

BAMSurgeon: Methods for spike-in mutations on BAM files

Adam D. Ewing (adam.ewing@mater.uq.edu.au)

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1 Introduction

1.1 Software Dependencies

BAMSurgeon requires the following software packages be available:

1. samtools/wgsim/tabix (<http://samtools.sourceforge.net/>)
2. pysam (<http://code.google.com/p/pysam/> or `pip install pysam`)
3. bwa (<http://bio-bwa.sourceforge.net/>)
4. velvet (<http://www.ebi.ac.uk/~zerbino/velvet/>)
5. exonerate (<https://github.com/nathanweeks/exonerate>)
6. picard (<http://http://picard.sourceforge.net/>)

For install instructions please see the included README.md or have a look at the Dockerfile.

1.2 Known bugs and limitations

BAMSurgeon is under continuous development driven by suggestions and bug reports from the mutation calling community.

1. If haplotype resolution is important for your application we suggest phasing BAMs beforehand and simulating mutations on a per-haplotype basis.

2 Adding SNVs to existing BAM alignments (addsnv)

2.1 Usage

```
usage: addsnv.py [-h] -v VARFILENAME -f BAMFILENAME -r REFFASTA -o OUTBAMFILE
                  [-s SNVFRAC] [-m MUTFRAC] [-n NUMSNVS] [-c CNVFILE]
                  [-d COVERDIFF] [-z HAPLOSIZE] [-p PROCS]
                  [--picardjar PICARDJAR] [--mindepth MINDEPTH]
                  [--maxdepth MAXDEPTH] [--minmutreads MINMUTREADS]
                  [--avoidreads AVOIDREADS] [--nomut] [--ignoresnps]
                  [--ignoreref] [--force] [--insane] [--single]
                  [--maxopen MAXOPEN] [--requirepaired] [--tagreads]
```

[--skipmerge] [--ignorepileup] [--aligner ALIGNER]
[--alignopts ALIGNOPTS] [--tmpdir TMPDIR] [--seed SEED]

adds SNVs to reads, outputs modified reads as .bam along with mates

optional arguments:

-h, --help show this help message and exit
-v VARFILENAME, --varfile VARFILENAME
Target regions to try and add a SNV, as BED
-f BAMFILENAME, --bamfile BAMFILENAME
sam/bam file from which to obtain reads
-r REFFASTA, --reference REFFASTA
reference genome, fasta indexed with bwa index _and_
samtools faidx
-o OUTBAMFILE, --outbam OUTBAMFILE
.bam file name for output
-s SNVFRAC, --snvfrac SNVFRAC
maximum allowable linked SNP MAF (for avoiding
haplotypes) (default = 1)
-m MUTFRAC, --mutfrac MUTFRAC
allelic fraction at which to make SNVs (default = 0.5)
-n NUMSNVS, --numsnvs NUMSNVS
maximum number of mutations to try (default: entire
input)
-c CNVFILE, --cnvfile CNVFILE
tabix-indexed list of genome-wide absolute copy number
values (e.g. 2 alleles = no change)
-d COVERDIFF, --coverdiff COVERDIFF
allow difference in input and output coverage
(default=0.9)
-z HAPLOSIZE, --haplosize HAPLOSIZE
haplotype size (default = 0)
-p PROCS, --procs PROCS
split into multiple processes (default=1)
--picardjar PICARDJAR
path to picard.jar, required for most aligners
--mindepth MINDEPTH minimum read depth to make mutation (default = 10)
--maxdepth MAXDEPTH maximum read depth to make mutation (default = 2000)
--minmutreads MINMUTREADS
minimum number of mutated reads to output per site
--avoidreads AVOIDREADS
file of read names to avoid (mutations will be skipped
if overlap)
--nomut dry run
--ignoresnps make mutations even if there are non-reference alleles
sharing the relevant reads
--ignoreref make mutations even if the mutation is back to the
reference allele

```

--force                force mutation to happen regardless of nearby SNP or
                        low coverage
--insane               ignore sanity check enforcing input read count =
                        output read count in realignment
--single               input BAM is single-ended (default is paired-end)
--maxopen MAXOPEN      maximum number of open files during merge (default
                        1000)
--requirepaired        skip mutations if unpaired reads are present
--tagreads             add BS tag to altered reads
--skipmerge            final output is tmp file to be merged
--ignorepileup         do not check pileup depth in mutation regions
--aligner ALIGNER      supported aligners:
                        backtrack,mem,novoalign,gsnap,STAR,bowtie2,tmap,bwakit
--alignopts ALIGNOPTS  aligner-specific options as comma delimited list of
                        option1:value1,option2:value2,...
--tmpdir TMPDIR        temporary directory (default=addsnv.tmp)
--seed SEED            seed random number generation

```

2.2 Description

Single nucleotide changes are introduced to an existing BAM alignment using `addsnv.py` as outlined in Figure 1. Input consists of a list of locations where SNVs will be made, a target BAM alignment, and a reference genome indexed using `bwa` (preferably the same genome used to align the reads in the BAM file).

2.3 Input

Mutation input (-v/-varfile) Input to `addsnv.py` (-v/-varfile) is a three column format plus an extra optional column for the desired variant allele fraction (VAF). Input coordinates are **1-based**, meaning they will match between input to -v/-varfile, VCF, and `samtools mpileup`. Note that `addindel.py` uses a 1-based coordinate scheme. The default VAF is specified via -m/-mutfrac. For example:

```

22 34166720 34166720 0.25
22 33908770 33908770 0.15
22 33714964 33714964 0.5
22 33769483 33769483 0.75
22 33958087 33958087 0.01
22 34264774 34264774 0.25
22 33702084 33702084 0.2

```

Specific bases can also be specified using a fifth input column:

```

22 34166720 34166720 0.25 A
22 33908770 33908770 0.15 T
22 33714964 33714964 0.5 G
22 33769483 33769483 0.75 C

```

The above input will cause addsnv to attempt to add SNVs at the specified locations, using the VAF specified in the fourth column if available, otherwise defaulting to 0.5 or whatever is specified by `-m/-mutfrac` (e.g. the last three lines). If a position is specified that spans more than one base, the position of the SNV input will be chosen at random from that interval.

BAM Alignment input (`-f/-bamfile`) BAMs ideally should adhere to SAM specification i.e. they should validate via picard's `ValidateSamFile`. BAMs should be sorted in coordinate order and indexed. BAMs should consist either of entirely paired-end reads or entirely single-end (fragment) reads using the `-single` option, mixing paired and single reads yields unpredictable results. Currently, bwa backtrack (`bwa aln`) and bwa mem are supported, although it should be straightforward enough to add further aligners through modification of the `remap_paired` function. In practice we have been able to spike mutations into RNA-seq MapSplice BAMs, for instance.

Reference genome (`-r/-reference`) The reference genome should be the **same as that used to create the target BAM file**, specifically the chromosome names and lengths in the reference FASTA must be the same as in the BAM header. The reference must be indexed for bwa (`bwa index`) and indexed with samtools (`samtools faidx`).

Avoiding existing SNPs with `-s/-snpfrac` (optional, default=1.0) In an attempt to preserve haplotype structure and avoid confusion by spiking mutations on top of existing heterozygous alleles, the `-s/-snpfrac` cutoff may be useful. For a selected mutation site S and specified cutoff f starting from the leftmost base L of the leftmost read containing S and ending at the rightmost base R of the rightmost read containing S , the minor allele frequency M for every intervening base is calculated for every base in the interval $[L, R]$. If $M < f$ for all bases in $[L, R]$ the mutation at S is allowed, and skipped otherwise. Note, do not confuse this with `-m/-mutfrac`, which is the target variant allele frequency for mutations unless overridden in the mutation input file. By default this function is turned off (threshold minor allele frequency set to 1.0). Setting this too low can yield few successful mutations as sequence errors will inhibit successful spike-in, we recommend a setting of 0.1 for Illumina GA/HiSeq reads.

CNV file (`-c/-cnvfile`) (optional) It is possible to simulate adding mutations to a single haplotype for copy-number variant regions. For example, if a region has absolute copy number 3 (instead of 2), a mutation into one haplotype should have an apparent VAF of roughly 0.33 instead of 0.5. This optional file should be in BED-3 format plus a fourth column representing the copy number fold-change for the region defined by the chromosome/contig, start, and end fields. A copy number fold-change of 1 will not adjust the variant allele fractions. Copy number changes below 1 will adjust VAFs higher and copy number fold-changes above 1 will adjust VAFs correspondingly lower.

Additional options

- `-d/-coverdiff` : threshold on output coverage divided by input coverage for mutation sites. Mutations can cause alignments to change, if this is undesired set this high, if this is part of the fun, set this low.
- `-z/-haplosize` : specifies size of haplotype block to consider when adding more than 1 proximal mutation. For instance, if two SNVs are spiked in 10bp apart and `-haplosize` is 10 or greater,

the two SNVs will be on the same haplotype (i.e. share the same reads for reads covering both positions)

- `-p/-procs` : BAMSurgeon programs currently spawn a new process for each spikein, to speed things along the processes can be run concurrently with the number of simultaneous jobs set by this parameter.
- `-picardjar` : path to `picard.jar` provided by picard tools, this is required for the some aligner options.
- `-mindepth` : minimum depth at sites for mutations, sites with fewer reads in pileup column will be dropped. Default is 10 reads.
- `-maxdepth` : sites with greater than this number of covering reads will be dropped. Default is 2000 reads. (May need to increase for targeted sequencing data)
- `-minmutreads` : minimum number of mutated reads per site. If this level of coverage cannot be obtained, mutations will be dropped.
- `-ignoresnps`: ignores existing non-reference bases at the mutation site, required if the goal is to change a non-reference allele back to reference
- `-ignoreref`: required if the goal is to change a non-reference allele back to reference
- `-avoidreads` : `addsnv` and `addindel` can be passed a list of read names to avoid when making mutations. Any sites selected that overlap these reads will be dropped. This feature exists as a means to ensure mutations across multiple runs do not overlap.
- `-force` : force the addition of a mutation even if it fails thresholds set by `-mindepth` and `-c/-coverdiff`. Not recommended for most use cases.
- `-insane`: ignores sanity checking number of input reads versus number of output reads. This seems to be required for some aligners or situations where unaligned reads are dropped or secondary alignments are added.
- `-single` : covered above, assume reads are single-end (i.e. fragment reads).
- `-maxopen` : set the maximum number of open files (check your system limit with `ulimit -a`)
- `-requirepaired` : drop mutations where a paired end in the region cannot be located. However, it is probably better to correct the problem in the BAM file than to resort to using this option.
- `-skipmerge` : instead of replacing the mutated and realigned reads back into the original BAM, which is normally the final step, leave the `addsnv`.
- `-aligner` : choose one of the supported aligners, default is `backtrack` (for `bwa backtrack`). Additional aligners can be added through modification of `bs/aligners.py`, and I try to accommodate requests for widely-used aligners if they are not already implemented. Aligner specific options are handled through `-alignopts`
- `-alignopts` : aligner-specific options are specified in a comma-delimited list as `option1:value1,option2:value2,et`. Required options are checked by the the function `checkoptions` in `bs/aligners.py`. For example, if `-aligner novoalign` is specified, `-alignopts novoref:path to novoalign reference;` should also be specified.

- `-tagreads` : adds a 'BS' tag to the reads altered by bamsurgeon, useful in debugging
- `-seed` : sets a random seed for reproducibility
- options not listed here are either new and the manual has not been updated as yet, or they are there for debugging and will be removed in the future.

3 Adding INDELs to existing BAM alignments (addindel)

3.1 Usage

```
usage: addindel.py [-h] -v VARFILENAME -f BAMFILENAME -r REFFASTA -o
                  OUTBAMFILE [-s SNVFRAC] [-m MUTFRAC] [-n NUMSNVS]
                  [-c CNVFILE] [-d COVERDIFF] [-p PROCS]
                  [--picardjar PICARDJAR] [--mindepth MINDEPTH]
                  [--maxdepth MAXDEPTH] [--minmutreads MINMUTREADS]
                  [--avoidreads AVOIDREADS] [--nomut] [--det] [--force]
                  [--insane] [--single] [--maxopen MAXOPEN] [--requirepaired]
                  [--aligner ALIGNER] [--alignopts ALIGNOPTS] [--tagreads]
                  [--skipmerge] [--ignorepileup] [--tmpdir TMPDIR]
                  [--seed SEED]
```

adds INDELs to reads, outputs modified reads as .bam along with mates

optional arguments:

```
-h, --help                show this help message and exit
-v VARFILENAME, --varfile VARFILENAME
                          Target regions to try and add a SNV, as BED
-f BAMFILENAME, --bamfile BAMFILENAME
                          sam/bam file from which to obtain reads
-r REFFASTA, --reference REFFASTA
                          reference genome, fasta indexed with bwa index _and_
                          samtools faidx
-o OUTBAMFILE, --outbam OUTBAMFILE
                          .bam file name for output
-s SNVFRAC, --snvfrac SNVFRAC
                          maximum allowable linked SNP MAF (for avoiding
                          haplotypes) (default = 1)
-m MUTFRAC, --mutfrac MUTFRAC
                          allelic fraction at which to make SNVs (default = 0.5)
-n NUMSNVS, --numsnvs NUMSNVS
                          maximum number of mutations to try (default: entire
                          input)
-c CNVFILE, --cnvfile CNVFILE
                          tabix-indexed list of genome-wide absolute copy number
                          values (e.g. 2 alleles = no change)
-d COVERDIFF, --coverdiff COVERDIFF
                          allow difference in input and output coverage
                          (default=0.1)
```

```

-p PROCS, --procs PROCS          split into multiple processes (default=1)
--picardjar PICARDJAR            path to picard.jar
--mindepth MINDEPTH             minimum read depth to make mutation (default = 10)
--maxdepth MAXDEPTH             maximum read depth to make mutation (default = 2000)
--minmutreads MINMUTREADS       minimum number of mutated reads to output per site
--avoidreads AVOIDREADS         file of read names to avoid (mutations will be skipped
                                if overlap)
--nomut                          dry run
--det                            deterministic base changes: make transitions only
--force                          force mutation to happen regardless of nearby SNP or
                                low coverage
--insane                         ignore sanity check enforcing input read count =
                                output read count in realignment
--single                        input BAM is single-ended (default is paired-end)
--maxopen MAXOPEN               maximum number of open files during merge (default
                                1000)
--requirepaired                 skip mutations if unpaired reads are present
--aligner ALIGNER               supported aligners:
                                backtrack,mem,novoalign,gsnap,STAR,bowtie2,tmap,bwakit
--alignopts ALIGNOPTS           aligner-specific options as comma delimited list of
                                option1:value1,option2:value2,...
--tagreads                      add BS tag to altered reads
--skipmerge                     final output is tmp file to be merged
--ignorepileup                  do not check pileup depth in mutation regions
--tmpdir TMPDIR                 temporary directory (default=addindel.tmp)
--seed SEED                     seed random number generation

```

3.2 Description

Short insertions and deletions (commonly referred to as INDELs) can be added to BAM files using addindel.py. The options are essentially the same as for addsnv.py, but the syntax of the input file is slightly different and, importantly is **0-based** (i.e. constant with BED files):

```

22 33694303 33694304 0.50 INS ATGGC
22 33815273 33815274 0.25 INS CG
22 33920490 33920491 0.33 INS ATTTGCTTAGCTGAGGGCTTAGGCTTAGCATGCGTA
22 33944542 33944543 0.50 DEL
22 34006643 34006653 0.50 DEL
22 33958087 33958090 0.40 DEL

```

The first four columns are the same as for addsnv.py input but the fourth column (VAF) is mandatory. The fifth column must be either 'INS' or 'DEL' (for insertion or deletion). For insertions there must be a sixth column specifying the sequence to be inserted at the position indicated

in the second column (the position in the third column does not matter for insertions). In the above example, the first three columns direct addindel.py to (1) add an insertion of “ATGGC” at chromosome 22 position 33694303 with 50% VAF, (2) add an insertion of “CG” at position 338152273 with 25% VAF, and (3) add an insertion of “ATTTCG...” with 33% VAF.

For deletions, the sequence from the start to the end (columns two and three) is deleted. Lines 4,5, and 6 above indicate deletions of 1 bp, 10bp, and 3 bp, respectively.

4 Adding SVs to existing BAM alignments (addsv.py)

4.1 Usage

```
usage: addsv.py [-h] -v VARFILENAME -f BAMFILENAME -r REFFASTA -o OUTBAMFILE
               [-l MAXLIBSIZE] [-k KMERSIZE] [-s SVFRAC] [--require_exact]
               [--minctglen MINCTGLEN] [-n MAXMUTS] [-c CNVFILE]
               [--donorbam DONORBAM] [--ismean ISMEAN] [--issd ISSD]
               [--simerr SIMERR] [-p PROCS] [--inslib INSLIB]
               [--aligner ALIGNER] [--alignopts ALIGNOPTS] [--tagreads]
               [--skipmerge] [--keepsecondary] [--debug] [--tmpdir TMPDIR]
               [--seed SEED] [--allowN]
```

adds SVs to reads, outputs modified reads as .bam along with mates

optional arguments:

```
-h, --help                show this help message and exit
-v VARFILENAME, --varfile VARFILENAME
                           whitespace-delimited target regions for SV spike-in,
                           see manual for syntax
-f BAMFILENAME, --bamfile BAMFILENAME
                           sam/bam file from which to obtain reads
-r REFFASTA, --reference REFFASTA
                           reference genome, fasta indexed with bwa index _and_
                           samtools faidx
-o OUTBAMFILE, --outbam OUTBAMFILE
                           .bam file name for output
-l MAXLIBSIZE, --maxlibsize MAXLIBSIZE
                           maximum fragment length of seq. library
-k KMERSIZE, --kmer KMERSIZE
                           kmer size for assembly (default = 31)
-s SVFRAC, --svfrac SVFRAC
                           allele fraction of variant (default = 1.0)
--require_exact           drop mutation if breakpoints cannot be made exactly as
                           input
--minctglen MINCTGLEN    minimum length for contig generation, also used to pad
                           assembly (default=4000)
-n MAXMUTS               maximum number of mutations to make
-c CNVFILE, --cnvfile CNVFILE
                           tabix-indexed list of genome-wide absolute copy number
```


	values (e.g. 2 alleles = no change)
--donorbam DONORBAM	bam file for donor reads if using BIGDUP mutations
--ismean ISMEAN	mean insert size (default = estimate from region)
--issd ISSD	insert size standard deviation (default = estimate from region)
--simerr SIMERR	error rate for wgsim-generated reads
-p PROCS, --procs PROCS	split into multiple processes (default=1)
--inslib INSLIB	FASTA file containing library of possible insertions, use INS RND instead of INS filename to pick one
--aligner ALIGNER	supported aligners: backtrack,mem,novoalign
--alignopts ALIGNOPTS	aligner-specific options as comma delimited list of option1:value1,option2:value2,...
--tagreads	add BS tag to altered reads
--skipmerge	do not merge spike-in reads back into original BAM
--keepsecondary	keep secondary reads in final BAM
--debug	output read tracking info to debug file, retain all intermediates
--tmpdir TMPDIR	temporary directory (default=addsv.tmp)
--seed SEED	seed random number generation
--allowN	allow N in contigs, replace with A and warn user (default: drop mutation)

4.2 Description

Larger structural variants (insertions, deletions, duplications, inversions, and compound rearrangements) are added to existing BAM alignments using `addsv.py` as described in Figure 3. Input consists of a list of regions where SVs will be made along with a specification of each variant, and as with `addsnv` and `addindel`, a target BAM alignment, and a reference genome indexed using `bwa` are required.

4.3 Input

The input mutation list consists of four columns: chromosome, start of region, end of region, and a controlled-vocabulary description of the mutation. A mutation will not be made if the largest contig obtained from local assembly of the specified region is less than 3 times the maximum expected library size (specified by `-l/--maxlibsize`). The mutation description starts with either `INS`, `DEL`, `DUP`, `TRN`, or `INV` for insertion, deletion, duplication, transduction, and inversion, respectively and is followed by mutation-specific options. An example follows.

```
22 34171055 34184700 INS ACTAACCTGCACAATGTGCACATGTACCCTAAACTTAGAGTATAATAAAAAAAAA 13 AA^TTTT 0.
22 33607508 33607508 TRN 22 34314981 34314981 ++ 1.0
22 33871043 33874754 DEL 0.75
22 33891043 33902689 BIGDEL 0.5
22 34071043 34084754 INV 0.5
22 34271043 34284754 DUP 3 0.9
```

For translocations (TRN), the above example will join the 6kbp region centered on 22:33607508 and the 6kbp region centered on 22:34314981. The variant allele fraction (VAF) is specified in the last column (0.9 in the above example).

For insertions, INS should be followed by either a FASTA file containing the sequence to be inserted, by the nucleotide sequence itself, or by RND if a library of potential insertion sequences is passed in FASTA format via the `-inslib` option. For example, `INS ATG` would insert the sequence `‘‘ATG’’` in the middle of the largest contig obtained from the specified region, while `INS LINE1.fa` would insert the sequence in the FASTA-formatted file `LINE1.fa` into the largest contig. Many insertions include target site duplications (TSDs), so to simulate this an integer value can be added in the fifth column of insertion entries specifying the TSD length. Endonuclease motifs are another common feature of insertion breakpoints, so to simulate this, a preferred cut site can be defined in the 6th column of an insertion entry with the syntax `NNN^NNN` where `'NNN'` denotes sequence of any length composed of characters (A, T, G, C) with the actual cut site in the motif specified by the caret (^). An optional seventh column can be used to specify the VAF, 0.8 in the example above.

For deletions DEL is optionally followed by specifying the per-mutation VAF (0.75 in the example above). Inversions (INV) have no further options aside from an optional VAF column - the region inside the largest contig will be inverted. Duplications (DUP) have two optional parameters, an integer specifying the number of times the sequence of the largest contig should be duplicated, e.g. `DUP 2` specifies the region is duplicated twice, and a VAF column. In general, we recommend that short insertions and deletions (those shorter than one read length) be created with `addindel.py` instead of `addsv.py` as `addindel.py` does not require reconstruction of the region through sequence assembly and is thus less error-prone.

Compound variants are also possible by chaining a number of mutations together in a semicolon-delimited list, e.g. `DUP 1;DEL 0.5;INS AAATCC;INV` would duplicate the region inside the largest contig once, delete half the width of the region, insert the sequence `AAATCC`, and invert the region (not currently supported with transductions). Good luck!

4.4 Mutation Size

Mutation types DEL, DUP, and INV are created within a single contig unless they are greater than 10kbp in length. Larger mutations are created using TRN mutations as the breakpoints with coverage adjustments to the intervening region. Internally these mutation types are named BIGDEL, BIGDUP, and BIGINV which can be specified in the variant input file but will be converted to DEL, DUP, and INV, respectively, if they are less than 5kbp in length. For duplications, the additional reads are supplied through a BAM file specified by the `--donorbam` parameter. If `--donorbam` is not given the BAM specified by `-f/--bamfile` is used instead (which is not ideal!). Ideally the donor BAM and the input BAM would be separate partitions of the same higher coverage BAM.

4.5 Exact Breakpoints

BAMSurgeon will prefer to create the user input breakpoints unless the assembled contig does not cover the breakpoint position. If `--require_exact` is specified, mutations that do not cover the user input breakpoint(s) will be dropped. In any case, the mutation positions output by the `makevcf_sv.py` script will reflect the actual breakpoints added.

4.6 Controlling SV VAF

Variant allele fraction for `addsv.py` can be controlled either via the `-c/--cnvfile` option (see Section 2.3) or through addition of a column in the input file (float value between 0.0 and 1.0). Note that all other optional columns must be specified for the VAF column to be effective, which means the input format for `addsv.py` really needs an overhaul.

4.7 Output

Where mutations add sequence (e.g. duplications), new reads are created that will be added to the original BAM, and where mutations remove sequence (e.g. deletions) those reads are marked as excluded (excluded read names are recorded in the file specified by `-x/--excluded`). Excluded reads are not carried over from the original BAM to the mutated BAM, creating the copy number effect associated with deletion.

4.8 Additional Options

Here are some extended descriptions for additional options not mentioned yet:

- `-s/-svfrac` : equivalent to `-m/-mutfrac` for `addindel.py` and `addsnv.py`
- `-k/-kmer` : kmer size to be fed to velvet, which is used for assembly
- `-n` : only attempt this many mutations
- `-ismean/-issd` : the mean and standard deviation of the library size should be estimated from the original bam and specified through these arguments. This can be accomplished a number of ways, I suggest `CollectInsertSizeMetrics` from the picard tools.
- `-simerr` : control sequencing error rate around breakends
- `-inslib` : a FASTA file can be specified and insertions can be tagged with RND instead of a sequence or file. If this is done, a sequence will be chosen at random from the FASTA file specified by `-inslib` and inserted.
- `-skipmerge` : same meaning as for `addsnv/addindel`
- `-allowN` : useful if desired mutations are being dropped due to one or more N bases present in the assembled contig

5 Output (all tools)

Output consists of a BAM file containing the spike-in mutations, a VCF file containing the results of the spike-in including expected variant positions and VAFs, and a directory of log files describing which bases were changed in which reads (one log file per mutation). For SVs this file contains the local assemblies for each region where a mutation was made. If needed, the directory containing output logs can be transformed into VCF format using the script `makevcf.py`, `makevcf_indel.py`, or `makevcf_sv.py` as appropriate, located in the `scripts/` subdirectory.

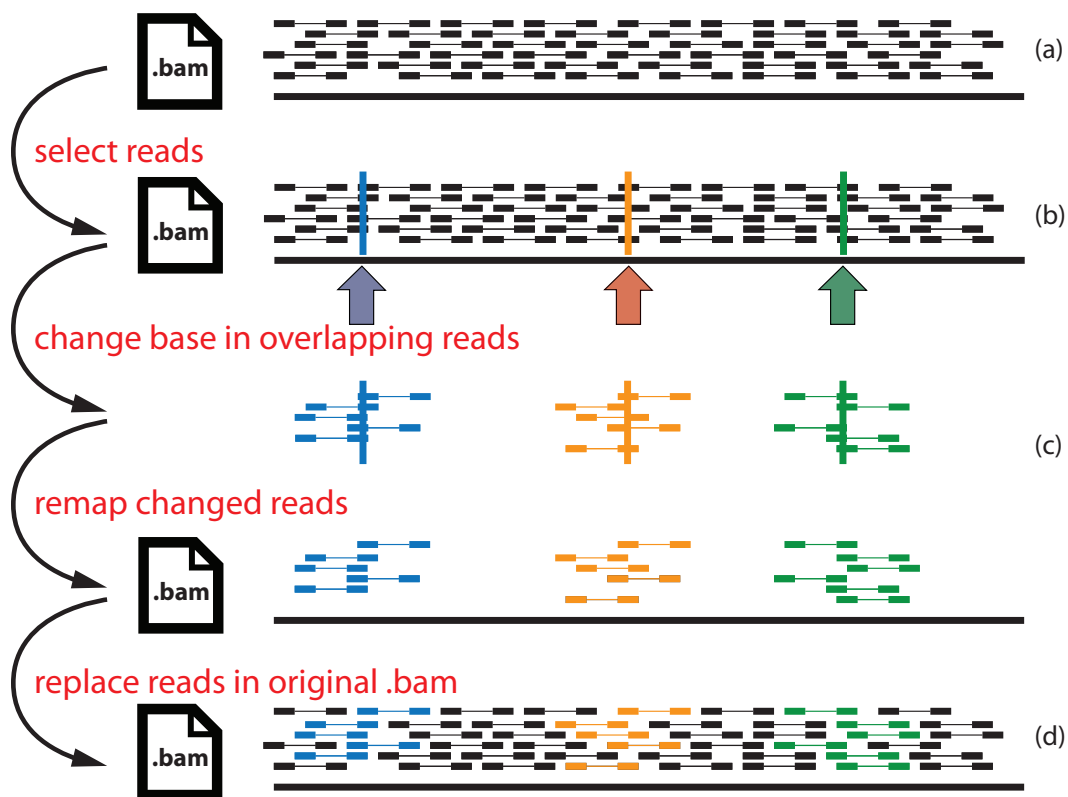


Figure 1: Method for adding SNVs to existing BAM alignments. Starting with the original BAM alignment (a) and a list of positions (b), reads overlapping the chosen positions are selected the target base change is made (c). Altered reads are re-mapped the the reference genome using bwa, and the modified, realigned reads replace the unmodified versions in the original BAM (d).

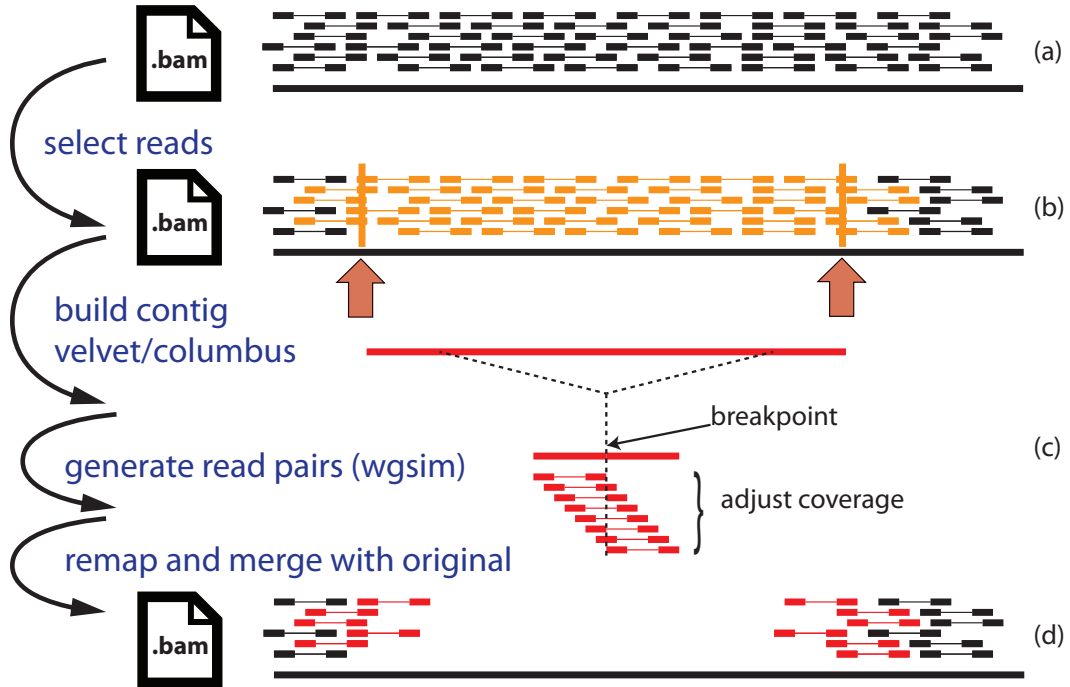


Figure 2: Method for adding multi-nucleotide variants (indels and SVs) to existing BAMs: deletion example. Starting with the original BAM alignment (a) regions, e.g. (b), are assembled using Velvet/Columbus (<http://www.ebi.ac.uk/zerbino/velvet/>) and the desired mutation(s) are created in the largest contig. Read coverage is simulated over the contig using wgsim (<https://github.com/lh3/wgsim>, also included with samtools) with parameters `-e 0 -r 0 -R 0` to suppress additional mutations, other parameters are set based on the input BAM and desired coverage. Simulated coverage is scaled based on whether the mutation adds sequence (duplication) or removes sequence (deletions), and replaced into the original BAM while excluded reads are removed from the original BAM (d).

6 Testing

The `test/` directory contains scripts useful for testing bamsurgeon's various modes, the primary ones are as follows:

6.1 Testing addsnv.py

The script `test/test_snv.sh` may be run with no parameters to display a usage statement:

```
usage: ./test_snv.sh <number of SNPs> <number of threads> <reference indexed with bwa index>
```

Or for testing with BWA MEM:

```
./test_snv_bwamem.sh <number of SNPs> <number of threads> <reference indexed with bwa index> <genome>
```

The number of SNPs can be up to 100 and are selected from the file `test_data/random_snvs.txt`, number of threads must be specified, use 1 to run on a single core, and the final argument must be a path to a human genome (GRCh37) reference indexed with `bwa index -a bwtsv`. The reference **should not** use the 'chr' prefix on the chromosome names.

6.2 Testing addindel.py

The script `test/test_sv.sh` may be run with no parameters to display a usage statement:

```
usage: ./test_indel.sh <number of threads> <reference indexed with bwa index>
```

6.3 Testing addsv.py

The script `test/test_sv.sh` may be run with no parameters to display a usage statement:

```
usage: ./test_sv.sh <number of threads> <reference indexed with bwa index>
```

7 Other Considerations

7.1 System Requirements

For each process (specified by `-p/--procs`) `add*.py` will require 4GB RAM. So for 8 concurrent mutation processes, you should have 32GB RAM. System requirements may decrease in the future as we find ways to improve efficiency. For more recent versions of `bwa`, it is possible to load the reference index into shared memory with `'bwa shm'` which seems to improve performance.

7.2 Alignments

Reads should be re-aligned using the same aligner and parameters as were used to create the original BAM file. By default, `bamsurgeon` assumes alignments were performed using `bwa aln`. Specific parameters that are not currently exposed on the command line may be altered through modification of the functions `remap_paired` (for paired reads) or `remap_single`. Supported aligners currently include: `bwa aln`, `bwa mem`, `novalign`, `gsnap`, `STAR`, `bowtie2`, `tmap`, `bwakit`.

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