# Table S1 Quality assessment and read processing

| **Name** | **OS** | **Input** | **Output** | **Supported platforms** | **Report** | **Tag (1) removal** | **Filtering** | **Trimming** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ContEST [1] | Lin, Mac, Win | BAM, VCF, FASTA (ref) | TXT | Illumina,  ABI SOLiD, 454 | no | no | no | no |
| FastQC [2] | Lin, Mac, Win | (CS) FASTQ, SAM, BAM | HTML | Illumina,  ABI SOLiD | yes | no | no | no |
| FASTX-Toolkit [3] | Lin, Mac, web interface | FASTA, FASTQ | FASTA, FASTQ | Illumina | yes | yes | yes | yes |
| Galaxy [4] | Lin, Mac, web interface, Cloud instance | FASTQ | FASTQ | Illumina | yes | yes | yes | yes |
| htSeqTools [5] | Lin, Mac, Win | FASTQ | Graphs | Illumina | yes | no | no | no |
| NGSQC [6] | Lin | FASTA (ref), FASTQ, CSFASTA, QUAL FASTA | HTML | Illumina, ABI SOLiD | yes | no | no | no |
| PIQA [7] | Lin, Mac, Win | FASTQ, bustard, output, SCARF | HTML, TXT | Illumina | yes | no | no | no |
| PRINSEQ [8] | Lin, Mac, Win, web interface | FASTA, FASTQ, QUAL FASTA | FASTA, FASTQ, QUAL FASTA, HTML | Illumina, 454 | yes | no | yes | yes |
| SolexaQA [9] | Lin, Mac | FASTQ | FASTQ, PNG | Illumina, 454 | yes | no | no | yes |
| TagCleaner [10] | Lin, Mac, web interface | FASTA, FASTQ | FASTA | 454 | no | yes | no | no |
| TileQC [11] | Lin, Mac | Eland output | Graphs | Illumina | yes | no | no | no |

(1) Artifacts such as multiplex identifiers, adapters, primers, and linkers

OS Operating system: Lin ... Linux, Mac ... Mac OS X, Win ... Windows

ref reference genome

BAM Binary SAM

Bustard Illumina’s base calling software

CSFASTQ color space (SOLiD encoded) FASTQ file

CSFASTA color space (SOLiD encoded) FASTA

Eland Illumina’s alignment algorithm

FASTA text-based format for representing nucleotide sequences

FASTQ text-based format for repesenting nucleotide sequences and their corresponding quality scores

QUAL FASTA text-based format for representing quality scores

SAM Sequence Alignment/Map

SCARF output of Illumina’s Gerald software (single colon separated file with one record per line containing read name, sequence, and quality)

VCF Variant Call Format

# Table S2 Alignment software

| **Name** | **OS** | **Input** | **Output** | **Supported platforms** | **Indexing method** | **Gapped alignment** |
| --- | --- | --- | --- | --- | --- | --- |
| BarraCUDA [12] | Lin | FASTQ | SAM | Illumina | FM index (BWT) | yes |
| BFAST [13] | Lin | FASTQ | SAM | Illumina, ABI SOLiD, 454 | Multiple (hash, tree, …) | yes |
| Bowtie [14] | Lin, Mac, Win | FASTQ, FASTA | SAM | Illumina, ABI SOLiD | FM index (BWT) | no |
| Bowtie2 [15] | Lin, Mac, Win | FASTQ, FASTA, QSEQ | SAM | Illumina, 454 | FM index (BWT) | yes |
| BWA [16] | Lin | (CS)FASTQ, FASTA | SAM | Illumina, ABI SOLiD(1) | FM index (BWT) | yes |
| BWA-SW [17] | Lin | FASTQ, FASTA | SAM | 454 | FM index (BWT) | yes |
| ELAND [18] | Lin | FASTQ, FASTA | SAM | Illumina | - | no |
| MAQ [19] | Lin | FASTQ, FASTA | Maq | Illumina | Hash based | yes |
| Mosaik [20] | Lin, Mac, Win | FASTQ, FASTA | SAM, BED, several others | Illumina, ABI SOLiD, 454 | - | yes |
| mrFAST [21] | Lin | FASTQ, FASTA | SAM, DIVET | Illumina | Hash based | yes |
| mrsFAST [22] | Lin | FASTQ, FASTA | SAM, DIVET | Illumina | Hash based | no |
| Novoalign [23] | Lin, Mac | FASTQ, (CS)FASTA | SAM, TXT | Illumina, ABI SOLiD | - | yes |
| SOAP2 [24] | Lin | FASTQ, FASTA | SOAP (2) | Illumina | FM index (BWT) | yes |
| SOAP3 [25] | Lin | FASTQ, FASTA | SAM | Illumina | FM index (BWT) | no |
| SSAHA2 [26] | Lin, Mac | FASTA | SAM, GFF | Illumina, ABI SOLiD, 454 | Tree index | yes |
| Stampy [27] | Lin, Mac (3) | FASTQ, FASTA | SAM | Illumina, 454 | FM index (BWT) | - |
| YOABS [28] | Lin | - | - | Illumina | FM & Tree index | yes |

(1) SOLiD support dropped with introduction of version 1.6.0

(2) Provides script for conversion to SAM

(3) Experimental

OS Operating system: Lin ... Linux, Mac ... Mac OS X, Win ... Windows

BED Browser Extensible Data, a text-based file format

CSFASTQ color space (SOLiD encoded) FASTQ file

CSFASTA color space (SOLiD encoded) FASTA

DIVET VariationHunter’s comma separated input file format

FASTA text-based format for representing nucleotide sequences

FASTQ text-based format for repesenting nucleotide sequences and their corresponding quality scores

GFF General Feature Format

Maq MAQ proprietary alignment format

QSEQ Illumina’s base calling result file format

SAM Sequence Alignment/Map

SOAP SOAP proprietary alignment format

Variant identification

## Table S3 Germline callers

| **Name** | **BAM/SAM input** | **Other inputs** | **VCF  output** | **Other outputs** | **Illumina** | **Solid** | **SNP** | **INDEL** | **Last update** | **Notes** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Atlas 2 [29] | yes | FASTA (ref. Genome) | yes |  | yes | yes | yes | yes | 2011-08-29 | Suite for variant analysis in WES data, which has been integrated into the Genboree Workbench; collects coverage information; |
| Bambino [30] | yes |  | no | CSV | yes | yes |  |  | 2012-03-09 |  |
| Beagle [31] | no | Beagle input format | no | Beagle format | yes | y/y | yes | yes | 2011-10-31 | Software for imputation, phasing and association; |
| CoNAn-SNV [32] | no | pileup, model parameters, segmentation boundaries | no | CSV | yes | yes | yes | no | 2010-06-23 |  |
| CORTEX [33] | no | fastq, FASTA | yes | FASTA-like | yes | yes | yes | yes | 2011-11-03 | Tool performs variant discovery by de novo assembly - no reference genome required; can also detect inversions, complex variants, and small haplotypes; |
| CRISP [34] | yes |  | yes |  | yes | yes | yes | yes | 2012-04-24 |  |
| Dindel [35] | yes | FASTA (ref. genome) | yes |  | yes | yes | no | yes | 2010-10-25 | Tool can also test if detected variants are real INDELs or sequencing or mapping errors; |
| FreeBayes [36] | yes |  | yes |  | yes | yes | yes | yes | 2012-05-30 | Software also finds MNPs (multi-nucleotide polymorphisms), and complex events (composite insertion and substitution events) smaller than the length of a short-read sequencing alignment; |
| GATK (UnifiedGenotyper) [37] | yes |  | yes |  | yes | yes | yes | yes | 2012-05-01 | Package includes SNP and genotype caller, SNP filtering, and SNP quality recalibration; |
| GSNP [38] | no | SOAP alignment result | no | CSV | yes | no | yes | no | 2011-11-11 | GPGPU (cuda) implementation of SoapSNP; |
| IMPUTE2 [39] | no | genotype-file-format, recombination-map | no | impute format | yes | yes | yes | yes | 2012-01-27 | Software for imputation and phasing, including a mode for genotype calling; good documentation is provided but a steep learning curve; |
| Indelocator [40] | yes |  | no | CSV | yes | yes | no | yes | 2012-02-22 | Calling short indels; uses inputs from normal and tumor samples; also runs with just one sample |
| Ion Variant Hunter [41] | yes |  | yes |  | - | - | yes | yes | 2012-04-04 |  |
| MaCH [42] | no | glf | no | MaCH format | yes | yes | yes | no | - |  |
| moDIL [43] | no | own CSV | no | CSV | yes | yes | no | yes | 2012-01-20 | No user guide could be found; |
| PolyScan [44] | no | Consed ace file | no | polyScan format | - | - | yes | yes | 2007-07-24 | May require some familiarization; |
| Qcall [45] | yes |  | - | - |  | - | yes | no | 2010-09-22 | No homepage, documentation, or binaries could be found |
| realSFS [46] | no | fastq | - | - | yes | no | yes | no | - |  |
| SAMtools [47] | yes | FASTA | yes |  | yes | yes | yes | yes | 2011-09-02 | Suite performs computation of genotype likelihoods and SNP and genotype calling; |
| SliderII [48] | no | prb files | no | CSV | yes | no | yes | no | 2009-06-09 |  |
| Sniper [49] | no | map file, reference genome, fastq | no | CSV | yes | no | yes | no | 2011-10-04 |  |
| SNVer [50] | yes |  | yes |  | yes | yes | yes | yes | 2012-04-21 | Statistical framework to find rare and common variants in individual and pooled sequencing data; |
| SNVMix [51] | no | pileup/mpileup | no | CSV | yes | yes | yes | no | 2012-03-21 |  |
| SOAPindel [52] | yes | SOAP, FASTA | no | CSV | yes | no | no | yes | 2012-03-29 |  |
| SOAPsnp [24] | no | SOAP alignment result | no | CSV, GLFv2, GPFv2 | yes | no | yes | y/n | 2009-05-25 |  |
| Syzygy [53] | yes | pool info file + target info file | no | CSV | yes | yes | yes | no | 2012-07-05 | Provided is a good user guide; Needs in addition to BAM/SAM pool info and target info file; |
| VarScan 2 | no | pileup/mpileup | yes | CSV | yes | yes | yes | yes | 2012-05-01 |  |
| VARiD [54] | yes | SAM, FASTA | no | CSV | yes | yes | yes | yes | 2010-10-05 |  |
| VipR [55] | no | pileup (from samtools) | yes |  | yes | yes | yes | yes | 2012-04-12 | Tool that uses data from multiple DNA pools; |

## Table S4 Somatic callers

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name** | **Input Format** | **Output Format** | **Illumina** | **Solid** | **SNP** | **INDEL** | **Last update** | **Notes** |
| GATK (SomaticIndelDetector) [37] | BAM | VCF | yes | yes | no | yes | 2012-05-01 | No SNP caller; |
| MutationSeq [56] | BAM | CSV | yes | yes | yes | yes | 2012-05-24 | Software requires matlab; |
| MuTect [57] | BAM | CSV |  |  | yes | no | 2012-04-10 | Identifies somatic point mutations and is suggested by the Broad Institute; currently only for registered beta-tester; |
| SAMtools [47] | BAM | VCF | yes | yes | yes | yes | 2011-09-02 | Requires merging pairs into one BAM file; no separate parameters for tumor and normal BAM files; |
| [SomaticCall](http://www.broadinstitute.org/node/1136" \l "SOMATICCALL) [58] | BAM | CSV | yes | yes | yes | yes | 2009-11-06 | The tool is no longer maintained; |
| SomaticSniper [59] | BAM | VCF / somatic sniper output | yes | yes | yes | no | 2012-06-12 |  |
| SPLINTER [60] | SCARF file | CSV | yes | no | yes | yes | 2010-07-01 | Registration is required; no dedicated download page is provided; |
| VarScan 2 [61] | pileup/mpileup | VCF / varscan CSV | yes | yes | yes | yes | 2012-05-01 | Can also predict cnv and be used as a germline variant caller; |

## Table S5 CNV identification

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Name** | **SAM/BAM** | **Other input** | **Output** | **Illumina** | **Solid** | **Last Update** | **Notes** |
| CNAseg [62] | yes | - | CSV | yes | yes | 2010-09-14 | Tool is using R; for tumor/normal pairs of cancer data; |
| CNVer [63] | yes | CSV | cnv files | yes | yes | 2011-07-11 | For Illumina data; authors recommend using the Bowtie with specific parameters; no paired-end reads supported; |
| cnvHMM [64] | no | cnsfile | CSV | yes | no | 2009-06-04 |  |
| CNVnator [65] | yes | FASTA | CSV | yes | yes | 2012-02-07 | Tool for identifying, genotyping and characterizing CNVs; not very easy to install and authors don't offer a comprehensive tutorial; |
| CNV-seq [66] | yes | psl | cnv summary file | yes | yes | 2011-07-15 | Currently supports BLAT psl ﬁle and SOLiD matching pipeline output as input; |
| CONTRA [67] | yes | 2 BAM files, BED | VCF, CSV | yes | yes | 2012-07-24 | For targeted resequencing data such as those from whole exome capture data; needs test and control BAM files; |
| CopySeq [68] | yes | - | CSV | yes | yes | 2011-04-06 |  |
| ExomeCNV [69] | yes | pileup + bed + FASTA |  | yes | yes | 2012-06-01 | Most suitable when paired samples (e.g. tumor-normal pair) are available; requires R; |
| RDXplorer [70] | yes | FASTA | CSV | yes | yes | 2012-01-13 | Tool for copy number variants (CNV) detection in whole human genome sequence data using read depth (RD) coverage. |
| readDepth [71] | no | BED, R | segmented CNVs, CSV | yes | yes | 2011-04-15 | Tool is reported to perform good on low and high coverage; R package; |
| Segseq [72] | no | CSV | CSV | yes | no | 2009-01-28 | Tool requires Matlab; |

## Table S6 SV identification

| **Name** | **BAM/SAM** | **Input Format** | **Output Format** | **Illumina** | **Solid** | **Last Update** | **Notes** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| APOLLOH [73] | no | CSV (infile, cnfile) | CSV | yes | yes | 2011-12-01 | Tool requires Matlab; predicts somatic loss of heterozygosity and allelic imbalance in whole tumour genome sequencing data; |
| BreakDancer [74] | yes | BAM + config file | ctx, BED | yes | yes | 2011-02-21 | Tool can detect deletions, insertions, inversions, intra, and inter chromosomal translocations; computes the copy number for each BAM file; |
| Breakpointer [75] | yes | BAM | GFF | yes | yes | 2012-01-20 | The tool can also call INDELs; |
| BreakSeq [76] | no | GFF | GFF, CSV, various ouput files |  |  | 2010-07-26 |  |
| Breakway [77] | yes | BAM | CSV | yes | yes | 2011-04-01 | Can also annotate the identified breakpoints; |
| CLEVER [78] | yes | BAM, FASTA | own CSV | yes | - | 2012-03-27 | Authors provide structured documentation; |
| ClipCrop [79] | yes | SAM, FASA | BED | yes | yes | 2012-01-27 | This is a tool for detecting structural variations using soft-clipping information. |
| CREST [80] | no | DIVET |  | yes | no | 2011-11-08 | The user can use his/her own programs in place of BLAT and CAP3, but needs to implement them by himself/herself; |
| FusionMap [81] | no | fastq | SAM, own format | yes | yes | 2012-04-17 | Aligns reads spanning fusion junctions directly to the genome without prior knowledge of potential fusion regions; |
| GASVPro [82] | yes | SAM/BAM | clusters file | yes | yes | 2012-06-14 | Software to detect SVs from paired-end mapping data; |
| Hydra-sv [83] | no | Hydra CSV file | BEDPE | - | - | 2010-08-20 |  |
| PEMer [84] | no | Several input files | multiple output files | yes | yes | 2009-02-02 | Megablast and Smith Waterman programs are required; tool requires several configuration steps; |
| Pindel [85] | yes | BAM + FASTA | CSV | yes | ?? | 2011-08-29 | Tool that detects breakpoints of large deletions, medium sized insertions, inversions, tandem duplications and other SVs; |
| SPLITREAD [86] | no | FASTA, mrFast SAM | BED | yes | no | 2011-10-13 | Identifies INDELs and SVs; |
| SVDetect [87] | yes | BAM/SAM or ELAND or Bioscope output | Txt, BED, Circos link | yes | yes | 2011-07-12 | Detects large deletions and insertions, inversions, intra- and inter-chromosomal rearrangements; |
| SVMerge [88] | yes | BAM + FASTA(ref) | bed | yes | - | 2012-02-16 | SVMerge integrates calls from several existing SV callers: BreakDancerMax, Pindel, RDXplorer, cnD and SECluster; |
| Tigra [89] | yes | BAM | CSV | yes | yes | - | Tool is not available anymore; |
| VariationHunter [90] | no | own CSV input file | txt for insertionas, deletions and inversions | yes | yes | 2010-01-12 |  |

# Table S7 Varaint annotation

| **Name** | **Input Format** | **Output Format** | **SNP** | **INDEL** | **CNV** | **GUI** | **CLI** | **Web** | **Notes** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ABSOLUTE [91] | HAPSEG output, sample level variance, precomputed models of cancer types, sigma values | Plot showing the Purity/Ploidy, R data file | yes | no | yes | no | yes | no | Comes bundles with HAPSEG; |
| Align-GVGD [92] | FASTA, substitutions list | Web report | yes | no | no | no | no | yes | Estimates SNP risk; |
| ANNOVAR [93] | VCF4, Complete Genomics, GFF3-SOLiD, CSV in Annovar format; | Gene-based annotation; Region-based annotations; Filter-based annotation. For all categories | yes | yes | yes | no | yes | no | Integrated tool providing gene annotation, db ids and various scores; |
| AnnTools [94] | VCF, pileup, CSV | VCF | yes | yes | yes | no | yes | no | Provides a set of helper tools for custom annotation; |
| Auto-mute [95] | PDB ID, Chain, Mutation | Web report | yes | no | no | no | no | yes | The tool performs stability and disease potential predictions. |
| CandiSNPer [96] | dbSNP ID, population | Web report | yes | no | no | no | no | yes |  |
| CHASM and SNVBox [97] | Passenger mutation rates, AA changes | CSV including CHASM score, p-value, and FDR | yes | no | no | no | yes | no | Predicts the functional significance of somatic missense mutations observed in the genomes of cancer cells and features prioritization of mutations; |
| CUPSAT [98] | PDB ID; PDB file format | Web report | yes | no | no | no | no | yes | Performs protein stability prediction; |
| dbNSFP [99] |  |  | yes | no | no | no | yes | no | Integrated SNP database; provides a simple JAVA CLI tool for searching; |
| VEP (Ensembl - Variant Effect Predictor) [100] | CSV, VCF, Pileup, HGVS, Variant Identifiers | Web report | yes | no | no | no | yes | yes |  |
| ESEfinder [101] | FASTA | Web report, CSV | - | - | - | no | no | yes | Analyzes sequences for the presence of ESE motifs; |
| ESRSearch [102] | plain sequence; FASTA | Web report | - | - | - | no | no | yes | Finds ESR sequences; |
| FANS [103] | FASTA format; or variation information via web interface | Web report, CSV | yes | no | no | no | no | yes | Prioritized variations based on risk levels; divided into: Genome View, Gene View, Transcript View, Variation View; |
| FastSNP [104] | Gene Symbol, dbSNP ID | Web report | yes | no | no | no | no | yes | Outputs prioritized list of SNPs with risk assessment; |
| FESD [105] | Gene name | Web report | yes | no | no | no | no | yes | Output includes regions: promoter, CpG, islands, translation start, splice site, translation stop, poly(A) signal, transcript; |
| FOLD-X [106] |  |  | yes | no | no | no | yes | yes | It performs protein stability analysis. |
| F-SNP [107] | SNP ID; disease; gene; chromosomal region |  | yes | no | no |  |  |  | The software integrates information obtained from 16 bioinformatics tools and databases about the functional effects of SNPs. |
| GERP++ [108] |  | Web report | yes | no | no | no | yes | yes | It produces evolutionary conservation scores. |
| GSITIC [109] | Segmentation File, Markers File, FASTA, (Array List File, CNV File) | Lesions, Amplification Genes, Deletion Genes, Gistic Scores, Plots | no | no | yes | no | yes | no | Identifies regions of the genome that are significantly amplified or deleted across a set of samples; |
| HOPE [110] | FASTA, accession code for protein | Web report on structural differences between wild type and mutations | yes | no | no | no | no | yes | The web-based tool offers a simple web interface for entering protein sequence and amino acid mutation. |
| Human Splicing Finder (HSF) [111] | Ensembl / RefSeq ID,  plain text sequences |  | yes | no | no | no | no | yes |  |
| I-Mutant2.0 [112] | One letter residue code, sequence residue number | Web report | yes | no | no | no | yes | yes | The tool is based on support vector machines. |
| LS-SNP [113] | SwissProt ID, dbSNP ID, Kegg Pathway ID, HUGO Gene ID | Web report | yes | no | no | no | no | yes | The tool offers prediction of disease association and confidence of prediction and is based on support vector machines (SVM). |
| MAPP [114] | FASTA | CSV in MAPP format | yes | no | no | no | yes | no |  |
| MuD [115] |  | Web report | yes | no | no | no | no | yes |  |
| MutaGeneSys [116] |  | Web report / CSV | yes | no | no | no | yes | yes | The query Interface is not working. |
| MutationAssessor [117] | CSV in MutationAssessor format, Uniprot ID, Refseq ID | CSV in MutationAssessor format | yes | no | no | no | no | yes |  |
| MutationTaster [118] | ORF, cDNA sequence, genomic sequence, alteration | Web report | yes | yes | no | no | yes | yes |  |
| MutPred [119] | FASTA sequence, CSV file of mutations | Web report | yes | no | no | no | no | yes | Calculates the impact of mutation on different protein properties; is based on SIFT and offers precomputed dbSNP results; |
| MutSig [120] | List of mutations, regions to investigate | CSV | yes | yes | no | no | yes | no | Still in beta testing – available upon request |
| NGS-SNP [121] | VCF, pileup, CSV | VCF | yes | no | no | no | yes | no |  |
| nsSNPAnalyzer [122] | FASTA, substitutions list | Web report | yes | no | no | no | no | yes | The tool outputs various SNP features and predicts the phenotypic class. |
| Oncotator [123] | Oncotator format | CSV | yes | yes | no | no | no | yes | Annotations with data relevant to cancer researcher; collects Genomic Annotations, Protein Annotations, Cancer Annotations |
| PANTHER [124] | Protein sequence and Substitution | subPSEC score | yes | no | no | no | yes | yes | Uses subPSEC score; |
| Parepro [125] | Protein sequence and Substitution | - | yes | no | no | no | yes | no | It is based on support vector machines (SVM). |
| PESX [126] | plain sequence; FASTA | Web report | - | - | - | no | no | yes | Finds ESE sequences; |
| pfSNP [127] | SNP ID; chromosome region; Gene ID; | Web report | yes | no | no | no | no | yes |  |
| PHAST [128] | FASTA, PHYLIP, MPM, MAF, SS | Conservation score | - | - | - | no | yes | no | Phylogenetic analysis toolbox, including phastCons and phyloP; |
| PhD-SNP [129] | One letter residue code, Swiss-Prot protein code, Sequence file | Effect preditction | yes | no | no | no | yes | no |  |
| PMUT [130] | FASTA sequence/file SWISSProt code | Web report | yes | no | no | no | no | yes | Offers different prediction modes and is able to output detailed mutation analysis reports; |
| PolyDoms [131] | Gene/protein symbol(s), RefSeqID dbSNP ID | Web report | yes | no | no | no | no | yes |  |
| PolyMAPr [132] | - | - | - | - | - | no | yes | no | No longer available; |
| PolyPhen-2 [133] | UniProt ID, FASTA, dbSNP ID | CSV in PolyPhen format | yes | no | no | no | yes | yes |  |
| PupaSNP Finder [134] | dbSNP ID, Gene/Transcript ID; PED format | Web report | yes | no | no | no | no | yes |  |
| QuickSNP [135] | genomic position; HUGO gene symbol | Web report | yes | no | no | no | no | yes |  |
| RescueESE [136] | plain text; multi-FASTA | predicts sequences with ESE activity | - | - | - | no | no | yes |  |
| SAPRED [137] | FASTA and mutation file |  | yes | no | no |  |  |  | The website is offline. |
| SCAN [138] |  | Web report | yes | no | yes | no | no | yes |  |
| SCONE [139] | MAF | Conservation score | - | - | - | no | yes | no |  |
| SeattleSeq Annotation [140] | Maq, GFFm CASAVA, VCF, GATK bed | VCF, own format | yes | yes | no | no | no | yes |  |
| SeqAnt [141] | FASTA sequence file | Web report | yes | yes | no | no | no | yes |  |
| SeqProfCod [142] | - | - | yes | no | no | - | - | - | Not available online; |
| SVA (Sequence Variant Analyser) [143] | VCF of variants, project file (for command line version) | --potential biological function --dbSNP/Kegg/GO/1000Genomes/DGV annotation --identifies protein-truncating variants --filtering by function | yes | yes | no | yes | yes | no |  |
| SIFT [144] | Multiple proteins, dbSNP ID, NCBI GI number, protein sequence, protein sequence alignment, Pileup, VCF4, maq, soap, gff3, casava, cg | XXX in SIFT format | yes | no | no | no | yes | yes |  |
| SIFT Indel [145] |  |  | no | yes | no | no | no | yes |  |
| SiPhy [146] | FASTA, MAF, PHYLIP |  | - | - | - | no | yes | no |  |
| SNAP [147] | AA in FASTA, substitutions format | Web report | yes | no | no | no | no | yes | This tool offers a user friendly web interface. |
| SNP Function Portal [148] | RefSNP Ids, OMIM Ids | Web report | yes | no | no | no | no | yes |  |
| [SNP@Domain](mailto:SNP@Domain) [149] |  |  | yes | no | no | - | - | - | Not available anymore; |
| SNPdbe [150] | Gene/protein symbol, FASTA | Web report | yes | no | no | no | no | yes | The protein function is predicted using SNAP and SIFT and entries are augmented with experimental information from public databases. |
| SNPeffect 4.0 [151] | FASTA, PDB file, PDB ID, UniProt ID | Web report | yes | no | no | no | no | yes | This tool mainly uses protein structure information. |
| SNPHunter [152] | Gene symbol; dbSNP ID; | Web report | yes | no | no | yes | no | no |  |
| SNPnexus [153] | [CSV](http://snp-nexus.org/guide.html" \l "input_format) in SNPnexus input format | CSV in SNPnexus output format | yes | yes | yes | no | no | yes | Outputs CNV, INDELs, inversions; |
| SNPper [154] | dbSNP ID, TSC ID, position | Web report | yes | no | no | no | no | yes |  |
| SNPs&GO [155] | One letter residue code; Swiss-Prot protein code; Sequence file; GO terms; CSV | Web report | yes | no | no | no | no | yes | Predicts neutral/deleterious; calculates reliability index and disease probability; |
| SNPs3D [156] | Gene symbol, SNP ID | Web report | yes | no | no | no | no | yes |  |
| SNPseek [157] | - | - | - | - | - | - | - | - | Tool that performs neural network based protein stability prediction which is not available anymore; |
| SNPselector [158] | - | - | - | - | - | no | no | yes | No longer available; |
| SnpSIFT + snpEff [159] | VCF, SNPs, insertions, deletions, and MNPs | CSV | yes | yes | no | no | yes | no | A collection of tools to manipulate VCF files; |
| SPOT [160] | SNPs and p-values, | Web report | yes | no | no | no | no | yes | Outputs various DB ids and scores; |
| StSNP [161] | protein sequence; protein name; dbSNP ID; gene symbol | Web report | yes | no | no | no | no | yes |  |
| TAMAL [162] | - | - | - | - | - | - | - | - | No longer available; |
| TopoSNP [163] | Protein ID, protein sequence | Web report | yes | no | no | no | no | yes | Predicts whether substitution is on surface of the protein structure;  conservation score based on Pfam protein alignments; |
| VARIANT [164] | VCF | Web report, text | yes | no | no | no | yes | yes |  |

CSV = comma separated value

Variant visualization

## Table S8 Genome Browsers

| **Name** | **BAM/SAM** | **VCF** | **Other formats** | **GUI** | **Web** | **Annotation** | **Notes** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ABrowse [165] | yes | no | GFF, WIG | no | yes (local) | yes | Shows tracks as large images similar to google maps; |
| AnnoJ [166] | no | no | own format | no | yes (local) | yes |  |
| Apollo [167] | no | no | DAS, GFF, GFF3, WIG | yes | no | yes |  |
| Argo / Combo [168] | no | no | FASTA, Genbank, GFF, BLAST, BED, Wiggle (WIG), Genscan files | yes | no | yes | Argo is a standalone genome viewer which integrates combo as a comparative genome browser. |
| Artemis [169] | yes | yes | BCF, FASTA | yes | no | yes | Standalone tool where BAMView has been integrated; |
| Bambino [30] | yes | no | FASTA, UCSC, 2bit, nib | yes(JWS) | no | yes |  |
| BamView [170] | yes | no | - | yes | no | no | This tool has been integrated into Artemis and is capable of calculating read counts and RPKM values. |
| Consed [171] | no | no | Newbler, Cross\_match, Phrap, MIRA, Velvet and PCAP | yes | no | no | The standalone tool has been designed to display genome assemblies. |
| DiProGB [172] | no | no | GenBank, FASTA, GFF PTT | yes | no | yes | Is able to display sequence graphs and a feature graphs; |
| EagleView [173] | no | no | ACE, READS, EGL, MAP | yes | no | no | A genome assembler viewer; |
| Ensembl [174] | yes | yes | BED, BedGraph, GFF, GTF, PSL, WIG, BigWig | no | yes | yes | Web-based tool with a variety of reference genome and integrated annotations; |
| Gaggle [175] | no | no | SQL, GFF | yes | no | yes | For systems biology; |
| Gap5 [176] | yes | no | ACE, BAF | yes | no | no | This standalone tool has been developed to facilitate the process of finishing assemblies. |
| GBrowse [177] |  |  | GFF | no | yes | no | This web-based tool is the precursor of JBrowse. |
| G-compass [178] | no | no | - | no | yes | yes | Web-based tool that shows comparison of different homolog genomes; |
| Genome Environment Browser [179] | no | no | - | yes | no |  | This tool has its strength for viewing repeat elements and other non-genic sequence features. |
| GenomeView [180] | yes | no | FASTA, GFF; BED, WIG, TDF, MAF, MAQ |  | no | yes |  |
| GenoViewer [181] | yes | no | FASTA, GFF | yes | no | yes | It is a standalone genome viewer that is not developed or supported anymore. |
| Hawkeye [182] | no | no | fastq, fastq | yes | no | no | A genome assembler viewer; |
| Integrated Genome Browser (IGB) [183] | yes | no | DAS, wig | yes (JWS) | no | yes | Standalone, Java tool with export feature into PDF, EPS, PNG, …; |
| Integrative Genomics Viewer (IGV) [184] | yes | yes | (> 30 formats) TDF, CN, SNP, GCT, RES, GFF, GFF3, BED, GISTIC, LOH, MUT, GCT, SEG, CBS, IGV, TAB, WIG | yes | no | yes | Can be started locally or from websites; offers lots of customization features; |
| JalView [185] | no | no | DAS | yes | no | no | This tools is capable of performing multiple sequence alignment. |
| JBrowse [186] | no | no | FASTA, BED, GFF, GFF3, WIG | no | yes (local) | yes | It is a web based tool where tracks are rendered on the client side. Tracks need to be prepared by the user in advance. |
| LookSeq [187] | yes | no | MAQ, CIGAR | no | yes | no | Web based alignment viewer; |
| MagicViewer [188] | no | no | ACE | yes | no | yes | This tool is aimed at users who work with DNA methylation data. |
| MapView [189] | no | no | MVF | yes | no | no |  |
| NGSView [190] | no | no | XML, BED, BLAST, Eland, mapview processed MAQ, Corona, GFF | yes | no | yes | Sequence alignment editor; |
| SAMSCOPE [191] | yes | no | BIP (specific file format) | yes | no | no |  |
| samtools tview [47] | yes | no | - | CLI | no | no |  |
| Savant [192] | yes | yes | FASTA, BED, GFF, WIG, any tab-delimited | yes | no | yes | Standalone, Java based genome viewer which allows users to create their own plug-ins; |
| SeqWord [193] |  |  |  | no | yes | no | A web-based tool to visualize the natural compositional polymorphism of DNA sequences. |
| SNUGB [194] |  |  |  | no | yes | no | The tool has been developed for comparative genomics. |
| Tablet [195] | yes | no | ACE, AFG, MAQ, SOAP2, FASTA, FASTQ, GFF3 | yes | no | yes |  |
| UCSC cancer genomics browser [196] | no | no | - | no | yes | yes | This tool displays cancer related datasets, but does not allow the upload of local data. |
| UCSC Genome Browser [197] | yes | yes | BED, bigBed, bedGraph, GFF, GTF, WIG, bigWig, MAF, BED, SNP, PSL | no | yes | yes | Web-based tool with a variety of public databases; It offers many customization features and allows the user to upload new tracks. |
| UTGB toolkit [198] | yes | no | FASTA, BED, WIG, DAS | no | yes (local) | yes | The tool is web-based and uses a dedicated database and web-server. It offers flexible customization possibilities and tracks can hold private or public data. |
| VEGA [199] | yes | yes | BED, bedGraph, BigBed, BigWig, GBrowse, GFF, GTF, PSL, WIG. | no | yes | yes | This application contains manually annotated genomes from different species. Large parts of the human genome are annotated. |
| Vista [200] | no | no | - | no | yes | no | The web-based viewer can be used to perform comparative genomics. |

## Table S9 CNV & SV visualization

| **Name** | **Input** | **Output** | **Types** | **GUI** | **Web** | **Notes** |
| --- | --- | --- | --- | --- | --- | --- |
| Circos [201] | 2D tracks, CSV | scatter, line, and histogram plots, heat maps, tiles, connectors, and text | CNV, INDEL, TRANS, INV | yes | no | Also runs on Windows; |
| Gremlin [202] | ? | ? | ? | no | yes | Currently Gremlin cannot be downloaded. |

# Table S10 Pipelines

| **Name** | **Input Format** | **Output Format** | **Illumina** | **Solid** | **Requirements** | **GUI** | **CLI** | **Cloud** | **Algn** | **Var** | **Anno** | **Notes** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Bcbio-nextgen [203] | fastq | fatsq, sam, BAM, bed, VCF, pdf | yes | ? | Linux, MaC OS X, Windows | yes | yes | no | yes | yes | no | Fully automated pipeline which includes alignment, SNP calling, summary collection, and integration into Galaxy; |
| Crossbow [204] | fastq, sra | SoapSNP output file | yes | yes |  | yes | yes | yes | yes | yes | no | Software pipeline which combines Bowtie and  SoapSNP; |
| Games [205] | BAM+FASTA(ref) | summary and annotation text  html files, output files that can in SIFT and PolyPhen | yes | yes | Linux | no | yes | no | no | yes | yes | Tool for identifying and predicting mutations; does not include alignment; |
| GATK [37] | BAM, FASTA(ref),  dbSNP(rod files),  refSeq table | CSV, VCF, txt | yes | yes | Linux | no | yes | no | no | yes | yes | Performs no alignment; |
| HugeSeq [206] | fastq, FASTA | VCF, gff, Annovar output | yes | - | Linux | no | yes | no | yes | yes | yes | Combines tools for alignment, variant calling, and annotation. Also identifies CNVs and SVs; |
| inGap [207] | fastq, FASTA | CSV | yes | no | Linux, MaC OS X, Windows | yes | no | no | yes | yes | no | Application with a graphical user interface integrating alignment and variant detection; it can be used for comparing genomes and simulating reads. |
| MutationTaster NGS pipeline [208] | FASTA, fastq, csFASTA (reads)  + FASTA (ref. Genome) + Annotation file in tab separated format with all Ensembl transcripts + bed (target region - optional) | MutationTaster snippets, read coverage, SNP positions, variation counts | yes | yes | Linux | no | yes | no | yes | yes | no | Performs mapping, variant calling, and variant annotation using MutationTaster and offers filtering options for the SNPs; requires basic knowledge of Linux. |
| Ngs-backbone [209] | fastq, FASTA | VCF, gff,text files | yes | no | Linux | no | yes | no | yes | yes | no (not functional) | Pipeline which includes read cleaning, mapping, transcriptome assembly, annotation and SNV calling; |
| RTG [210] | fastq, FASTA, Complete  Genomics format | SAM, BAM, BED, VCF, snp files | yes | no | Linux, MAC OS X, Windows | no | yes | no | yes | yes | no | This tool performs alignment, variant detection, and calculation of various summary statistics. |
| SeqGene [211] | SAM | snpa, wig, svg | yes | yes | Linux, Windows, Mac OS X | no | yes | no | no | yes | yes | This pipeline supports SNP/INDEL detection, SNP filtering, and performing SNP-expression association tests but has no alignment capability. It offers pre-built annotation packages for latest Ensembl human, mouse, and rat genomes. |
| SHORE [212] | fastq, CSFASTA | various text files (for SNPs, SVs, CNVs) | yes | yes | Linux or MacOS; Dual Core; 2GB RAM; 500GB storage | no | yes | no | yes | yes | no | The pipeline covers alignment and performs its own statistical analysis to detect variants. |
| Simplex [213] | fastq | sam, BAM, gtf, amt, xls | yes | yes | Linux | no | yes | yes | yes | yes | yes |  |
| Treat [214] | fastq, BAM or variant file | summary files | yes | yes (annotation module) | Linux; 4-cores - 16 GB of RAM, ~175 GB storage space | no | yes | yes | yes | yes | yes | A tool for analyzing and interpreting NGS data covering alignment, variant calling, and variant annotation; It provides four different categories of variant annotations and links variants to a genome viewer. Currently, it provides no hg19 reference. |

# Table S11 Workflow systems

| **Name** | **Illumina** | **Solid** | **Requirements** | **GUI** | **CLI** | **Online** | **Cloud** | **Notes** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ergatis [215] | yes | yes | Linux, MAC OS X, Windows | yes | no | yes | Yes | Web-based workflow management system for configuring and monitoring pipelines; offers components for BWA, bowtie without executables and is due to its complexity aimed at bioinformaticians; |
| Galaxy [216] | yes | yes | Linux, MAC OS X | yes | no | yes | yes | Web-based platform for performing, reproducing, and sharing complete analyses; The system offers graphical workflow editing and includes many built-in NGS tools. Users can add new tools and share them with the community. |
| Genboree Workbench [217] | yes | yes | Linux, MAC OS X, Windows | yes | no | yes | Yes | It is a platform for deploying genomic tools as a service and offers a web-based drag and drop interface. Tasks are executed on a compute cluster and data can be uploaded as tracks. The system cannot be installed locally. |
| GenePattern [218] | yes | yes | Linux, MAC OS X, Windows | yes | no | yes | No | Scientific workflow system that provides access to more than 150 genomic analysis tools; |
| GeneProf [219] | yes | yes | Linux (it is not tested on  Others yet) | yes | no | yes | No | A web-based, graphical software suite for the analysis of NGS data; To complete the installation IT experience is required. |
| Kepler (bioKepler) [220] | yes | yes | Linux, MAC OS X, Windows;  > 1 GB RAM,  2 GHz CPU | yes | no | no | No | Free software with a graphic user interface system for managing scientific workflows; supports hierarchy in workflows to create modular components.; |
| KNIME [221] | yes | - | Linux, MAC OS X, Windows | yes | yes | no | Yes | Open source platform for graphically building and editing workflows and data analysis pipelines; includes some NGS analysis programs, which are mostly for filtering and manipulating VCF files; |
| LONI Pipeline [222] | yes | yes | Linux, MAC OS X, Windows | yes | yes | no | No | Workflow processing application where executables can be used by creating dedicated wrappers; provides a few NGS analysis pipelines and users can apply for an account to download and use the software; |
| Moa [223] | yes | yes | Linux | yes | yes | no | No | The command line based management system allows users to write and executes workflows; offers some NGS support, mainly for aligning reads to a reference genome; |
| Tavaxy [224] | yes | yes | Linux | yes | no | yes | Yes | The tool combines the web interface of Galaxy, with the complexity of Taverna. |
| Taverna [225] | yes | yes | Linux, MAC OS X, Windows | yes | yes | no | yes | Open source, web-service based workflow management application with a large library of existing tools that operate on genomic sequences; does not ship with any prepackaged sequence analysis tools and integrating the tools requires some programming experience; |
| Yabi [226] | - | - | Linux | yes | yes | yes | yes | Currently, only a trial version is available and users need to request for an account. The public available version does not contain predefined workflows for NGS analysis. |

# Supplementary Information I1 Additional information about evaluated software tools

## Crisp

The tool requires an existing Python installation and provides a tool to convert BAM files into their pileup format. The pileup format created by SAMtools is not supported.

## GATK

It requires Ant and Java to build and execute the tools. GATK offers an extensive documentation and wiki system as well as a very active community.

## SAMtools

The included software suite “BCFtools” has the ability to call SNPs and short INDELs from a single alignment file in the pileup format, which in turn can easily be generated from multiple BAM or SAM files, using SAMtools’ mpileup. SAMtools is self-contained and has no further dependencies. It needs to be compiled from source code, and the steps required to do so are listed in its detailed documentation.

## SNVer

The tool is written in Java and hence, it is operating system independent and requires no dependencies besides a Java runtime environment. SNVer provides a straightforward command-line interface, which is well documented. Additionally, the tool includes sample data for quick testing.

## VarScan 2

It takes input files in pileup format, thus requiring a preparation step using SAMtools to convert BAM input files. Installation is only dependent on an existing Java environment and extensive usage documentation is provided.

## SomaticSniper

The required input is a tumor BAM file, a normal BAM file, and the corresponding reference genome in FASTA format. SomaticSniper outputs results either in VCF format or in a format similar to the SAMtools consensus format. The program is available as a Debian APT package or can be manually installed. Both the installation and user manual are detailed and standard usage is straightforward.

## CNVnator

It uses read-depth analysis based on mean-shift to detect CNVs. In order to compile the tool, the ROOT data analysis framework is required, which has to be compiled and configured as well. Though it provides a script for setting the necessary environment variables, the script did not work on our test system and consequently the variables had to be set manually. CNVnator provides a command-line interface for analysis. However, multiple separate commands are necessary in order obtain CNV calls.

## CONTRA

The tool requires Python and R and is dependent on BEDtools (which is included in the installation package) and SAMtools. Installation of the tool is straightforward and well documented and test data to check correct configuration is provided. It is important that BEDtools is correctly configured as CONTRA will not run without it and does not check for its correct configuration on startup. The application takes as input SAM/BAM files for test and control samples and outputs results in VCF and CSV format. The control sample can additionally be provided as a baseline file in BED format.

## ExomeCNV

It takes as input BAM files, which need to be converted into a proprietary pileup format. The conversion can be done using a provided script or GATK’s *DepthOfCoverage* function, where the latter is preferred due to superior runtime performance. Moreover, BAM files need to be sorted canonically and require appropriate read group information. Further required input is an exome definition file in *chr#:start-end* format and a conservative approximation of sample admixture rate (for tumor samples only). ExomeCNV is dependent on R including the package DNAcopy.

## RDXplorer

The tool is distributed as a stand-alone program but can also be used on a high performance computer cluster. It accepts as input sorted BAM files where duplicates need to be either marked or removed, e.g.: using Picard’s *MarkDuplicates* function (http://picard.sourceforge.net). Installation of the program is dependent on multiple programs (blas, lapack, nose, SAMtools), requires Python including multiple packages, and an R installation with special configuration parameters. The installation is challenging for non-experienced users. To start the analysis, a configuration file and the run file need to be adapted.

## BreakDancer

The tool depends on Perl and the installation of five Perl modules. As BreakDancer has only been compiled for MacOSX, manual compilation for Linux systems is required, which depends on the installation of SAMtools and manual adaptation of the make file.

## Breakpointer

It requires aligned BAM files as input and outputs predicted regions containing breakpoints in GFF format. As the tool does not investigate SV content, additional methods for the classification of SVs are required. Breakpointer depends on the BamTools API (https://github.com/pezmaster31/bamtools), which needs to be installed before compilation. Installation and usage is documented in a readme file. No test data is provided and no further information how to interpret the output is given.

## CLEVER

The command-line tool provides easy to use compilation and installation scripts. CLEVER offers an intuitive script with default parameters called "clever-all-in-one", but parameters can also be fine-tuned. It takes BAM files as input and delivers as output detected variations in a proprietary text file format. BAM files need to be sorted by read name which can be achieved using the SortSam command of the Picard suite with *SORT\_ORDER=queryname*.

## GASVPro

It requires the installation of the GASV software, which is dependent on Perl, Java, and Ant. The tool provides an extensive user manual, example data, and the scripts *GASVPro.sh* and *GASVPro-HQ.sh*, which facilitate the streamlined use of the pipeline. *GASVPro-HQ* works with datasets containing high-quality unique read mappings in BAM format whereas *GASVPro* requires a combination of a high-quality unique read BAM and a lower-quality possibly multiple mapping BAM file.

## SVMerge

SVMerge requires a multitude of dependencies that need to be installed before usage, including Perl, LSF queuing system, and all integrated tools. The software does not offer ready-to-use virtual box or cloud implementations, which would enhance the usability of the installation.

## ANNOVAR

The tool includes a separate script called *auto\_annovar.pl*, which implements an iterative filter workflow that has been used to identify two causal mutations for Miller syndrome. If the user wants to use all features of ANNOVAR, numerous manual commands need to be executed to download all databases. The tool requires its own text-based input format, though there are easy to use scripts provided to convert most popular genotype calling formats.

## AnnTools

Although the authors provide an easy to use installation script, the program requires a MySQL database, which has to be installed and configured manually. Once the database is configured, the tool provides a straightforward command-line interface.

## NGS-SNP

The scripts have numerous dependencies which all have to be installed manually. To facilitate the usage of the tool, the authors provide a pre-configured Linux virtual image, which requires only one simple configuration script. While testing, the regular annotation script lost connection to the Ensembl database several times, which aborted the annotation process. However, the authors provide a separate script, which should fix this issue.

## SeattleSeq

The output can be provided either in VCF format or SeattleSeq’s own text output format. The latter can then be displayed in a simple web interface providing overview metrics, as well as some basic sorting and filtering options.

## SVA

Variation lists can be exported in CSV format. Required input parameters are list of variants in VCF format, the reference genome, and binary coverage files. Furthermore, a project configuration file has to be written, defining the input files, the reference genome, and various other parameters or data sources. The required database files have to be downloaded manually and are denoted in the script file.

## snpEff

The program can be downloaded as a jar file and besides downloading the desired reference databases, no additional set up steps are required. The results are provided either in VCF or text format, and additionally, in an easy to read html summary file, including various diagrams, is generated.

## VARIANT

The tool requires that VCF files use GRCh references and even though it recognizes inputs with a UCSC hg version as valid VCF files it does not annotate mutations based on a UCSC hg reference genome.

## VEP

The command-line version includes a simple installation script that installs missing modules and downloads a minimal set of the Ensembl API. For the annotation process the online database is used per default, but database files can be cached locally. The Perl API addresses developers who want to integrate the functionality of VEP into their own programs.

## UCSC Genome Browser

The tested BAM and VCF files needed to be sorted and indexed. However, given the correct format, the tool offers great usability and supports many different data formats.

## Vega

BAM and VCF files can be easily uploaded into Vega, and the usage is similar to the Ensemble Genome Browser.

## Artemis

It is important that users load the appropriate reference genome first, otherwise uploading BAM and VCF files is not possible. The tool offers many different features and customization options.

## IGV

Furthermore, MATLAB users can load files directly into IGV and jump to specific loci. Upon startup, IGV automatically loads a specified reference genome. All test files could be loaded into the genome browser and the interface is intuitive and responsive.

## Savant

Savant features a plug-in mechanism where users are encouraged to develop new features. All tested files could be loaded into Savant. The interface is responsive, and additional information can be directly loaded from the UCSC web server.

## Circos

The tool requires an existing Perl installation and is dependent on several Perl modules. A test script is included in order to determine which modules are still missing or are not configured properly. The visual appearance of the output is defined using configuration files.

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