

Operations Manual

RTG Tools 3.4.4

Real Time Genomics

Edition: April 2015



ABSTRACT

This manual documents the use of RTG Tools software from Real Time Genomics. It describes both product use and administration.

Notice

This publication and its contents are proprietary to Real Time Genomics, and are intended solely for the contractual use of its customers and for no other purpose than to operate the system described herein.

This publication and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Real Time Genomics.

Real Time Genomics does not assume any liability arising out of the application or use of any software described herein. Further, Real Time Genomics does not convey any license under its patent, trademark, copyright, or common-law rights nor the similar rights of others.

Real Time Genomics reserves the right to make any changes in any processes, products, or parts thereof, described herein, without notice. While every effort has been made to make this guide as complete and accurate as possible as of the publication date, no warranty of fitness is implied.

© 2015 Real Time Genomics All rights reserved.

Illumina, Solexa, Complete Genomics, Ion Torrent, Roche, ABI, Life Technologies, and PacBio are registered trademarks and all other brands referenced in this document are the property of their respective owners.

Table of Contents

Chapter 1: Overview	5
1.1 Introduction	5
1.2 RTG software description	5
1.9 Installation and deployment	5
1.9.1 Quick start instructions	6
1.9.2 License Management	7
1.10 Technical assistance and support	7
Chapter 2: RTG Command Reference	8
2.1 Command line interface (CLI)	8
2.2 RTG command syntax	8
2.3 Data Formatting Commands	13
2.3.1 format	13
2.3.3 sdf2fasta	16
2.3.4 sdf2fastq	17
2.11 Utility Commands	18
2.11.1 bgzip	18
2.11.2 index	19
2.11.3 extract	20
2.11.5 sdfstats	20
2.11.7 sdfsubset	22
2.11.8 sdfsubseq	23
2.11.15 mendelian	24
2.11.16 vcfstats	25
2.11.17 vcfmerge	27
2.11.18 vcffilter	28
2.11.19 vcfannotate	31
2.11.20 vcfsubset	32
2.11.21 vcfeval	33
2.11.22 pedfilter	35
2.11.23 pedstats	36
2.11.25 rocplot	38
2.11.30 version	39
2.11.31 license	40
2.11.32 help	40
Chapter 5: Administration & Capacity Planning	41
5.1 Advanced installation configuration	41
5.2 Run-time performance optimization	42
5.3 Alternate configurations	42
5.4 Exception management - TalkBack and log file	43
5.5 Usage logging	43

5.5.1	<i>Single-user, single machine</i>	44
5.5.2	<i>Multi-user or multiple machines</i>	44
5.5.3	<i>Advanced configuration</i>	45
Chapter 6: Appendix		46
6.3	RTG reference file format	46
6.5	Pedigree PED input file format	49
6.6	RTG commands using indexed input files	50
6.9	Distribution Contents	50
6.10	README.txt	50

1 Overview

This chapter introduces the features, operational options, and installation requirements of the RTG Tools data analysis software.

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 each command is independently enabled through a license key.

Usage:

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

NOTE: For detailed information about RTG command syntax and usage, refer to Command Reference.

1.9 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 Table 2 below.

Table 2: 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).

1.9.1 Quick start instructions

These instructions are intended for an individual to install and operate the RTG software without the need to establish root / admin 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 1.7 that has undergone quality assurance testing. RTG may be used on other operating systems for which a JVM version 1.7 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 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.
- 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`

Windows:

- Unzip the RTG distribution to the desired location.
- If your distribution requires a license, copy the license file from 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.
- 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.

1.9.2 License Management

Some RTG products require the presence of a valid license file for operation.

The license 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
```

NOTE: For more data center deployment and instructions for editing scripts, see Section 5 *Administration*.

1.10 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>

2 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. 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, as shown in the following:

User-specified	Description
DIR, FILE	File or directory name(s)
INT	Integer value
FLOAT	Floating point decimal value
STRING	A sequence of characters for comments, filenames, or labels

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, as shown below.

```
$ rtg format --help
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

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

Filtering

	<code>--duster</code>	Treat lower case residues as unknowns.
	<code>--exclude=STRING</code>	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.
	<code>--select-read-group=STRING</code>	Set to only include only reads with this read group ID when formatting from SAM/BAM files.
	<code>--trim-threshold=INT</code>	Set to trim the read ends to maximise the base quality above the given threshold.

Utility

	<code>--allow-duplicate-names</code>	Set to disable duplicate name detection. Use this if you need to use less memory and you are certain there are no duplicate names in the input.
<code>-h</code>	<code>--help</code>	Prints help on command-line flag usage.
	<code>--no-names</code>	Do not include sequence names in the resulting SDF.
	<code>--no-quality</code>	Do not include sequence quality data in the resulting SDF.
	<code>--sam-rg=STRING FILE</code>	Specifies a 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
```

Alternatively, use the abbreviated character version of a full command parameter with only a single dash, as is typical for a command flag (`--output` is the same as command option as 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:

```
Product: RTG
Core Version: v3.4 build 6236f4e (2014-10-31)
RAM: 40.0GB of 47.0GB RAM can be used by rtg (84%)
License: Expires on 2015-03-30
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

Citation:
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.

(c) Real Time Genomics Inc, 2014

To see what commands you are licensed to use, type `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
<i>Data formatting:</i>		
format	Licensed	GA
sdf2fasta	Licensed	GA
sdf2fastq	Licensed	GA
<i>Utility:</i>		
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:

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

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
addpachio	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
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
```

NOTE: 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.

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 -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). (Must be
```

		one of [fasta, fastq, cgfastq, 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+). (Must be one of [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

	<code>--allow-duplicate-names</code>	Set to disable duplicate name detection. Use this if you need to use less memory and you are certain there are no duplicate names in the input.
<code>-h</code>	<code>--help</code>	Prints help on command-line flag usage.
	<code>--no-names</code>	Do not include sequence names in the resulting SDF.
	<code>--no-quality</code>	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:READGROUP1\tSM:BACT_SAMPLE\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. When using FASTQ 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.

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 files that represent mate pair data, indicate each side respectively using the `--left=FILE` and `--right=FILE` flags.

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).

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. The SAM input format is the same as the output format of Picards `FastqToSam` command line tool.

See: <http://picard.sourceforge.net/command-line-overview.shtml#FastqToSam>.

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.

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

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 reference file typically begins with the characters chr.

See also: cg2sdf, map, sdf2fasta, sdf2fastq, sdfstats, sdfsplit

2.3.3 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	Specifies the SDF data to be converted.
-o	--output=FILE	Specifies the file name used to write the resulting FASTA output.

Filtering

--end-id=INT	Only output sequences with sequence id less than the given number. (Sequence ids start at 0).
--start-id=INT	Only output sequences with sequence id greater than or equal to the given number. (Sequence ids start at 0).

Utility

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

See also: `format`, `cg2sdf`, `sdf2fastq`, `sdfstats`, `sdfsplint`

2.3.4 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

<code>-i</code>	<code>--input=SDF</code>	Specifies the SDF data to be converted.
<code>-o</code>	<code>--output=FILE</code>	Specifies the file name used to write the resulting FASTQ output.

Filtering

<code>--end-id=INT</code>	Only output sequences with sequence id less than the given number. (Sequence ids start at 0).
<code>--start-id=INT</code>	Only output sequences with sequence id greater than or equal to the given number. (Sequence ids start at 0).

Utility

<code>-h</code>	<code>--help</code>	Prints help on command-line flag usage.
<code>-q</code>	<code>--default-quality=INT</code>	Set the default quality to use if the SDF does not contain sequence quality data (0-63).
<code>-l</code>	<code>--line-length=INT</code>	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).
<code>-Z</code>	<code>--no-gzip</code>	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. 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.

See also: `format`, `cg2sdf`, `sdf2fasta`, `sdfstats`, `sdfsplint`

2.11 Utility Commands

2.11.1 `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

<code>-l</code>	<code>--compression-level=INT</code>	the compression level to use, between 1 (least but fast) and 9 (highest but slow) (Default is 5)
<code>-d</code>	<code>--decompress</code>	Set to decompress the input file.
<code>-f</code>	<code>--force</code>	Overwrite the output file if it already exists.
	<code>--no-terminate</code>	if set, do not add the block gzip termination block
<code>-c</code>	<code>--stdout</code>	Write output to standard output, keep the original files unchanged. Implied when using standard input.
	<code>FILE+</code>	Specifies the file to be compressed or decompressed. Use '-' to read from standard input. Must be specified 1 or more times.

Utility

<code>-h</code>	<code>--help</code>	Prints 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`

2.11.2 `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]... -f FORMAT FILE+
```

Multi-file input specified in a text file:

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

Example:

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

Parameters:**File Input/Output**

<code>-f</code>	<code>--format=FORMAT</code>	Specifies format of the input files to be indexed. (Must be one of [sam, bam, sv, coveragets, bed, vcf]).
<code>-I</code>	<code>--input-list-file=FILE</code>	Specifies a file containing a list of block compressed files (1 per line) containing data in the specified genome position format.
	<code>FILE+</code>	Specifies a block compressed file containing data in the specified genome position format to be indexed. May be specified 0 or more times.

Utility

<code>-h</code>	<code>--help</code>	Prints 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 and the output from `sv`, `discord`, `coverage` and `snp` commands. The `index` command can also be used to produce BAM indexes for BAM files with no index.

See also: `map`, `coverage`, `snp`, `sv`, `discord`, `extract`, `bgzip`

2.11.3 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 'chr1:10000+10'
```

Parameters:

File Input/Output

FILE	The indexed block compressed genome position data file to extract.
------	--

Filtering

STRING+	Specifies the region to display. The format is one of <sequence_name>, <sequence_name>:start-end or <sequence_name>:start+length. May be specified 0 or more times.
---------	---

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.

See also: `map`, `coverage`, `snp`, `sv`, `index`, `bgzip`

2.11.5 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
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 be specified 1 or more times.

Reporting

--lengths Set to print out the name and length of each sequence. (Not recommended for read sets).

-p --position Set to include information about unknown bases (Ns) by read position.

-q --quality Set to display mean of quality.

 --sex=SEX Set to display the reference sequence list for the given sex. (Must be one of [male, female, either]). May be specified 0 or more times.

 --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`, `cg2sdf`, `sdf2fasta`, `sdf2fastq`, `sdfstats`, `sdfsplitt`

2.11.7 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

-I	--id-file=FILE	Specifies a file containing sequence ids, or sequence names if the names flag is set, (one per line) to extract from the input SDF.
-n	--names	Set to specify sequence names instead of sequence ids.
	STRING+	Specifies the sequence id, or sequence name if the names flag is set to extract 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`

2.11.8 `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

<code>-i</code>	<code>--input=FILE</code>	Specifies the input SDF.
-----------------	---------------------------	--------------------------

Filtering

<code>-I</code>	<code>--sequence-id</code>	Set to use sequence id instead of sequence name in region flag (0-based).
-----------------	----------------------------	---

<code>STRING+</code>	Specifies the region to display. The format is one of <code><sequence_name></code> , <code><sequence_name>:start-end</code> or <code><sequence_name>:start+length</code> . Must be specified 1 or more times
----------------------	--

Utility

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

2.11.15 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

<code>-i</code>	<code>--input=FILE</code>	VCF file containing the multiple sample variant calls. Use '-' to read from standard input.
	<code>--output=FILE</code>	Set to output annotated calls to this VCF file.
	<code>--output-consistent=FILE</code>	Set to output only consistent calls to this VCF file.
	<code>--output-inconsistent=FILE</code>	Set to output only non-Mendelian calls to this VCF file.
<code>-t</code>	<code>--template=SDF</code>	SDF containing template to which was used to create the VCF.

Sensitivity Tuning

<code>-l</code>	<code>--lenient</code>	Set to allow homozygous diploid variant calls in place of haploid calls and assume missing values are equal to the reference.
	<code>--all-records</code>	Use all records, regardless of filters. Default is to only process records where FILTER is "." or "PASS".
	<code>--pedigree=FILE</code>	Specify a genome relationships PED file. The default is to extract pedigree information from the VCF header fields.

Utility

<code>-h</code>	<code>--help</code>	Prints help on command-line flag usage.
<code>-Z</code>	<code>--no-gzip</code>	Set this flag to create the VCF output file without compression. By default the output file is compressed with blocked gzip.

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 template and ensure the sex of each sample is supplied as part of the pedigree information. While it is best to give the template used in the creation of the VCF, for checking third-party outputs any template containing the same chromosome names and an appropriate `reference.txt` file will work.

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, you can end up with situations where calls are not available for every member of the family. Under normal circumstances these will be reported as an allele count constraint violation. It is possible 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 mislabelled. 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`

2.11.16 `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 to include
                        all samples). May be specified 0 or more
                        times.

FILE+                 VCF file from which to derive statistics.
                        Use '-' to read from standard input. 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.

See also: `snp`, `family`, `somatic`, `vcfmerge`, `discord`

2.11.17 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

-a	--add-header=STRING	Add the supplied text to the output VCF header. May be specified 0 or more times.
-o	--output=FILE	The output VCF file name. Use '-' to write to standard output.
	FILE+	VCF files to be merged. Must be specified 1 or more times.

Utility

	--force-merge=STRING	Set to allow merging of specified header ID even when descriptions do not match. May be specified 0 or more times.
-h	--help	Prints help on command-line flag usage.
-Z	--no-gzip	Set this flag to create the VCF output file without compression. By default the output file is compressed with blocked gzip.
	--no-index	Set this flag to not produce the index for the VCF output file.
	--preserve-formats	If set, variants with different ALTs and unmergeable FORMAT fields will be kept unmerged (Default is to remove those FORMAT fields so the variants can be combined).
	--stats	Set to output statistics for the merged VCF file.

Usage:

The vcfmerge command takes a list of VCF files and outputs to a single VCF file. The input files must have consistent header lines, although similar header lines can be forced to merge using the --force-merge parameter. 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). If the input VCFs contain multiple calls at the same position for the same sample, a warning is issued and only the first is kept.

When multiple records occur at the same position and the length on the reference is the same, the records will be merged into a single record. If the merge results in a change in the set of ALT alleles, any VCF `FORMAT` fields declared to be of type 'A', 'G', or 'R' will be set to the missing value ('.'), as they cannot be meaningfully updated. The `--preserve-formats` flag prevents this loss of information by refusing to merge the records (separate records will be output).

See also: `snps`, `family`, `population`, `somatic`, `discord`, `bgzip`, `index`

2.11.18 `vcffilter`

Synopsis:

Filter VCF output files to include or exclude records based on various criteria.

Syntax:

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

Example:

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

Parameters:

File Input/Output

<code>--all-samples</code>	Set to apply sample-specific criteria to all samples contained in the input VCF.
<code>--bed-regions=FILE</code>	If set, only read VCF records that overlap the ranges contained in the specified BED file. Requires the input VCF to be tabix indexed.
<code>-i --input=FILE</code>	Specifies the VCF file containing variants to be filtered. Use '-' to read from standard input.
<code>-o --output=FILE</code>	Specifies the output VCF file. Use '-' to write to standard output.
<code>--region=STRING</code>	if set, only read VCF records within the specified range. The format is one of <code><template_name></code> , <code><template_name>:start-end</code> .
<code>--sample=STRING</code>	Set to apply sample-specific criteria to the named sample contained in the input

VCF. May be specified 0 or more times.

Filtering

-w	--density-window=INT	Set a window length in which multiple called variants are discarded.
	--exclude-bed=FILE	Set to discard all variants within the regions contained in the BED file.
	--exclude-vcf=FILE	Set to discard all variants that overlap with the ones in this VCF file.
	--include-bed=FILE	Set to only keep variants within the regions contained in the BED file.
	--include-vcf=FILE	Set to only keep variants that overlap with the ones in this VCF file.
-k	--keep-filter=STRING	Set to only keep variants with this FILTER tag. May be specified 0 or more times.
-K	--keep-info=STRING	Set to only keep variants with this INFO tag. May be specified 0 or more times.
-A	--max-ambiguity-ratio=FLOAT	Set the maximum allowed ambiguity ratio.
	--max-avr-score=FLOAT	Set the maximum allowed AVR score.
-C	--max-combined-read-depth=INT	Set the maximum allowed combined read depth.
	--max-denovo-score=FLOAT	Set the maximum allowed de novo score.
-G	--max-genotype-quality=FLOAT	Set the maximum allowed genotype quality.
-Q	--max-quality=FLOAT	Set the maximum allowed quality.
-D	--max-read-depth=INT	Set the maximum allowed sample read depth.
	--min-avr-score=FLOAT	Set the minimum allowed AVR score.
-c	--min-combined-read-depth=INT	Set the minimum allowed combined read depth.
	--min-denovo-score=FLOAT	Set the minimum allowed de novo score.
-g	--min-genotype-quality=FLOAT	Set the minimum allowed genotype quality.
-q	--min-quality=FLOAT	Set the minimum allowed quality.
-d	--min-read-depth=INT	Set the minimum allowed sample read depth.

	<code>--non-snp-only</code>	Set to output MNPs and INDELs only.
	<code>--remove-all-same-as-ref</code>	Set to remove records where all the samples are same as the reference.
<code>-r</code>	<code>--remove-filter=STRING</code>	Set to remove variants with this FILTER tag. May be specified 0 or more times.
<code>-R</code>	<code>--remove-info=STRING</code>	Set to remove variants with this INFO tag. May be specified 0 or more times.
	<code>--remove-overlapping</code>	Set to remove records that overlap with previous records.
	<code>--remove-same-as-ref</code>	Set to remove variants where the sample is the same as reference.
	<code>--snps-only</code>	Set to output simple SNPs only.

Reporting

	<code>--clear-failed-samples</code>	Set to have the GT field of failing samples set to the missing value instead of removing the record.
	<code>--fail=STRING</code>	Set to have the filter field of a failed record set to the provided value instead of removing it.

Utility

<code>-h</code>	<code>--help</code>	Prints help on command-line flag usage.
<code>-Z</code>	<code>--no-gzip</code>	Set this flag to create the output file without compression. By default the output file is compressed with tabix compatible blocked gzip.
	<code>--no-index</code>	Set this flag to not produce the tabix index for the output file.

Usage:

Use `vcffilter` to get a subset of the results from snp calling based on the filtering criteria supplied by the filter flags. When filtering on multiple samples, if any of the specified samples fail the criteria, the record will be filtered.

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.

See also: snp, family, somatic, population, vcfannotate, vcfssubset

2.11.19 vcfannotate

Synopsis:

Used to add annotations to a VCF file.

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

-i	--input=FILE	Specifies the VCF file containing variants. Use '-' to read from standard input.
-o	--output=FILE	Specifies the output VCF file for the annotated variants. Use '-' to write to standard output.

Reporting

--bed-ids=FILE	Specifies a file in BED format containing variant ids in the name column to be added to the VCF id field. May be specified 0 or more times.
--bed-info=FILE	Specifies a file in BED format containing annotations in the name column to be added to the VCF info field. May be specified 0 or more times.
--fill-an-ac	Set to add or update the AN and AC info fields to the VCF.
--vcf-ids=FILE	Specifies a file in VCF format containing variant ids to be added to the VCF id field. May be specified 0 or more times.

Utility

-h	--help	Prints help on command-line flag usage.
-Z	--no-gzip	Set this flag to create the output file without compression. By default the output file is compressed with tabix compatible blocked gzip.
	--no-index	Set this flag to not produce the tabix index for the output file.

Usage:

Use `vcfannotate` to add text annotations to variants that fall within ranges specified in a BED file. The annotations from the BED file are added as an INFO field in the output VCF file.

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 4.1 specification) created or updated.

See also: `snp`, `family`, `somatic`, `population`, `vcffilter`, `vcfsubset`

2.11.20 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

<code>-i</code>	<code>--input=FILE</code>	Specifies the VCF file containing variants to manipulate. Use '-' to read from standard input.
<code>-o</code>	<code>--output=FILE</code>	Specifies the output VCF file for the subset records. Use '-' to write to standard output.

Filtering

<code>--keep-filter=STRING</code>	Specifies a VCF FILTER tag to keep in the output. May be specified 0 or more times.
<code>--keep-format=STRING</code>	Specifies a VCF FORMAT tag to keep in the output. May be specified 0 or more times.
<code>--keep-info=STRING</code>	Specifies a VCF INFO tag to keep in the output. May be specified 0 or more times.
<code>--keep-sample=STRING</code>	Specifies a sample to keep in the output. May be specified 0 or more times.
<code>--remove-filter=STRING</code>	Specifies a VCF FILTER tag to remove from the output. May be specified 0 or more times.

<code>--remove-filters</code>	Set to remove all of the FILTER tags from the output.
<code>--remove-format=STRING</code>	Specifies a VCF FORMAT tag to remove from the output. May be specified 0 or more times.
<code>--remove-info=STRING</code>	Specifies a VCF INFO tag to remove from the output. May be specified 0 or more times.
<code>--remove-infos</code>	Set to remove all of the INFO tags from the output.
<code>--remove-sample=STRING</code>	Specifies a sample to remove from the output. May be specified 0 or more times.
<code>--remove-samples</code>	Set to remove all of the sample data from the output.

Utility

<code>-h</code>	<code>--help</code>	Prints help on command-line flag usage.
<code>-Z</code>	<code>--no-gzip</code>	Set this flag to create the output file without compression. By default the output file is compressed with tabix compatible blocked gzip.
	<code>--no-index</code>	Set this flag to not produce the tabix index for the output file.

Usage:

Use the `vcfssubset` 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.

See also: `snmp`, `family`, `somatic`, `population`, `vcffilter`, `vcfannotate`

2.11.21 `vcfeval`

Synopsis:

Use the `vcfeval` command to evaluate called variants for agreement with a known baseline variant set.

Syntax:

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

Example:

```
$ rtg vcfeval -b simulated.vcf.gz -c snps.vcf.gz -t HUMAN_reference
--sample simsample -f AVR -o eval
```

Parameters:

File Input/Output

-b	--baseline=FILE	The VCF file containing baseline variants.
-c	--calls=FILE	The VCF file containing called variants.
-o	--output=DIR	The name of the output directory.
-t	--template=SDF	The reference SDF on which the variants were called.

Filtering

--all-records	Set to use all records regardless of filters. Default is to only process records where FILTER is . or PASS.
--sample=STRING	Set the name of the sample to select. (Required when using multi-sample VCF files).
--squash-ploidy	Treat heterozygous variants as homozygous ALT in both baseline and calls.

Reporting

	--baseline-tp	Also output a file containing the baseline version of true positive variants (baseline-tp.vcf). This is in addition to the tp.vcf which contains the calls version of true-positive variants.
-O	--sort-order=STRING	Set the order in which to sort the ROC scores so that "good" scores come before "bad" scores. (Must be one of [ascending, descending]). (Default is descending).
-f	--vcf-score-field=STRING	Set the VCF format field to sort the ROC using. Also valid are "QUAL" or "INFO=<name>" to select the named VCF INFO field. (Default is GQ).

Utility

-h	--help	Prints help on command-line flag usage.
-Z	--no-gzip	Set this flag to create the output files without compression.
-T	--threads=INT	Specify the number of threads to use in a multi-core processor. (Default is all 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.

The `vcfeval` command outputs the following primary files:

- `tp.vcf` – contains those variants from the calls VCF which agree with variants in the baseline VCF
- `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.
- `weighted_roc.tsv` – contains data for ROC analysis, which can be plotted with the `rocplot` command.

It is also possible to output a true-positive VCF file that contains the baseline version of correct variants with the `--baseline-tp` option. This can be used to successively refine a highly sensitive baseline variant set to produce a consensus from several call sets.

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. The `--squash-ploidy` flag treats heterozygous genotypes from both baseline and calls as homozygous ALT to provide a more lenient comparison, however multiallelic sites where the genotype for the baseline or call employs multiple ALTs may still result in disagreement.

See also: `snp`, `popsim`, `samplesim`, `childsim`, `rocplot`

2.11.22 `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 stdin.
------	--

Filtering

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

Usage:

The `pedfilter` comand can be used to perform manipulations on pedigree information and convert pedigree information between PED and VCF header 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 stdout, which can be redirected to a file or another pipeline command as required.

See also: `family`, `population`, `mendelian`, `pedstats`

2.11.23 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

```

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

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

For a larger pedigree:

```
$ dot -Tpdf -o mypedigree.pdf <(rtg pedstats --dot "Study" mypedigree.ped)
```

To output a list of all founders:

```

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

```

Parameters:

File Input/Output

FILE	The pedigree file to process, may be PED or VCF, use '-' to read from stdin.
------	--

Reporting

--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.

Utility

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

Usage:

Used to show pedigree summary statistics or select groups of individual Ids. In particular, it is possible to generate a simple pedigree visualization.

The VCF files output by the `family` and `population` commands contain full pedigree information represented as VCF header lines, and the `pedstats` command can also take these VCFs as input.

See also: `family`, `population`, `pedfilter`

2.11.25 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

<code>--curve=STRING</code>	Specify a ROC data file with title optionally specified (path[=title]). May be specified 0 or more times.
<code>--png=FILE</code>	Set to output a PNG image to the given file instead of loading the interactive plot.
<code>FILE+</code>	Specify the ROC data file to plot. May be specified 0 or more times.

Reporting

<code>--hide-sidepane</code>	Set to hide the sidepane from the GUI on startup.
<code>--line-width=INT</code>	Set the line width for the plots. (Default is 2).
<code>--scores</code>	Set to show scores on the plot.
<code>-t --title=STRING</code>	Set the title for the plot.

Utility

<code>-h --help</code>	Prints help on command-line flag usage.
------------------------	---

Usage:

Used to produce ROC plots from the ROC files produced by `readsimeval` 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 a PNG file using the `--png` parameter.

Some quick tips for the interactive GUI:

- Select regions within the graph to zoom in. Right click to bring up a context menu that allows resetting the zoom.
- 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 clear 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.
- Additional ROC data files can be loaded by clicking on the '+' button.
- Each ROC curve can be shown/hidden, renamed, and reordered in it's widget area on the right hand side of the UI.
- Each ROC 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.

See also: readsimeval, vcfeval

2.11.30 version

Synopsis:

The RTG version display utility.

Syntax:

```
$ rtg version
```

Example:

```
$ rtg version
Product: RTG Core 3.4
Core Version: v3.4 build 4586490 (2014-12-04)
RAM: 3.5GB of 3.8GB RAM can be used by RTG (91%)
License: Expires on 2015-03-30
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

Citation:
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.

(c) Real Time Genomics, 2014
```

Parameters:

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

Usage:

Use the `version` command to instantiate the RTG product and display release and version information.

See also: `help`, `license`

2.11.31 `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 (stdout) shows command name, licensed status, and command release level. It is possible to have access to commands prior to general availability (GA) release with certain support contracts from Real Time Genomics.

See also: `help`, `version`

2.11.32 `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`

5 Administration & Capacity Planning

5.1 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. As described, 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.: <code>RTG_MEM=48G</code> . 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 <code>RTG.jar</code> executable (default assumes current path).
RTG_JAVA_OPTS	Provide any additional Java JVM options.
RTG_DEFAULT_THREADS	By default any RTG module with a <code>--threads</code> parameter will automatically use the number of cores as the number of threads. This setting makes the specified number the default for the <code>--threads</code> 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).

Variable	Description
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 <code>username</code> , <code>hostname</code> and <code>commandline</code> 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.

5.2 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 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 (gzip), 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.

5.3 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, 3MB 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.

5.4 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 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, pflows, -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.

5.5 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, hostname 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.

5.5.1 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.

5.5.2 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.

5.5.3 Advanced 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
```

6 Appendix

6.3 RTG reference file format

Additional information about the structure of a reference genome can be provided for RTG mapping and variant calling by creating a `reference.txt` file in the reference genome's SDF directory. This file specifies information about the structure of the chromosomes in the reference genome including sex information. 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.

```
version 1
```

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 "diploid", "haploid", "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

```

Several reference.txt files for common human reference versions are included as part of the RTG distribution in the scripts subdirectory.

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 POLYPOID 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
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 XO sex determination also.

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.

6.5 Pedigree PED input file format

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

Column	Definition
Family ID	Alphanumeric ID of a family group.
Individual ID	Alphanumeric ID of an individual. This corresponds to the Sample ID specified in the read group of the individual.
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.
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.
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
```

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.

6.6 RTG commands using indexed input files

Several RTG commands require indexed input files to operate and several more require them when the `--region` or `--bed-regions` parameter is used.

The commands that always require indexed input files are `snp`, `family`, `somatic`, `population`, `vcfmerge` and `extract`. The commands that only require indexed input files if the `--region` or `--bed-regions` parameter is set are `coverage`, `cnv`, `sv`, `discord` and `sammerge`.

The RTG commands which produce the inputs used by these commands will by default produce them with 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.

6.9 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.

6.10 README.txt

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

```
=== RTG Software ===

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 RTG Operations Manual.

Quick Start Instructions
=====

RTG software is delivered as a Java application accessed via a wrapper
script that allows a user to customize initial memory allocation and
other configuration options.

For individual use, follow these quick start instructions.

Linux/MacOS X:
```

Unzip the RTG distribution to the desired location.

If your RTG distribution does not already include a license (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 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 does not already include a license (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.

Using 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.

Terms of Use =====

This proprietary software program is the property of Real Time Genomics, Inc. All use of this software program is subject to the terms of an applicable end user license agreement.

Third Party Software Used =====

RTG software uses the open source Picard library (<https://sourceforge.net/projects/picard/>) for reading and writing SAM files, under the terms of following license:

The MIT License

Copyright (c) 2009 The Broad Institute

Permission is hereby granted, free of charge, to any person obtaining a copy of this software and associated documentation files (the "Software"), to deal in the Software without restriction, including without limitation the rights to use, copy, modify, merge, publish, distribute, sublicense, and/or sell copies of the Software, and to permit persons to whom the Software is furnished to do so, subject to the following conditions:

The above copyright notice and this permission notice shall be included in

all copies or substantial portions of the Software.

THE SOFTWARE IS PROVIDED "AS IS", WITHOUT WARRANTY OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO THE WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE AND NONINFRINGEMENT. IN NO EVENT SHALL THE AUTHORS OR COPYRIGHT HOLDERS BE LIABLE FOR ANY CLAIM, DAMAGES OR OTHER LIABILITY, WHETHER IN AN ACTION OF CONTRACT, TORT OR OTHERWISE, ARISING FROM, OUT OF OR IN CONNECTION WITH THE SOFTWARE OR THE USE OR OTHER DEALINGS IN THE SOFTWARE.

RTG software uses the bzip2 library included in the open source Ant project (<http://ant.apache.org/>) for decompressing bzip2 format files, under the following license:

Copyright 1999-2010 The Apache Software Foundation

Licensed under the Apache License, Version 2.0 (the "License"); you may not use this file except in compliance with the License. You may obtain a copy of the License at

<http://www.apache.org/licenses/LICENSE-2.0>

Unless required by applicable law or agreed to in writing, software distributed under the License is distributed on an "AS IS" BASIS, WITHOUT WARRANTIES OR CONDITIONS OF ANY KIND, either express or implied. See the License for the specific language governing permissions and limitations under the License.

RTG Software uses a modified version of java/util/zip/GZIPInputStream.java (available in the accompanying gzipfix.jar) from OpenJDK 7 under the terms of the following license:

This code is free software; you can redistribute it and/or modify it under the terms of the GNU General Public License version 2 only, as published by the Free Software Foundation. Oracle designates this particular file as subject to the "Classpath" exception as provided by Oracle in the LICENSE file that accompanied this code.

This code is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. See the GNU General Public License version 2 for more details (a copy is included in the LICENSE file that accompanied this code).

You should have received a copy of the GNU General Public License version 2 along with this work; if not, write to the Free Software Foundation, Inc., 51 Franklin St, Fifth Floor, Boston, MA 02110-1301 USA.

Please contact Oracle, 500 Oracle Parkway, Redwood Shores, CA 94065 USA or visit www.oracle.com if you need additional information or have any questions.

RTG Software uses hierarchical data visualization software from <http://sourceforge.net/projects/krona/> under the terms of the following license:

Copyright (c) 2011, Battelle National Biodefense Institute (BNBI); all rights reserved. Authored by: Brian Ondov, Nicholas Bergman, and Adam Phillippy

This Software was prepared for the Department of Homeland Security (DHS) by the Battelle National Biodefense Institute, LLC (BNBI) as part of contract HSHQDC-07-C-00020 to manage and operate the National Biodefense Analysis and Countermeasures Center (NBACC), a Federally Funded Research and Development Center.

Redistribution and use in source and binary forms, with or without modification, are permitted provided that the following conditions are met:

- * Redistributions of source code must retain the above copyright notice, this list of conditions and the following disclaimer.
- * Redistributions in binary form must reproduce the above copyright notice, this list of conditions and the following disclaimer in the documentation and/or other materials provided with the distribution.
- * Neither the name of the Battelle National Biodefense Institute nor the names of its contributors may be used to endorse or promote

products derived from this software without specific prior written permission.

THIS SOFTWARE IS PROVIDED BY THE COPYRIGHT HOLDERS AND CONTRIBUTORS "AS IS" AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE DISCLAIMED. IN NO EVENT SHALL THE COPYRIGHT HOLDER OR CONTRIBUTORS BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY OUT OF THE USE OF THIS SOFTWARE, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGE.