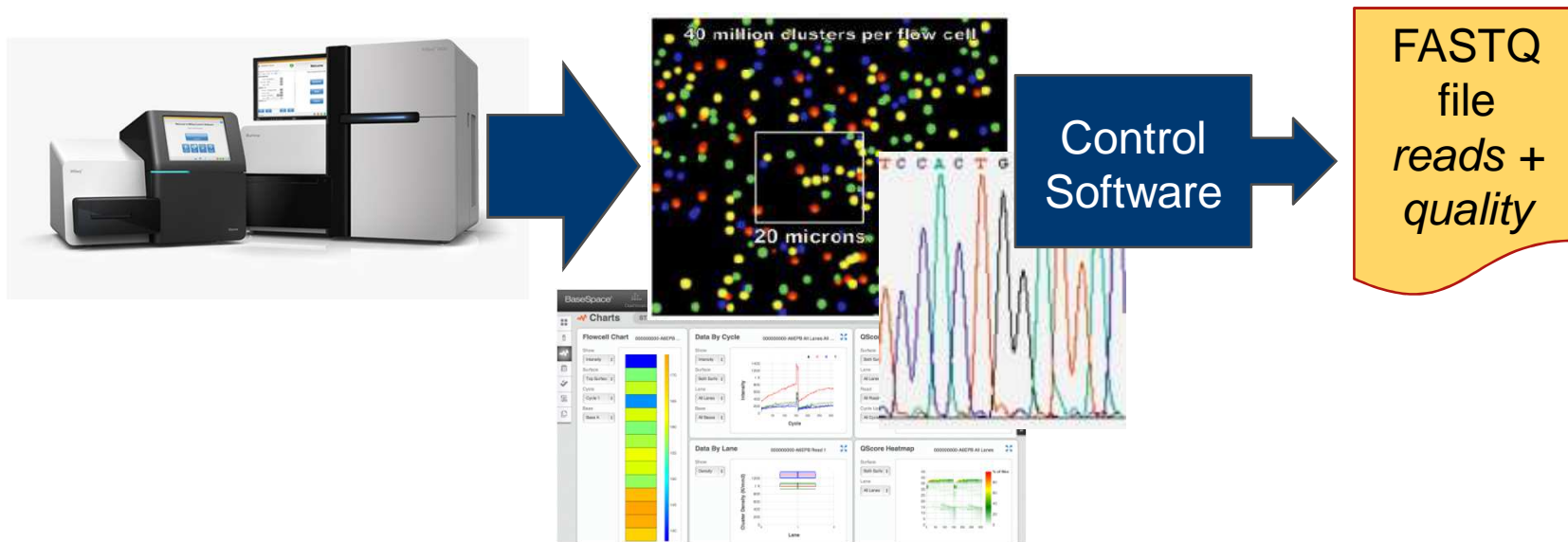




Analysis with SureCall 2.1

Danielle Fletcher
Field Application Scientist
July 2014

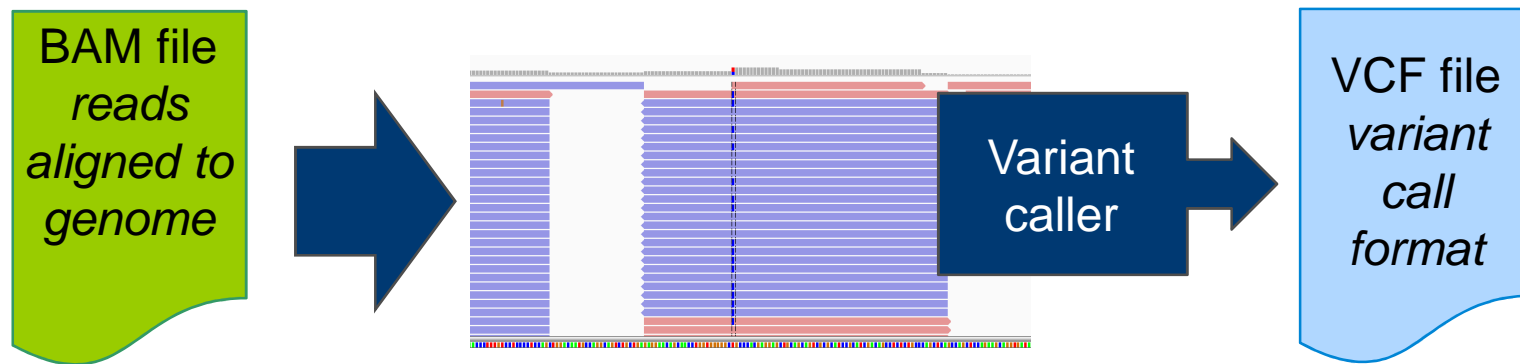
Stages of NGS Analysis – Primary analysis, base calling



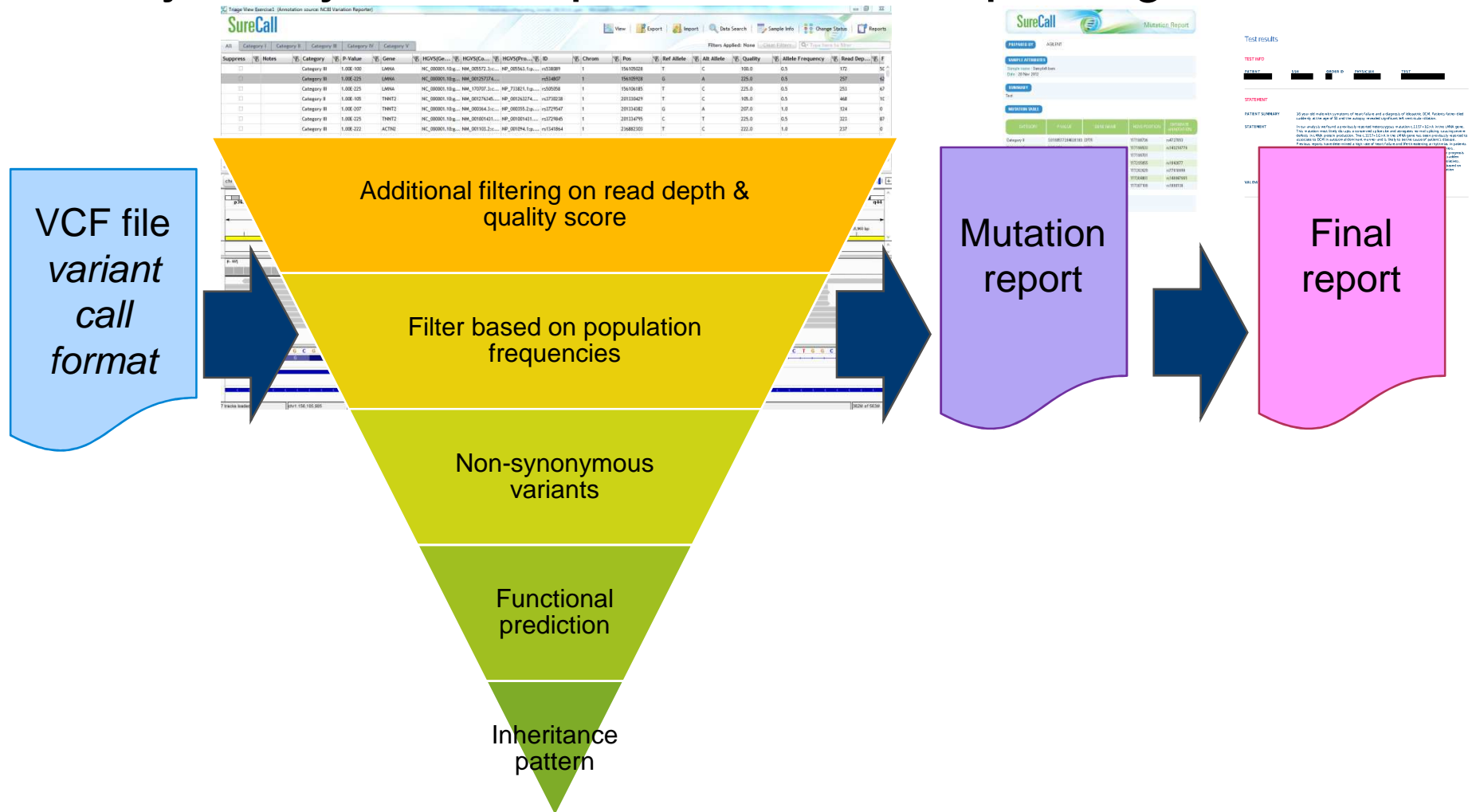
Stages of NGS Analysis – Secondary analysis part 1, read alignment



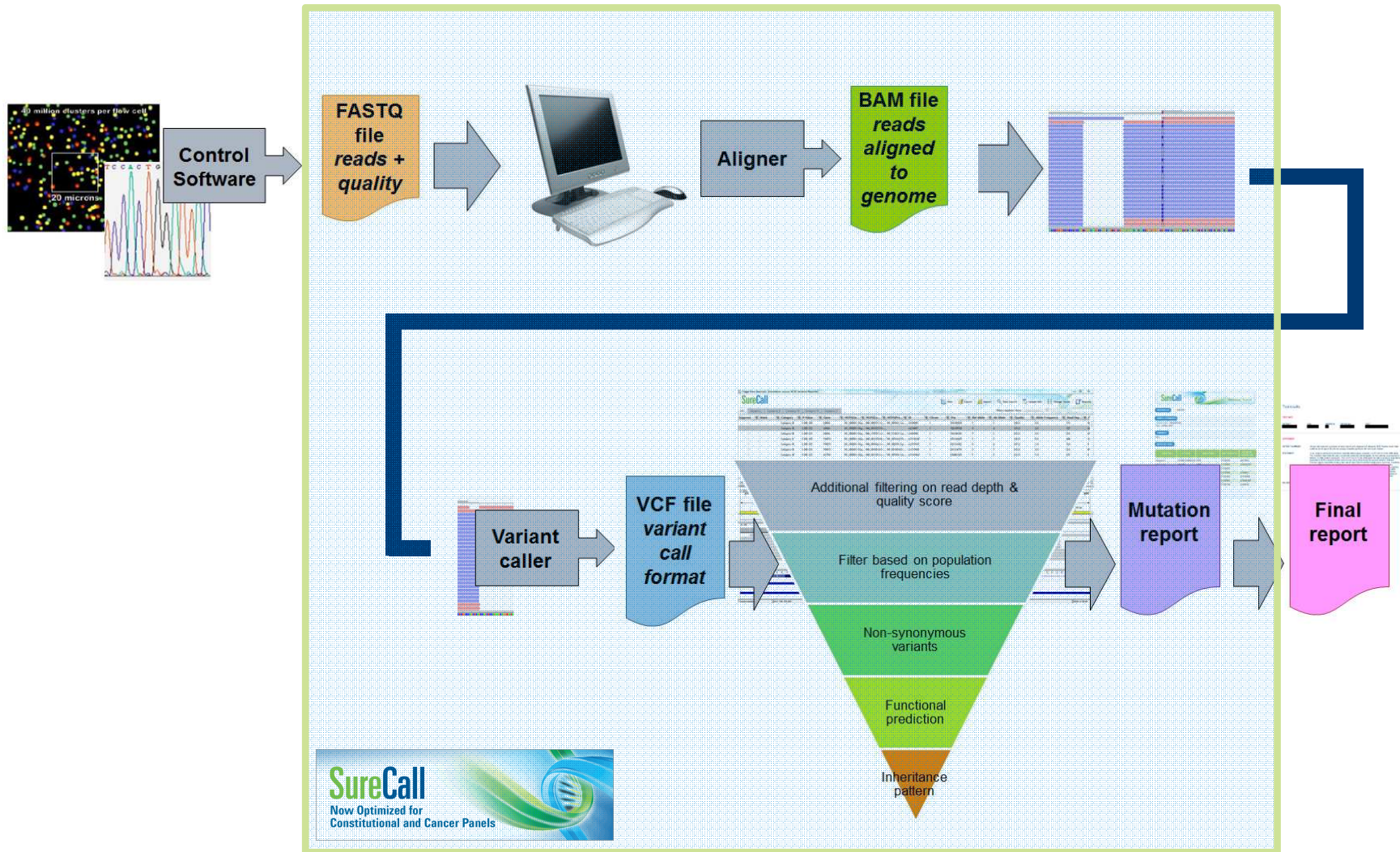
Stages of NGS Analysis – Secondary analysis part 2, variant calling



Stages of NGS Analysis – Tertiary analysis, interpretation and reporting



SureCall – from alignment to mutation report



Agilent SureCall Software

Resolves data analysis bottleneck

An easy-to-use software for end-to-end data analysis from alignment to categorization of mutations

- Easy to Implement
- Easy to Use with a Streamlined Workflow
- Fast Time to Results
- Designed for clinical research
- Free of Charge

www.agilent.com/genomics/surecall

The image displays two screenshots of the Agilent SureCall software. The top screenshot shows a 'Mutation Report' page with fields for 'PREPARED BY', 'SAMPLE ATTRIBUTES' (including sample name, ERIC, TE/TC, and median base quality), a 'SUMMARY' section, and a 'MUTATION TABLE'. The table has columns for LOCUS, GENE NAME, TYPE (SNP/INDEL/MP), P-VALUE, CATEGORY, and NOTES. The bottom screenshot shows the 'Mutation Report - Checked Out' window with a 'SureCall' header, a search bar, and a table with columns: SUPP..., NOTES, CHROM, POS, ID, REF/Alt, ALT, AIL..., Allele..., and p-Value. Below the table is a genomic browser view for chromosome 7, showing a 65 bp region with a mutation at position 117,199,500 bp. The browser displays read alignments and a sequence view: TGGCTTTATTTCAGACTTCCTCTAATGGTGATTATGGGAGAACTGGACCTCAGAGGTA.

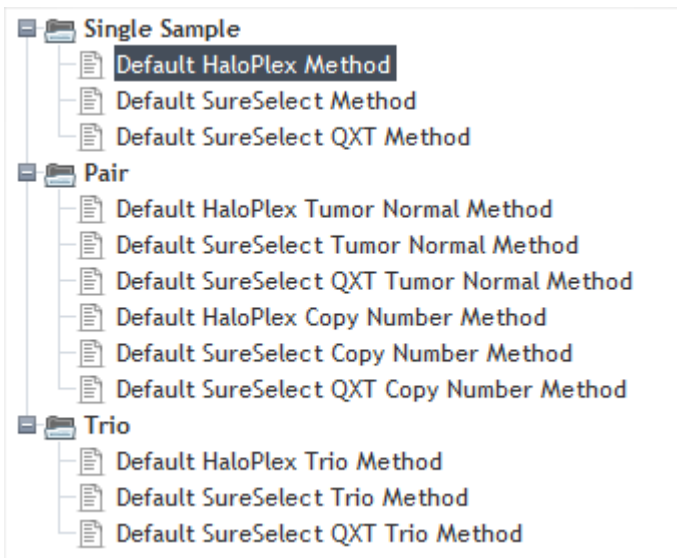
SureCall makes NGS data analysis easy, fast, cheap

Choose one of the three analysis types available in SureCall

- Single Sample Analysis
- Pair Analysis
- Trio Analysis

	Description	Result
Single Sample Analysis	<ul style="list-style-type: none">For individual samples	<ul style="list-style-type: none">SNPs and indels
Pair Analysis	<ul style="list-style-type: none">To determine copy number changes (use a normal reference).To determine somatic mutations in tumor-normal samples	<ul style="list-style-type: none">SNPs and indelsCNVsSomatic mutations
Trio Analysis	<ul style="list-style-type: none">For trios, typically mother, father and child	<ul style="list-style-type: none">SNPs and indelsDe novo mutations

Default analysis methods for single samples, tumor-normal pairs, copy-number, and trio analysis



	HaloPlex	SureSelect	SureSelect QXT
Trimmer	Yes	No	Yes
Remove Duplicates	No	Yes	Yes
Region Padding	No	Yes - 100 bp	Yes - 100 bp

Instead of using default analysis method, create your own analysis method

Create your own analysis method by choosing your own settings

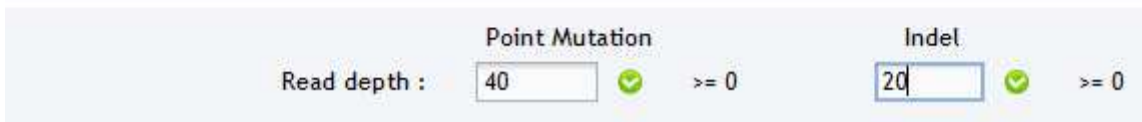
- Trimmer
- Aligner
- Remove duplicates (yes/no)
- Fix mates (yes/no)
- Variant caller
- Filters
- Mutation impact
- Version of annotations
- Add your own annotations

The image shows a screenshot of a software interface with several panels of settings. The panels are arranged in a grid-like fashion. The 'Alignment' panel has two checked options: 'Trimmer' and 'Aligner'. The 'Post Alignment Processing' panel has three checked options: 'Remove Duplicates', 'Fix Mates', and 'Region Padding'. The 'SNP Call' panel has two radio button options: 'BAQ SNP Caller' (selected) and 'SNPPET SNP Caller'. The 'Filter' panel has one checked option: 'Filter'. The 'Mutation Impact' panel has one checked option: 'Mutation Impact'. The 'Version of Annotations' panel has one checked option: 'Version of Annotations'. The 'Track Based Annotations' panel has one unchecked option: 'Track Based Annotations'.

Five examples when to use your own settings

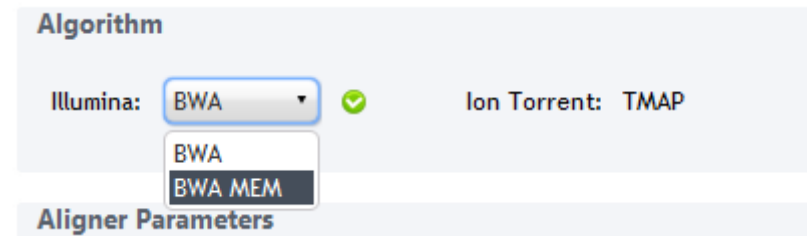


Example 1: Only analyze a subset of regions from the original design



Example 2: Filter mutations on read depth

Example 3: Choose a different aligner



Example 4: Add your own custom annotations to variants

Track Based Annotations

SureCall allows you to use tracks that contain custom annotations for mutations. To import a track go to Supporting Files, Tracks. The tracks can be used to attach custom annotations to mutations. These custom annotation will be added as additional columns in the Triage View. The tracks can also be used to only analyze a subset of regions from the original design instead of the entire set of analyzable target regions (for HaloPlex) or covered regions (for SureSelect).

Select Track:



Example 5: Change category names and supporting evidence

Agilent SureCall

SureCall

Home Analysis Workflow Sample Review **Configure Settings** Supporting Files Admin

Analysis Methods

Categorization

For Research Use Only. Not for Use in Diagnostic Procedures.

Delete Save As Cancel

Default Categorization

View Unused Attribut...

[+] Add a Category

Category Name: Category I [-]

Description:

> Known in literature to be clinically significant and causative. Evidence for pathogenicity in locus specific databases as being associated with disease in GWAS catalog. Is associated with a tumor site in COSMIC. Is validated in clinical study in NCBI SNP

>>

<

<<

Category Name: Category II [-]

Description:

> Introduction of a stop codon
In-frame exon deletion
Mutates the initiation codon (ATG)
Missense mutation of the normal stop codon
Annotated probable pathogenic in NCBI SNP database
Deletes nucleotide(s) that lead(s) to a shift of reading frame
Deletes exon which results in shift of reading frame

Categorization Name: Default Categorization

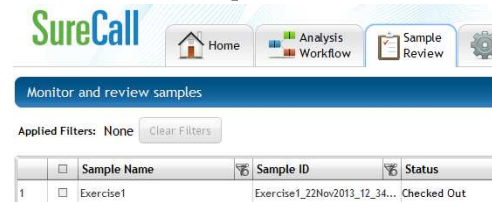
Follow the simple 3-step Analysis Workflow

The screenshot displays the SureCall software interface with a vertical navigation bar on the left and a main content area. The navigation bar has three steps: 1. Import Samples, 2. Describe Samples, and 3. Run Analysis. The main content area is divided into three sections corresponding to these steps:

- 1. Import Samples:** A section titled "Import samples and assign analysis method" with instructions to import BAM files and assign an analysis method.
- 2. Describe Samples:** A section titled "Describe samples" with a table for "Sample Name" and a "BAM File" column. A "Run summary" section below it contains fields for "Job Name" (Job_31Oct2012_23_00_45), "Description" (Job_31Oct2012_23_00_45, Please Write job description here), and "Selected Sample(s) & Analysis Method(s)" (B907_2_Sorted.bam, Default Analysis Method).
- 3. Run Analysis:** A section at the bottom right with a "Run Analysis" button.

Navigation buttons for "Back" and "Run Analysis" are located at the bottom right of the interface.

After the sample is analyzed open the sample in the Sample Review tab

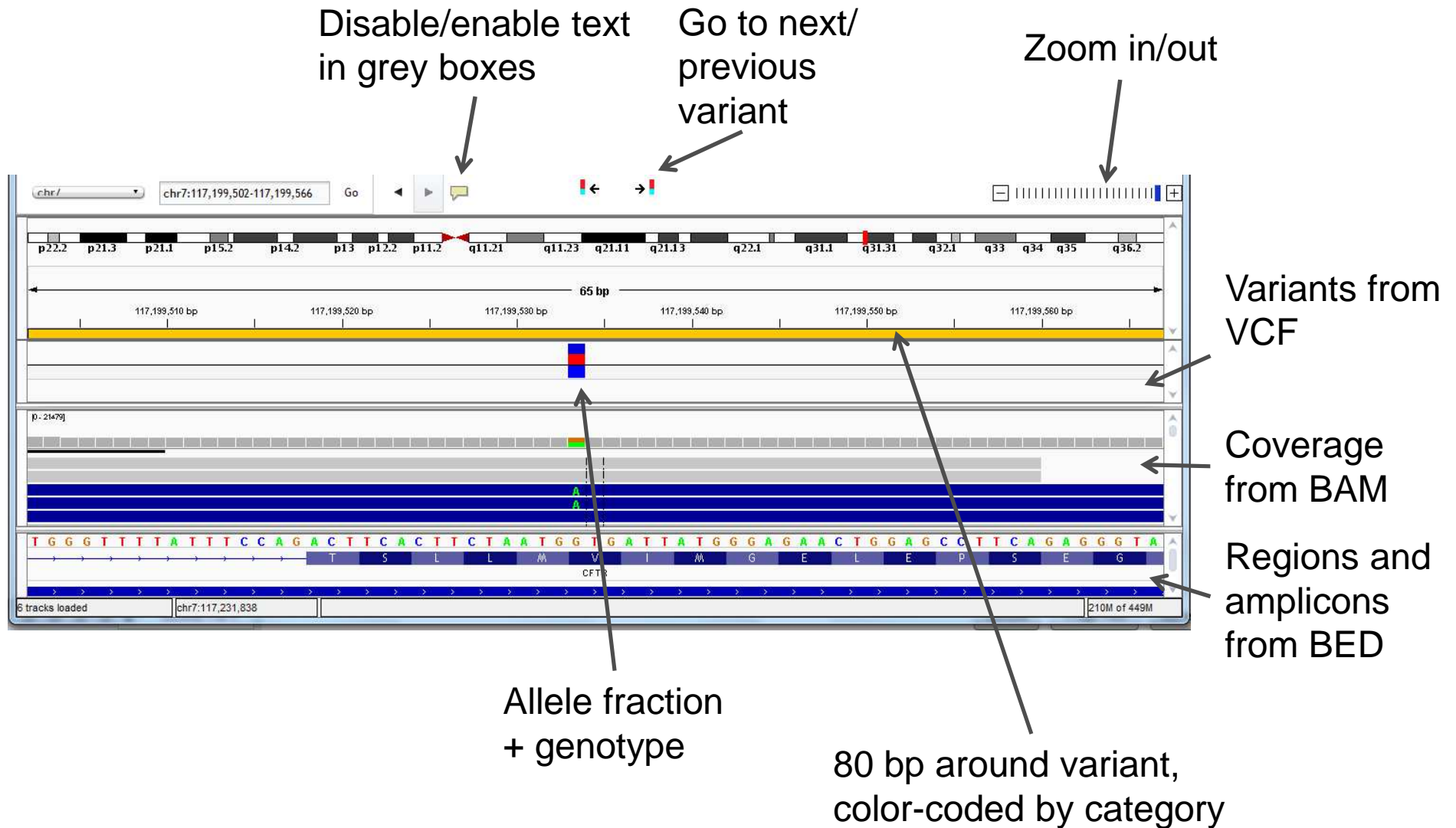


SUPPR...	NOTES	CHROM	POS	ID	REFAllele	ALT Alle...	Allele ...	p-Value	ReadD...	Mappi...	Effect	Primar...	Category	Functi...	CODON	AA
<input type="checkbox"/>		7	117199533		G	A,T,C	1	142.0	5613	50	NON_SYN...	NON_SYN...	Category II	MISSENSE	Gtg/Atg	V47C
<input type="checkbox"/>		7	117231852		T	C	0.5	207.0	2247	47	INTRON(M...	INTRON	Category III			
<input type="checkbox"/>		7	117251543		T	C	0.5	70.0	204	50	INTRON(M...	INTRON	Category III			
<input type="checkbox"/>		7	117267511		C	A	1	87.8	862	50	INTRON(M...	INTRON	Category III			

mutation table

genome viewer

Use the Genome Viewer to review the variants



Display regions and exons without sufficient read depth

1

QC Metrics

QC Metrics

Alignment

Trimmer

QC Metrics

With each analysis job, SureCall creates two bed files that contain the exons and the analyzable target regions (for HaloPlex) or covered regions (for SureSelect) with one or more bases of coverage below the specified read depth. The exons and the regions can be shown in the Triage View by going to View. The bed files are also saved to the job's output folder. The names of the files end with ExonsBelowThreshold and RegionsBelowThreshold.

Report exons and regions that have a Read Depth below: 1 - 10000

2

View | Export | Import

Hide Genome Viewer

Show/Hide Mutation Attributes

Show RegionsBelowThreshold track

Show ExonsBelowThreshold track

IGV (Advanced users)

3

21.1 q21.2 q21.3 q22.1 q22.3 q23.2 q24.1 q24.3 q31.1 q3

1,543 bp

23,859,400 bp 23,859,600 bp 23,859,800 bp

MYH6

AM_PGRI0310138_7429

MYH6

4

Total count: 18

A: 0

C: 0

G: 18 (100%, 9+, 9-)

T: 0

N: 0

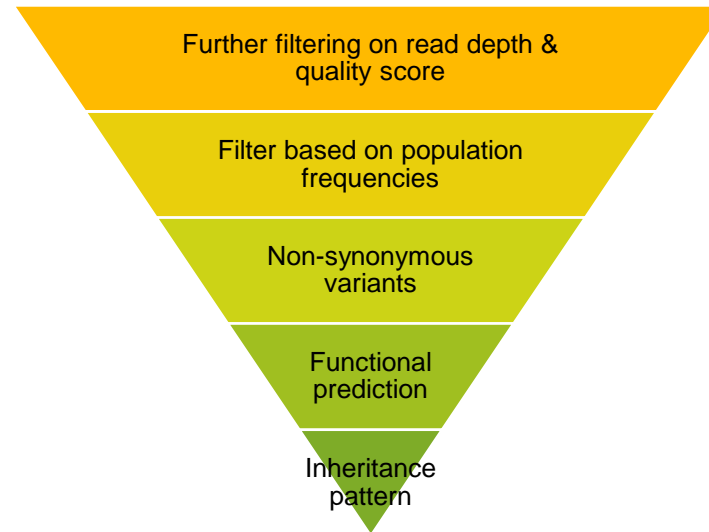
Analyzable target region

Amplicons

Exons Below Threshold

Use the Mutation Table to do the following

1. Filter mutations
2. Link out to databases: OMIM, GeneCard, dbVar, NCBI, Ensembl, Uniprot
3. Select transcript
4. Change categorization
5. Add notes
6. Suppress mutations



1

Filters Applied: None Clear Filters Q- myh

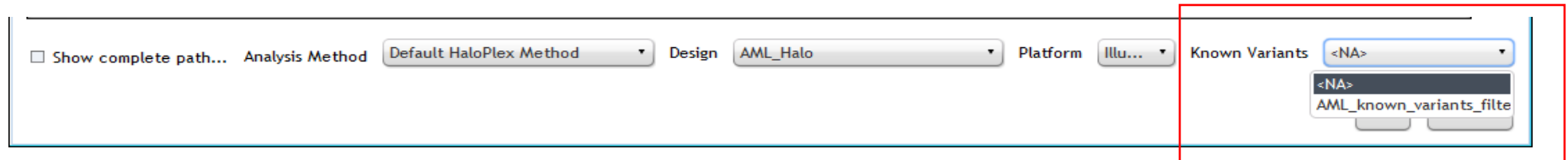
Result	6	5	4										
Suppress	Notes	Chrom	Category	Pos	ID	Ref Allele	Alt Allele	Quality	Allele Fre...	HGVS(Genomic)	HGVS(Coding)		
<input type="checkbox"/>		14	Category IV	23857097	rs74039310		T	255.0	0.415		NR_047545.1:n.749T>C		
<input type="checkbox"/>		14	Category III	23888494	rs45501694		C	255.0	0.545	NC_000014.8:g.2388...	NR_047545.1:n.749T>C		
<input type="checkbox"/>		14	Category III	23899027	rs735711	C	T	255.0	0.425	NC_000014.8:g.2389...	NR_047544.1:n.1502T>C NM_001257374.1:c.525T>C NM_005572.3:c.861T>C NM_170707.3:c.861T>C NM_170708.3:c.861T>C		

2

3

Perform 'known variant' analysis – NEW

- Known variant list selected during analysis set up



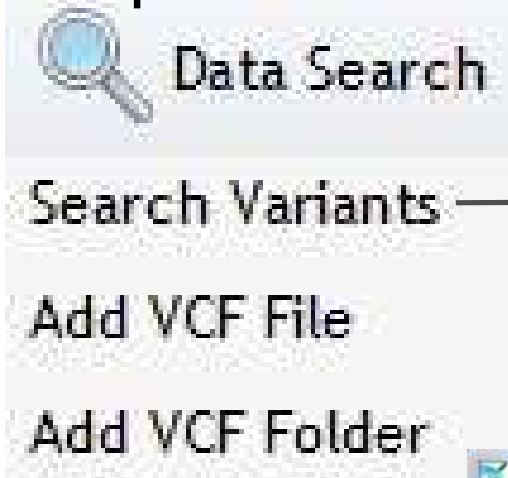
- Known variants found in sample will be highlighted in results table

Suppress	Notes	Category	P-Value	Gene	HGVS(Ge...)	HGVS(Co...)	HGVS(Pro...)	ID	Chrom	Pos	Ref Allele	Alt Allele	Quality
<input type="checkbox"/>		Category III	1.00E-255	NRAS	NC_000001.10:g...	NM_002524.4:c...		rs969273	1	115256669	G	A	255.0
<input type="checkbox"/>		Category III	1.00E-255	TET2	NC_000004.11:g...	NM_001127208....		rs2647243	4	106196092	C	T	255.0
<input type="checkbox"/>		Category II	1.00E-255	TET2	NC_000004.11:g...	NM_001127208....	NP_001120680....	rs2454206	4	106196951	A	G	255.0
<input type="checkbox"/>		Category III	1.00E-255	NPM1		NM_199185			5	170837513	CTT	TT	255.0
<input type="checkbox"/>		Category III	1.00E-255	FLT3	NC_000013.10:g...	NM_004119.2:c...		rs17086226	13	28592546	T	C	255.0

- Known Variant table lists reason for not finding variants i.e. low coverage, filtered, not significant

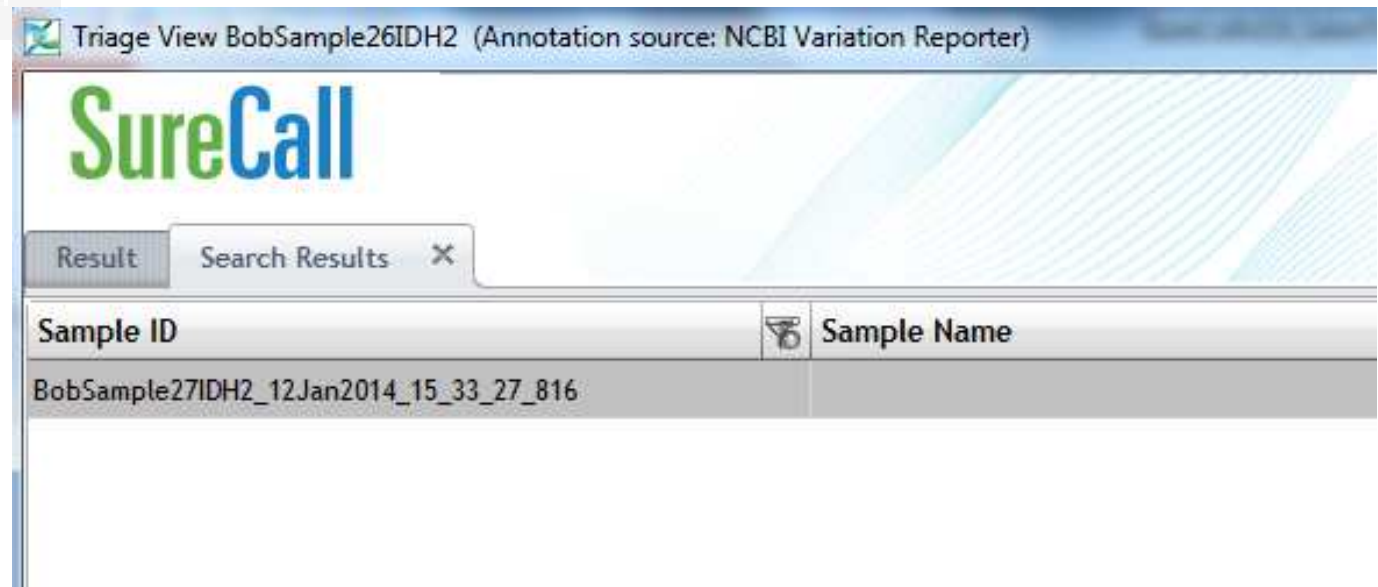
Known Variants
Observation Details
FILTERED_ON_3_PRIME_DIST
FILTERED_ON_3_PRIME_DIST
FILTERED_ON_3_PRIME_DIST
NOT_CALLED_SIGNIFICANT
FILTERED_ON_READ_DEPTH

Use the Database Search functionality to find other samples with the same variants



Find other samples containing the same mutation(s) stored in the system

List of samples with same variants will show up in Search Results



Generate Reports

Variant report & Known variant report

QC report

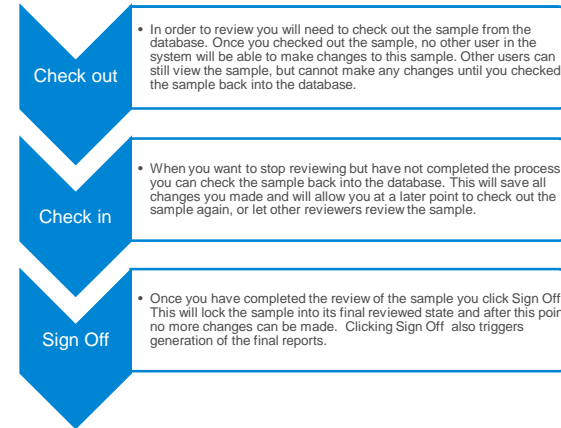
Make your own report

Export Table

Export Viewer Snapshot

Other important features

- Check out, check in, sign off of samples



- Audit trail
- User roles: technician, scientist, administrator



Role	Capabilities
Technician	<ul style="list-style-type: none"> • Run analysis workflows • Add sample information • Monitor workflow jobs • Triage samples <ul style="list-style-type: none"> ◦ Check in/out samples ◦ Add notes ◦ Suppress mutations ◦ Change category assigned to mutations ◦ Compare mutations across samples
Scientist	Technician tasks, plus: <ul style="list-style-type: none"> • Configure analysis methods • Configure categorizations • Sign-off results and generate reports • Unlock results
Administrator	Complete system access, including all Technician and Scientist tasks, plus: <ul style="list-style-type: none"> • Add users and roles • Change database connection settings for client systems

Demo...

