integer,' +
  'Description="Coverage threshold that was applied">');
```

ensureFilterHeader({FILTER_HEADER_STRING})

Add a new `FILTER` field to the VCF header if it is not already present. This will add an `FILTER` declaration line to the header.

```
ensureFilterHeader('##INFO=<ID=FAIL_VAL,' +
  'Description="Failed validation">');
```

4.5.4 Testing for overlap with genomic regions

One common use case is to test whether any given VCF record overlaps or is contained within a set of genomic regions. Regions may be loaded from either an external BED file or VCF file in the run-once portion of the JavaScript by either of the following:

Regions.fromBed({FILENAME})

Load the specified BED file into a *regions* object. For example:

```
var myregions = Regions.fromBed('/path/to/regions.bed');
```

Regions.fromVcf({FILENAME})

Load the specified VCF file into a *regions* object. For example:

```
var myregions = Regions.fromVcf('/path/to/regions.vcf');
```

Having loaded a set of genomic regions, this can be used to test for region overlaps using the following methods:

{REGIONS_OBJECT}.overlaps({CHROM}, {START}, {END})

Return true if the loaded regions overlap the specified interval. This function is typically used within the `record` function to test the coordinates of the current VCF record, e.g.:

```
function record() {
    if (myregions.overlaps(CHROM, POS, POS + REF.length)) {
        // do something if the record overlaps any region
    }
}
```

{REGIONS_OBJECT}.encloses({CHROM}, {START}, {END})

Return true if the loaded regions entirely encloses the supplied interval. This function is typically used within the `record` function to test the coordinates of the current VCF record, e.g.:

```
function record() {
    if (myregions.encloses(CHROM, POS, POS + REF.length)) {
        // do something if the record is fully enclosed by any region
    }
}
```

4.5.5 Additional information and functions

SAMPLES

This variable contains an array of the sample names in the VCF header.

```
SAMPLES; // ['NA12877', 'NA12878']
```

print({STRING})

Write the provided string to standard output.

```
print('The samples are: ' + SAMPLES);
```

error({STRING})

Write the provided string to standard error.

```
error('The samples are: ' + SAMPLES);
```

checkMinVersion(RTG_MINIMUM_VERSION)

Check the version of RTG that the script is running under, and exits with an error message if the version of RTG does not meet the minimum required version. This is useful when distributing scripts that make use of features that are not present in earlier versions of RTG.

```
checkMinVersion('3.9.2');
```

See also:

For javascript filtering usage and examples see *vcffilter*

4.6 Distribution Contents

The contents of the RTG distribution zip file should include:

- The RTG executable JAR file.
- RTG executable wrapper script.
- Example scripts and files.
- This operations manual.
- A release notes file and a readme file.

Some distributions also include an appropriate java runtime environment (JRE) for your operating system.

4.7 README.txt

For reference purposes, a copy of the distribution `README.txt` file follows:

```
=== RTG.VERSION ===

RTG software from Real Time Genomics includes tools for the processing
and analysis of plant, animal and human sequence data from high
throughput sequencing systems.  Product usage and administration is
described in the accompanying RTG Operations Manual.


Quick Start Instructions
========================

RTG software is delivered as a command-line Java application accessed
via a wrapper script that allows a user to customize initial memory
allocation and other configuration options. It is recommended that
these wrapper scripts be used rather than directly accessing the Java
JAR.

For individual use, follow these quick start instructions.

No-JRE:

  The no-JRE distribution does not include a Java Runtime Environment
  and instead uses the system-installed Java.  Ensure that at the
  command line you can enter "java -version" and that this command
  reports a java version of 1.7 or higher before proceeding with the
  steps below. This may require setting your PATH environment variable
  to include the location of an appropriate version of java.

Linux/MacOS X:

  Unzip the RTG distribution to the desired location.

  If your RTG distribution requires a license file (rtg-license.txt),
  copy the license file from Real Time Genomics into the RTG
  distribution directory.

  In a terminal, cd to the installation directory and test for success
  by entering "./rtg version"

  On MacOS X, depending on your operating system version and
  configuration regarding unsigned applications, you may encounter the
  error message:
```

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```
  -bash: rtg: /usr/bin/env: bad interpreter: Operation not permitted

  If this occurs, you must clear the OS X quarantine attribute with
  the command:

  xattr -d com.apple.quarantine rtg

  The first time rtg is executed you will be prompted with some
  questions to customize your installation. Follow the prompts.

  Enter "./rtg help" for a list of rtg commands.  Help for any individual
  command is available using the --help flag, e.g.: "./rtg format --help"

  By default, RTG software scripts establish a memory space of 90% of
  the available RAM - this is automatically calculated.  One may
  override this limit in the rtg.cfg settings file or on a per-run
  basis by supplying RTG_MEM as an environment variable or as the
  first program argument, e.g.: "./rtg RTG_MEM=48g map"

  [OPTIONAL] If you will be running rtg on multiple machines and would
  like to customize settings on a per-machine basis, copy
  rtg.cfg to /etc/rtg.cfg, editing per-machine settings
  appropriately (requires root privileges).  An alternative that does
  not require root privileges is to copy rtg.example.cfg to
  rtg.HOSTNAME.cfg, editing per-machine settings appropriately, where
  HOSTNAME is the short host name output by the command "hostname -s"

Windows:

  Unzip the RTG distribution to the desired location.

  If your RTG distribution requires a license file (rtg-license.txt),
  copy the license file from Real Time Genomics into the RTG
  distribution directory.

  Test for success by entering "rtg version" at the command line.  The
  first time rtg is executed you will be prompted with some
  questions to customize your installation. Follow the prompts.

  Enter "rtg help" for a list of rtg commands.  Help for any individual
  command is available using the --help flag, e.g.: "rtg format --help"

  By default, RTG software scripts establish a memory space of 90% of
  the available RAM - this is automatically calculated.  One may
  override this limit by setting the RTG_MEM variable in the rtg.bat
  script or as an environment variable.


The scripts subdirectory contains demos, helper scripts, and example
configuration files, and comprehensive documentation is contained in
the RTG Operations Manual.

Using the above quick start installation steps, an individual can
execute RTG software in a remote computing environment without the
need to establish root privileges.  Include the necessary data files
in directories within the workspace and upload the entire workspace to
the remote system (either stand-alone or cluster).

For data center deployment and instructions for editing scripts,
please consult the Administration chapter of the RTG Operations Manual.
```

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```
A discussion group is now available for general questions, tips, and other
discussions. It may be viewed or joined at:
https://groups.google.com/a/realtimegenomics.com/forum/#!forum/rtg-users

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