CEITEC

Central European Institute of Technology

BRNO | CZECH REPUBLIC

Modern Genomic Technologies (LF:DSMGT01)

Lecture 4 : Structural variants

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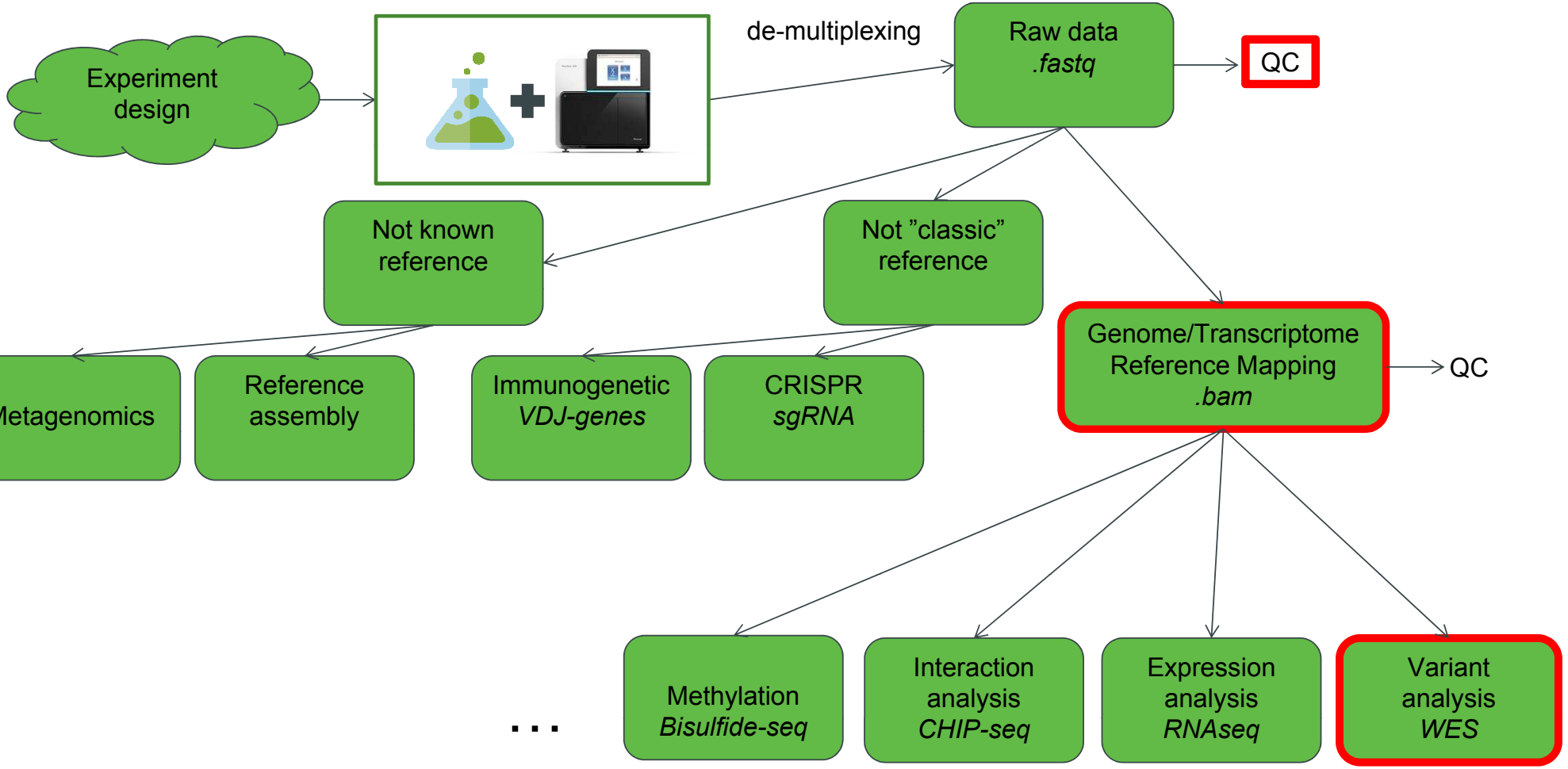
NGS data analysis



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DNA re-sequencing

- Variant Calling
- Medical purposes
- Cancer genomics

- Small variants (SNV + small indels) vs. Structural Variants

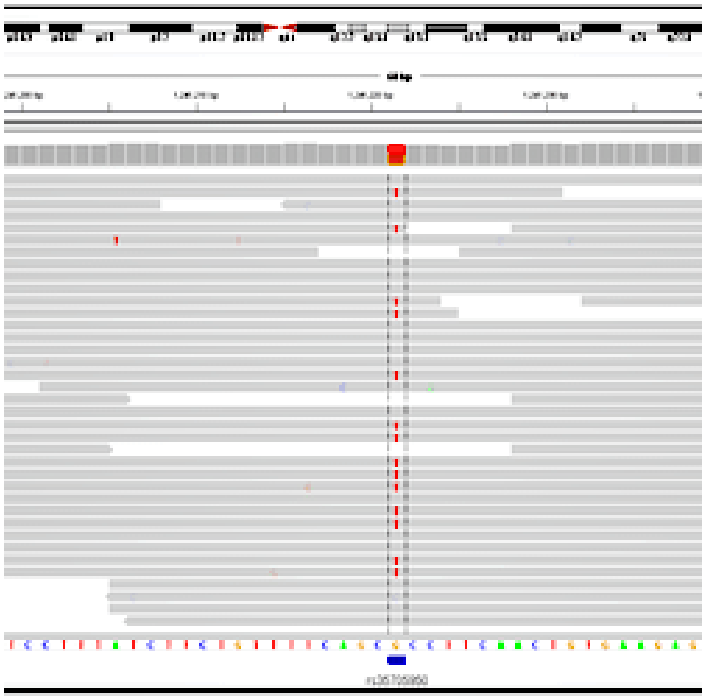
- Germline vs. Somatic

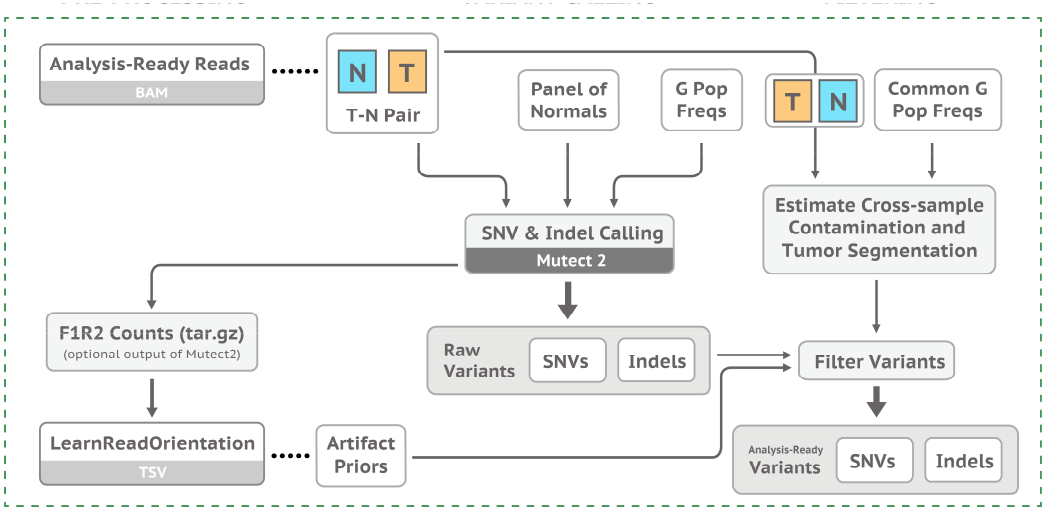
Mapping

- Computationally most demanding
- More or less standardized
- Output .bam
  - .bam = binary (ziped) .sam
  - .sam = Sequence Alignment Map DNA re-sequencing

- Tools
  - BWA - DNA
  - STAR - RNA

Small Variant calling





Mapping QC

General Statistics

Copy table | Configure Columns | Plot | Showing 12/12 rows and 16/24 columns.

	K Reads Mapped	% GC	Ins. size	≥ 100X	≥ 500X	≥ 20X	≥ 30X	Median cov	Mean cov	% Aligned	Fold Enrichment	Target Bases 30X	% Dups	% Dups	% GC	K Seqs
	100 827.9	48%	176	43.3%	0.8%	93.2%	88.7%	89.0X	111.8X	99.6%	43	83%				
Dups													4.7%			
														26.8%	47%	50 603.8
														25.4%	47%	50 603.8
	100 523.1	48%	178	42.8%	0.8%	93.2%	88.8%	88.0X	111.2X	99.6%	43	84%				
Dups													4.6%			
														26.7%	47%	50 460.3
														25.5%	47%	50 460.3
	84 081.9	48%	172	33.7%	0.5%	92.1%	86.4%	75.0X	94.4X	99.6%	44	80%				
Dups													4.5%			
														24.4%	47%	42 202.7
														23.3%	47%	42 202.7

Mapping QC

Qualimap Report: BAM QC

Summary

Globals

Reference size	3,101,804,739
Number of reads	84,405,388
Mapped reads	84,038,132 / 99.56%
Unmapped reads	367,256 / 0.44%
Mapped paired reads	84,038,132 / 99.56%
Mapped reads, first in pair	42,129,277 / 49.91%
Mapped reads, second in pair	41,908,855 / 49.65%
Mapped reads, both in pair	83,774,794 / 99.25%
Mapped reads, singletons	263,338 / 0.31%
Secondary alignments	0
Supplementary alignments	7,807 / 0.01%
Read min/max/mean length	30 / 80 / 80.02
Clipped reads	2,065,102 / 2.45%

Globals (inside of regions)

Regions size/percentage of reference	45,326,818 / 1.46%
Mapped reads	63,363,519 / 75.07%
Mapped reads, only first in pair	31,877,600 / 37.77%
Mapped reads, only second in pair	31,485,919 / 37.3%
Mapped reads, both in pair	63,167,455 / 74.84%
Mapped reads, singletons	196,064 / 0.23%
Correct strand reads	0 / 0%
Clipped reads	2,065,102 / 2.45%
Duplicated reads (flagged)	2,968,557 / 4.68%

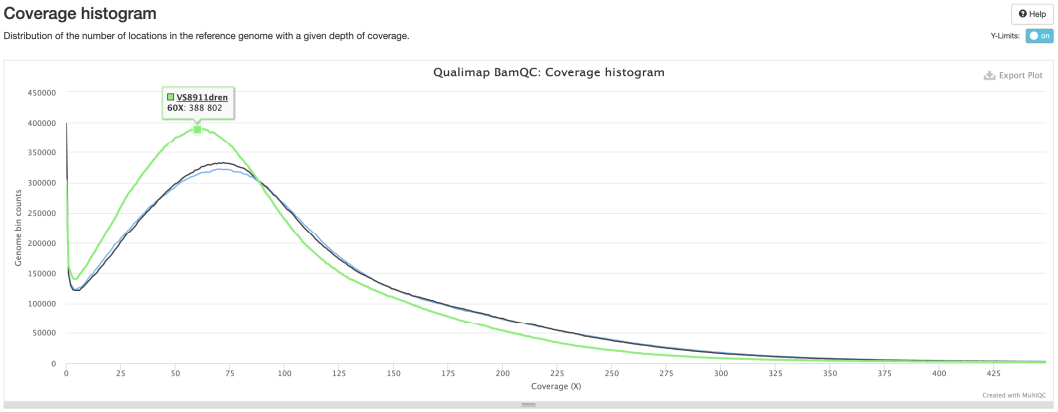
ACGT Content (inside of regions)

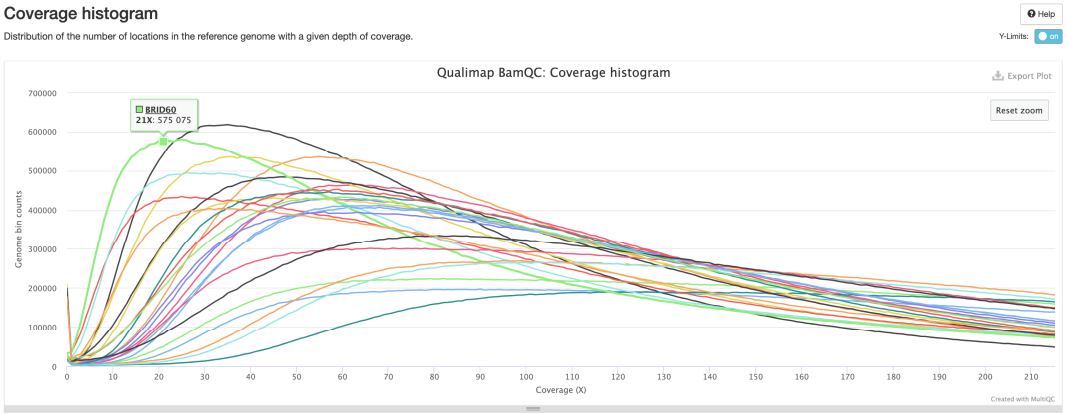
Number/percentage of A's	1,090,175,822 / 25.48%
Number/percentage of C's	1,048,730,118 / 24.52%
Number/percentage of T's	1,108,474,060 / 25.91%
Number/percentage of G's	1,030,171,088 / 24.08%
Number/percentage of N's	237,846 / 0.01%
GC Percentage	48.6%

Coverage (inside of regions)

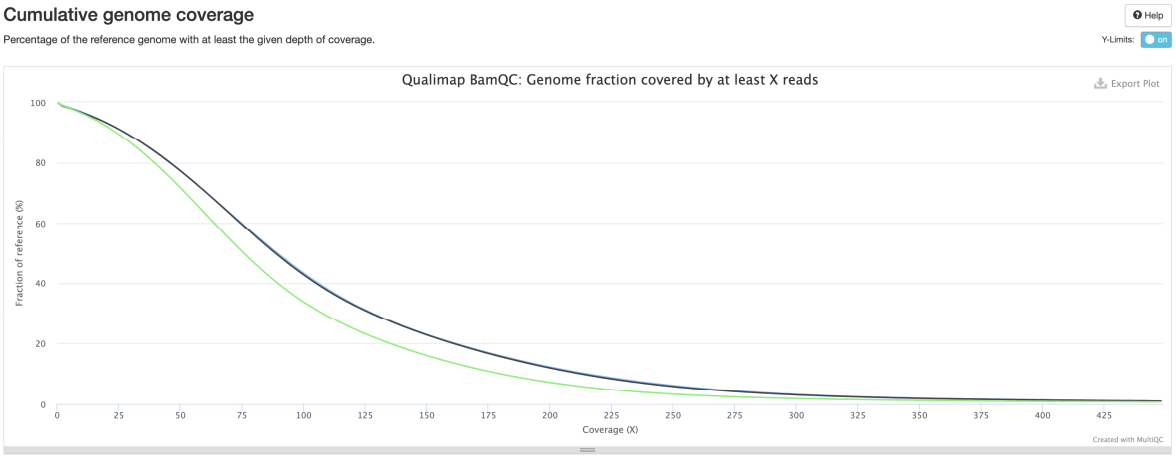
Mean	94.3822
Standard Deviation	97.2737

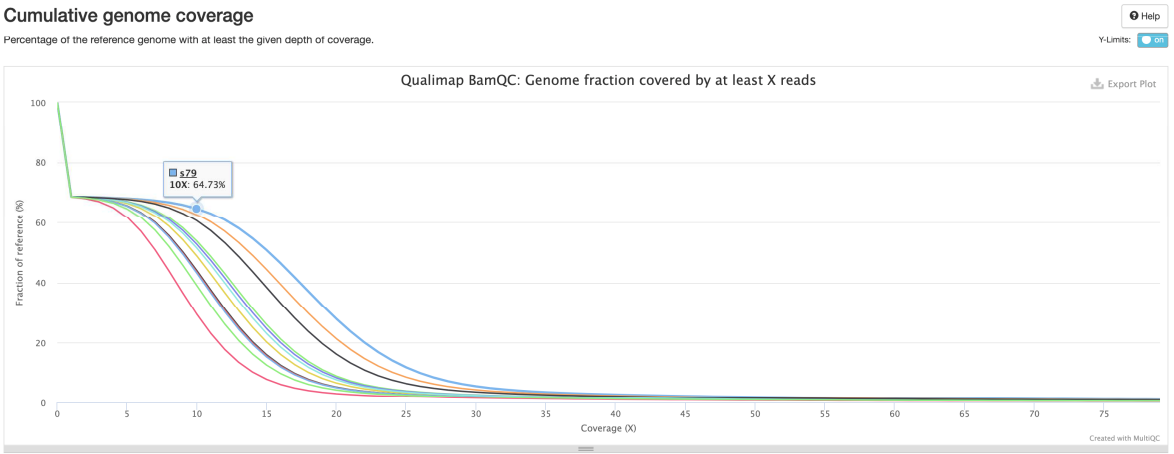
Mapping QC - coverage





Mapping QC – cumulative coverage





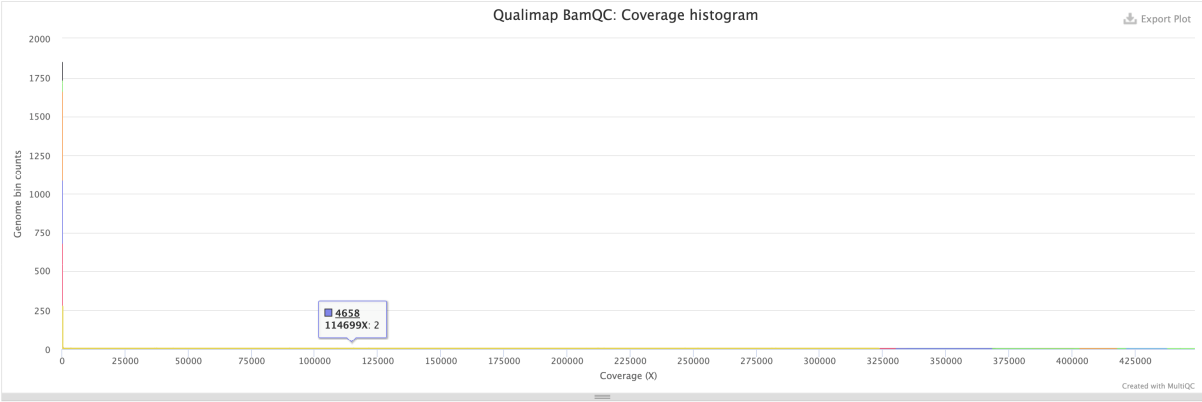
Mapping QC

Coverage histogram

Distribution of the number of locations in the reference genome with a given depth of coverage.

Help

Y-Limits: on

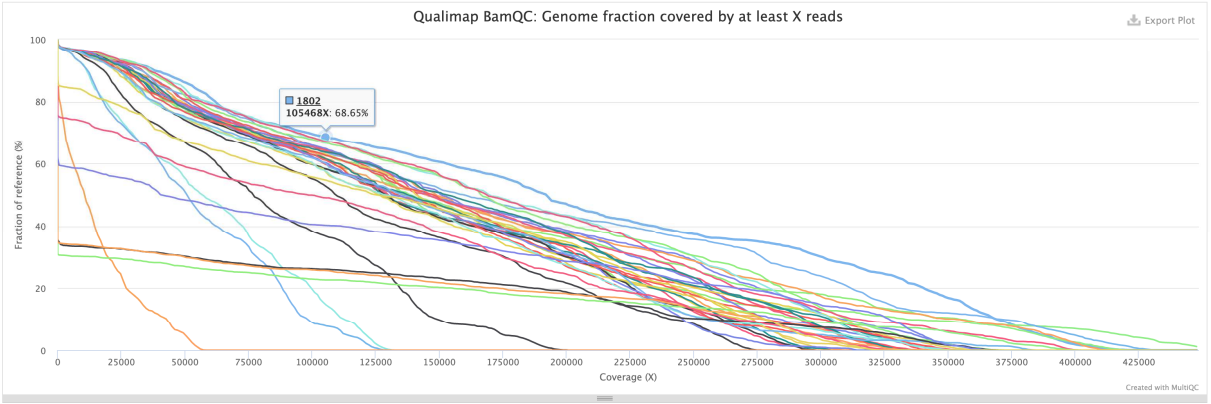


Cumulative genome coverage

Percentage of the reference genome with at least the given depth of coverage.

Help

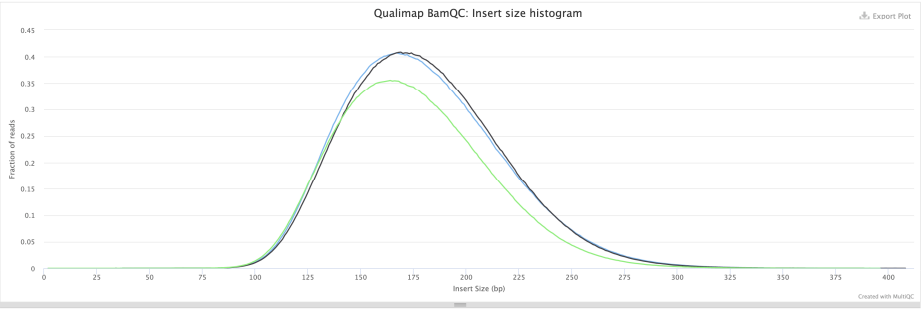
Y-Limits: on



Mapping QC

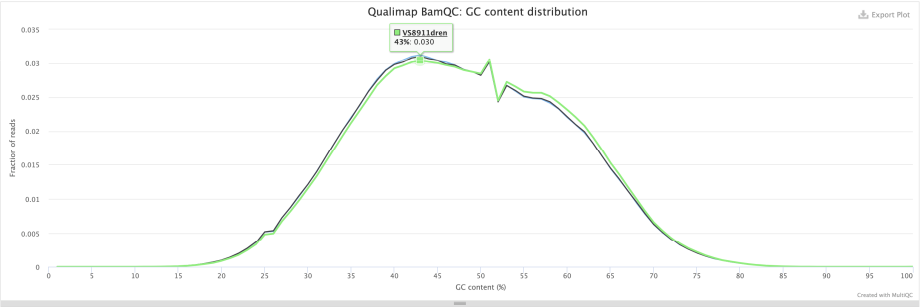
Insert size histogram

Distribution of estimated insert sizes of mapped reads.



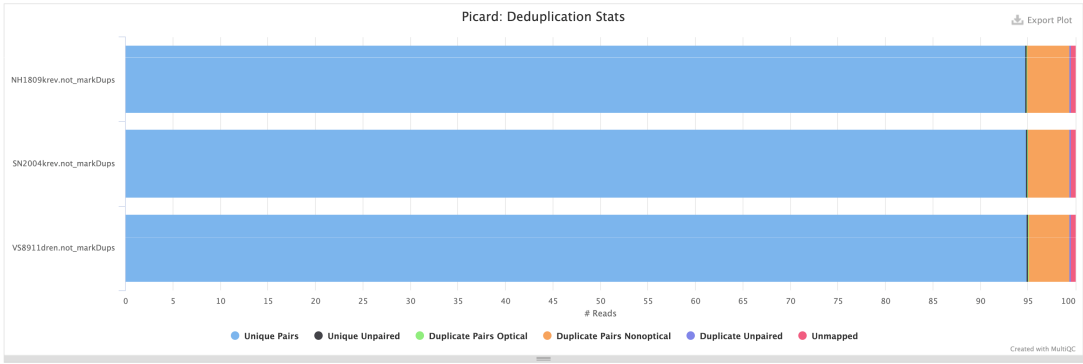
GC content distribution

Each solid line represents the distribution of GC content of mapped reads for a given sample.

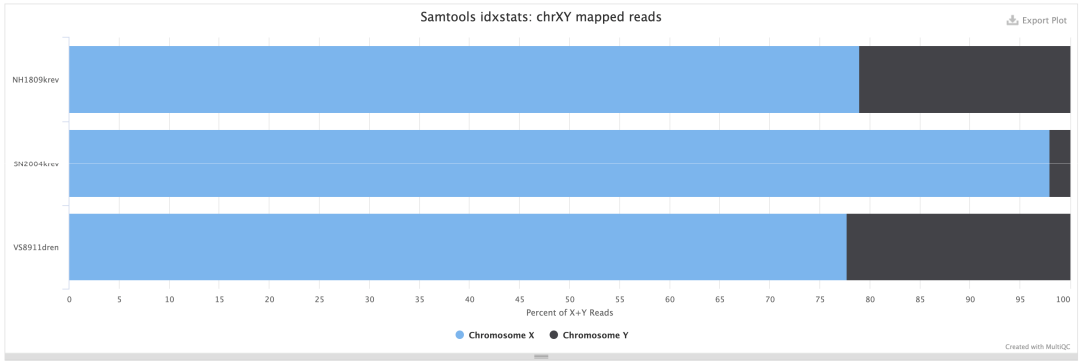


Mark Duplicates

Number of reads, categorised by duplication state. **Pair counts are doubled** - see help text for details.

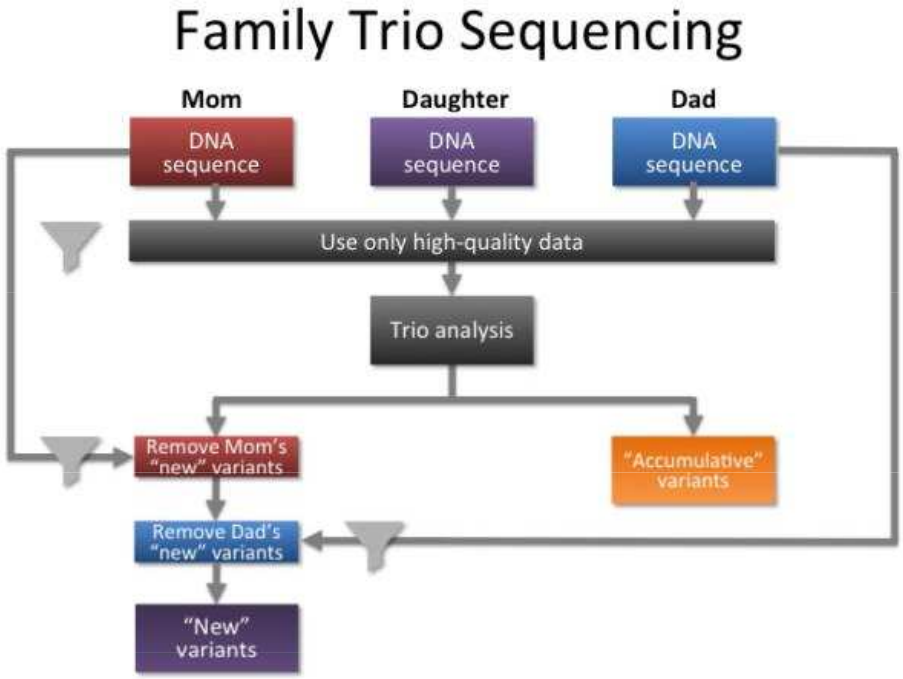


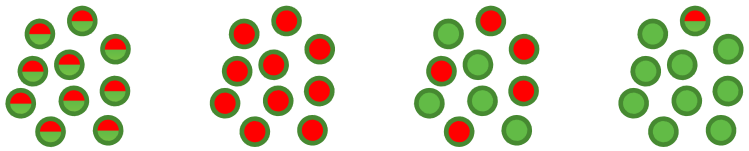
XY counts



Variant Calling - Germline

- What you have from birth
- Family trio sequencing
- Predispositions





Variant Calling - Germline

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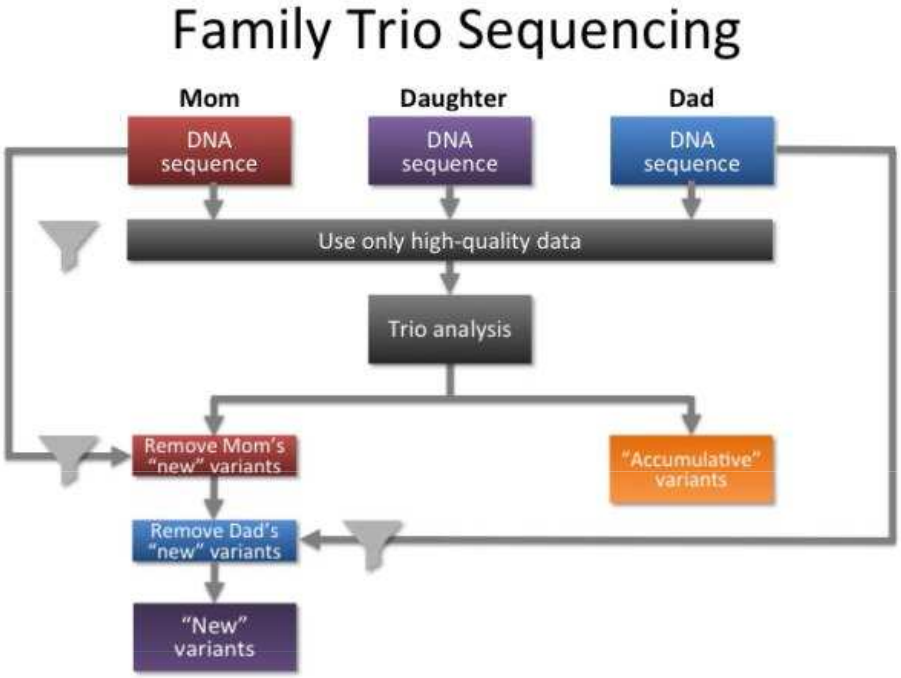
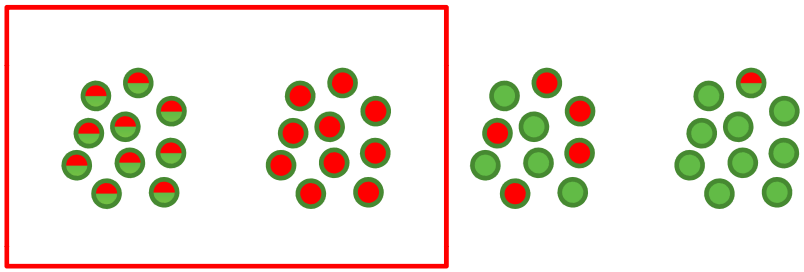


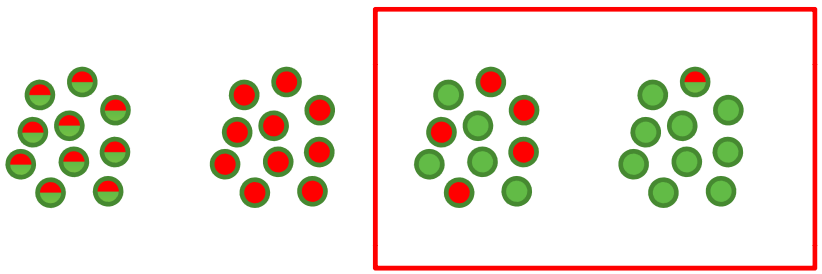


Variant Calling - Germline

- Tools:

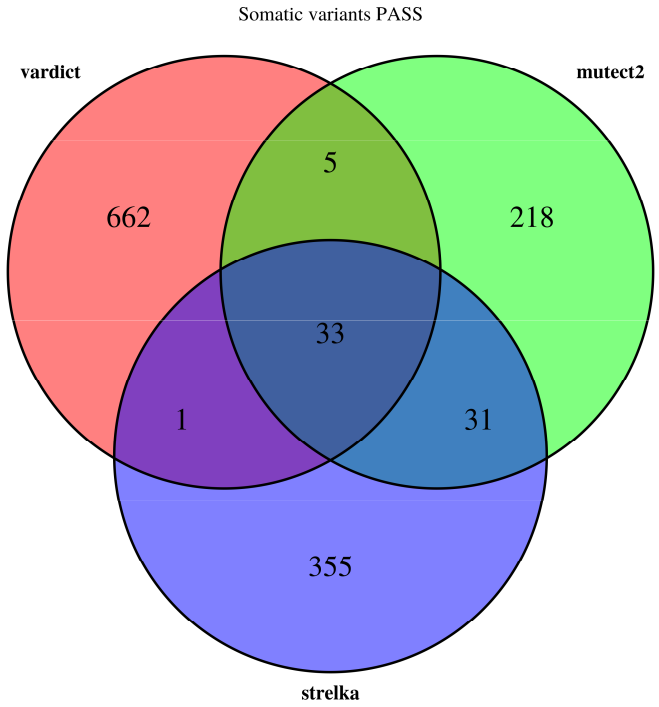
Variant Calling - Somatic

- Diagnostics / prognostic / therapy decision
- Tumor – normal paired
  - Somatic variant calling without normal needs high coverage
- Expected variant heterogeneity
- Indirectly corelates to the necessary coverage



Variant Calling - Somatic

- Multiple tools:
  - strelka2, verdict, mutect2, somaticsniper, lofreq, muse, varscan
- Ensemble caller
  - SomaticSeq
  - Use machine learning to detect TP from FP
- Sensitivity vs. specificity
  - Preferred sensitivity
  - Preferred accuracy for derived information



Small Variant annotation

- VEP – variant effect predictor
- Transcript ”selection”
  - Refseq vs. ensemble
- Population frequency
  - 1000 genome project
  - Gnomad
- Many clinical variant DBs
  - Gene based vs. variant based
  - snpDB
  - COSMIC
  - clinvar
  - CGC

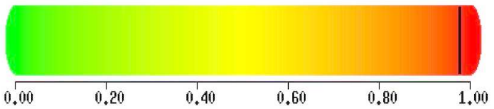
Small Variant annotation – functional prediction

- General variant consequence
  - Based on the position
  - Impact
- Effect of the variant on protein structure
  - PolyPhen
  - SIFT

POLYPHEN-2

This mutation is predicted to be **PROBABLY DAMAGING** with a score of **0.976**

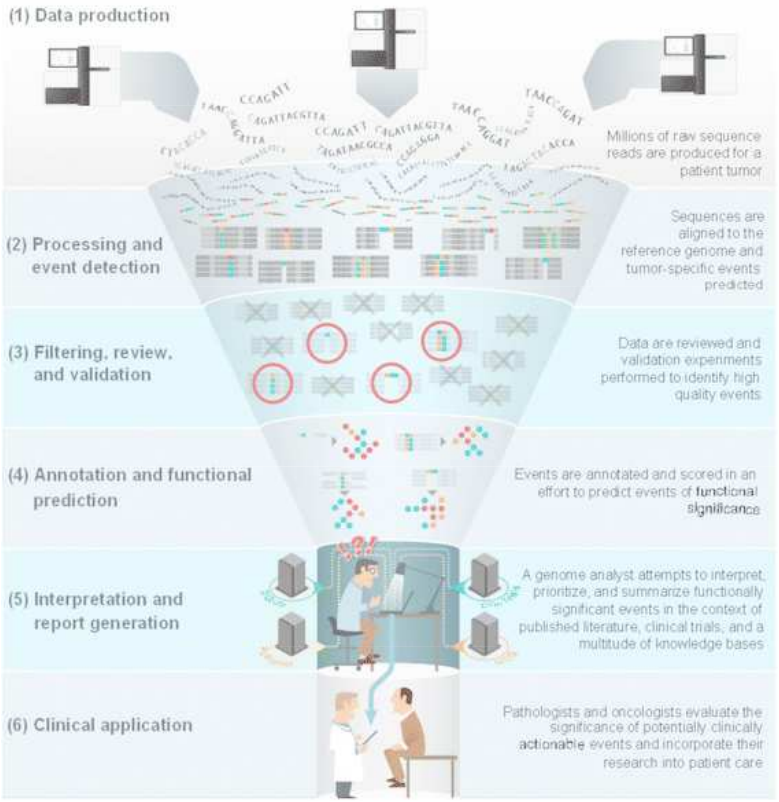
(sensitivity: **0.76**; specificity: **0.96**)



*	SO term	SO description	SO accession	Display term	IMPACT
	transcript_ablation	A feature ablation whereby the deleted region includes a transcript feature	SO:0001893	Transcript ablation	HIGH
	splice_acceptor_variant	A splice variant that changes the 2 base region at the 3' end of an intron	SO:0001574	Splice acceptor variant	HIGH
	splice_donor_variant	A splice variant that changes the 2 base region at the 5' end of an intron	SO:0001575	Splice donor variant	HIGH
	stop_gained	A sequence variant whereby at least one base of a codon is changed, resulting in a premature stop codon, leading to a shortened transcript	SO:0001587	Stop gained	HIGH
	frameshift_variant	A sequence variant which causes a disruption of the translational reading frame, because the number of nucleotides inserted or deleted is not a multiple of three	SO:0001589	Frameshift variant	HIGH
	stop_lost	A sequence variant where at least one base of the terminator codon (stop) is changed, resulting in an elongated transcript	SO:0001578	Stop lost	HIGH
	start_lost	A codon variant that changes at least one base of the canonical start codo	SO:0002012	Start lost	HIGH
	transcript_amplification	A feature amplification of a region containing a transcript	SO:0001889	Transcript amplification	HIGH
	inframe_insertion	An inframe non synonymous variant that inserts bases into in the coding sequenc	SO:0001821	Inframe insertion	MODERATE
	inframe_deletion	An inframe non synonymous variant that deletes bases from the coding sequenc	SO:0001822	Inframe deletion	MODERATE
	missense_variant	A sequence variant, that changes one or more bases, resulting in a different amino acid sequence but where the length is preserved	SO:0001583	Missense variant	MODERATE
	protein_altering_variant	A sequence_variant which is predicted to change the protein encoded in the coding sequence	SO:0001818	Protein altering variant	MODERATE
	splice_region_variant	A sequence variant in which a change has occurred within the region of the splice site, either within 1-3 bases of the exon or 3-8 bases of the intron	SO:0001630	Splice region variant	LOW
	incomplete_terminal_codon_variant	A sequence variant where at least one base of the final codon of an incompletely annotated transcript is changed	SO:0001626	Incomplete terminal codon variant	LOW
	stop_retained_variant	A sequence variant where at least one base in the terminator codon is changed, but the terminator remains	SO:0001567	Stop retained variant	LOW
	synonymous_variant	A sequence variant where there is no resulting change to the encoded amino acid	SO:0001819	Synonymous variant	LOW

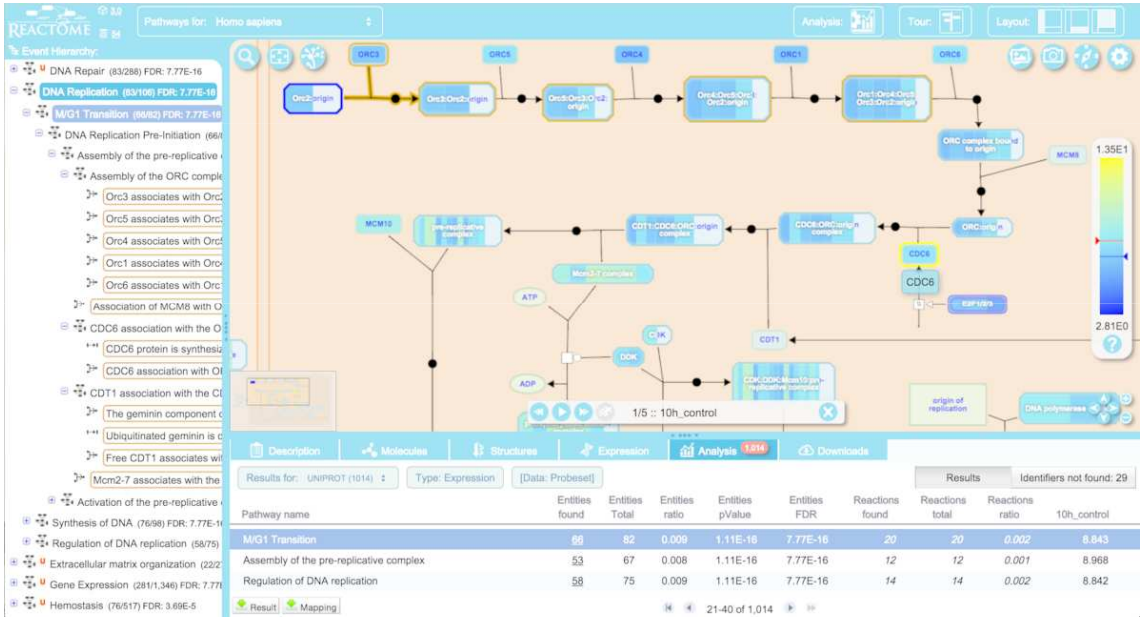
Small Variant interpretation

- Hardest part
- Usually manual work
  - Clinical genetics
  - Select 5 probable causal from ~1000
- Bioinformatics can help



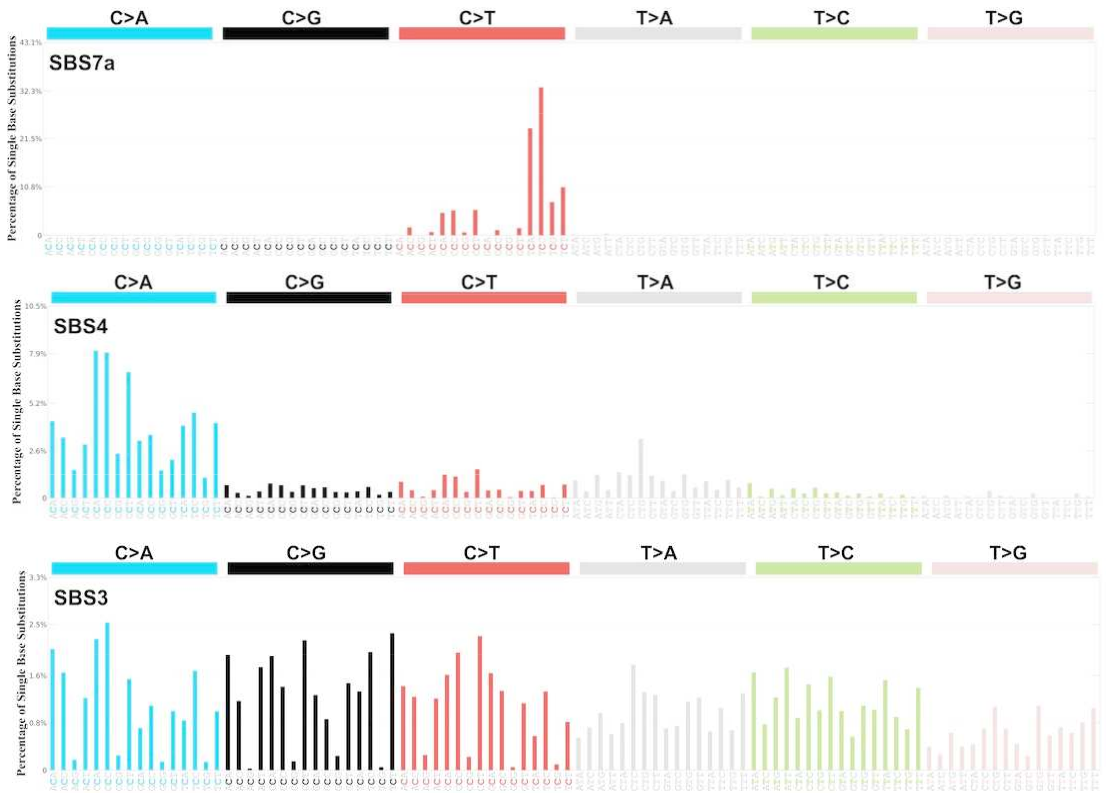
Variant interpretation – gene networks

- Gene ontology
- Biological pathway DB
  - KEGG
  - Reactome
  - WikiPathways



Variant interpretation – derived informations

- Tumor mutational burden
  - Several definitions
  - Mutations per million bases
- Mutational Signatures
  - COSMIC
  - exposure to ultraviolet light
  - Tabacco smoking
  - Defective DNA damage repair



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Thank you for your attention!

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