Supplementary materials: Reproducibility of SNV-calling in multiple sequencing runs from single tumors

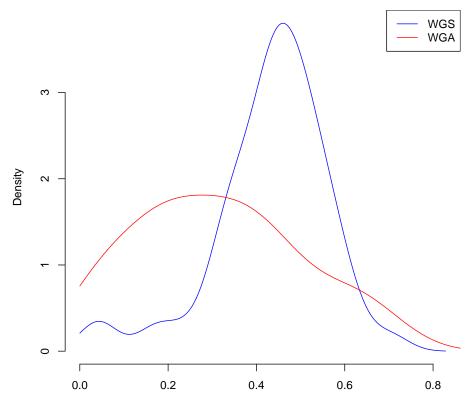
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Percentage of putative SNVs in a sample also recovered in the samples's technical replication

Figure 1: One third (WGA) to one half (WGS) of putative SNVs were recovered in technical replicates. Density of the percentage of each WGS (blue) and WGA (red) sample that is present in the overlap between replicates for each patient. The WGS distribution is higher and narrower, showing that the WGS samples overall have a higher percentage overlap than the WGA samples, and less range in this parameter.

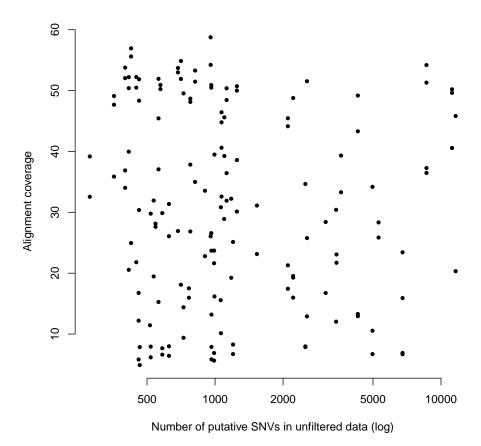


Figure 2: Number of putative SNVs in a sample does not correlate with coverage. The number of SNVs called in a sample does not correlate with the coverage of that sample (Spearman $\rho = -0.1255027$, S = 671817.1, P = 0.1222).

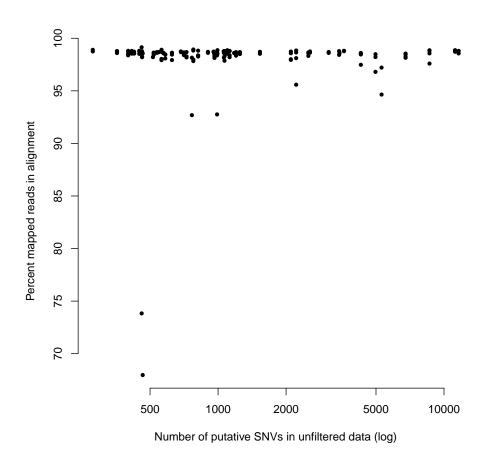


Figure 3: Number of putative SNVs in a sample does not correlate with percentage of mapped reads. The number of SNVs called in a sample does not correlate with the percentage of mapped reads in the alignment of that sample (Spearman $\rho = -0.06771965$, S = 637326.1, P = 0.4056).

Table 1: Back-end Processing. This table shows the software packages we used in data processing, what we used each piece of software for, and the command associated with it. The rows are in order of use.

| software | purpose | command |
|---------------|--------------------------------------|---|
| picard | regenerate fastq files from BAM file | java -d64 -Xmx4g -jar SamToFastq.jar I=\$pfx.bam |
| | aligned to hg18 | F=\$pfx.1.fastq F2=\$pfx.2.fastq $2>$ &1 |
| bwa | align fastq files to hg19 | bwa aln -q 30 -t 8 \$hgReference \$fastq > \$fastq.aln.sai |
| bwa, samtools | convert aligned fastq files into new | bwa sampe -a 600 -P -r "\$RG" \$hgReference \$fastq1.aln.sai |
| | BAM file | \$fastq2.aln.sai \$fastq1 \$fastq2 samtools view -bSh -o |
| | | <pre>\$outprefix.bam -</pre> |
| samtools | sort and index new BAM file | samtools sort -@ 16 \$outprefix.bam \$outprefix.sorted 2, |
| | | samtools index \$outprefix.sorted.bam 2 |
| samtools | remove duplicate reads from BAM | <pre>samtools rmdup/\$tumorpfx/\$tumorpfx.out.sorted.bam</pre> |
| | files | <pre>\$tumorpfx.dedup.bam</pre> |
| GATK | indel realignment | java -d64 -jar \$gatkJar -R \$hgReference -T IndelRealigner |
| | | -rf BadCigar -I \$tumorpfx.dedup.bam -known \$G1000.Mills |
| | | -known \$G1000.Phase1.Indels -targetIntervals |
| | | <pre>\$tumorpfx.intervals -o \$tumorpfx.realn.bam</pre> |
| GATK | base recalibration | java -d64 -jar \$gatkJar -nct 8 -T BaseRecalibrator -rf |
| | | BadCigar -I \$tumorpfx.realn.bam -R \$hgReference -knownSites |
| | | <pre>\$dbSNP -o \$tumorpfx.recal.grp</pre> |
| samtools | index recalibrated BAM file | samtools index \$tumorpfx.realn.recal.bam |
| SomaticSniper | call somatic mutations, generate | bam-somaticsniper -q 40 -Q 40 -J -s 0.001 -F vcf -f |
| | VCF | <pre>\$hgReference \$tumorbam \$normalbam \$tumorpfx.SS.vcf</pre> |