Analysis with SureCall 2.1
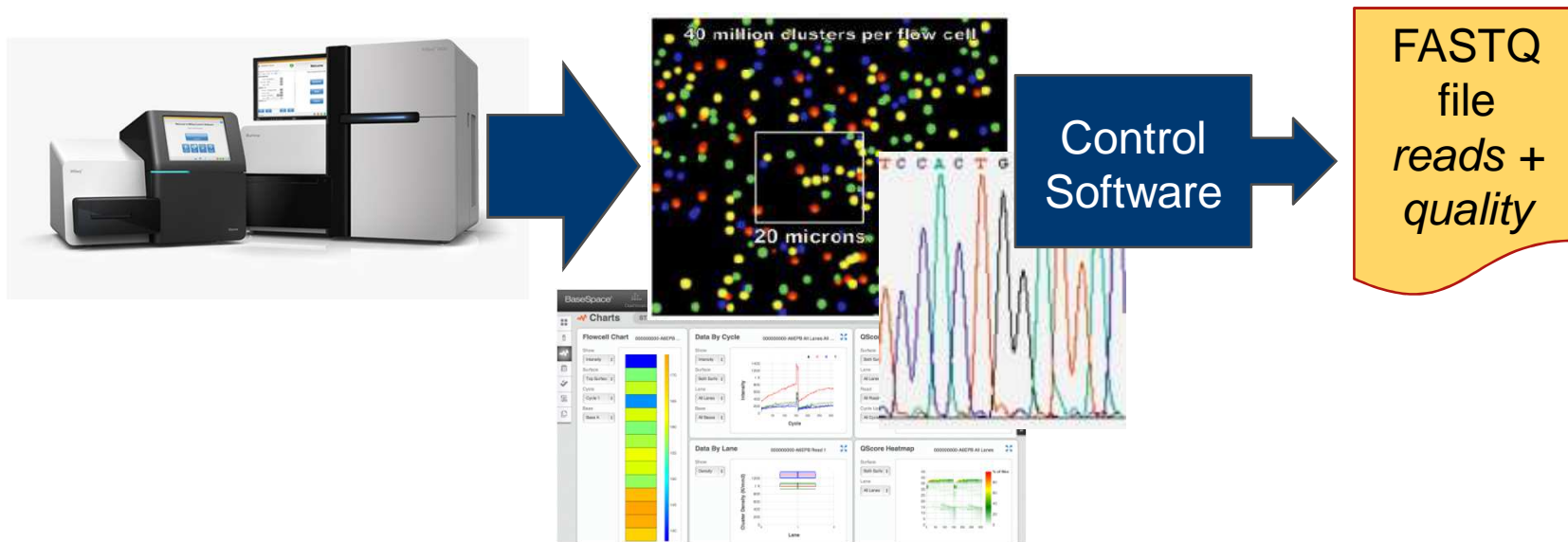
Danielle Fletcher

Field Application Scientist

July 2014
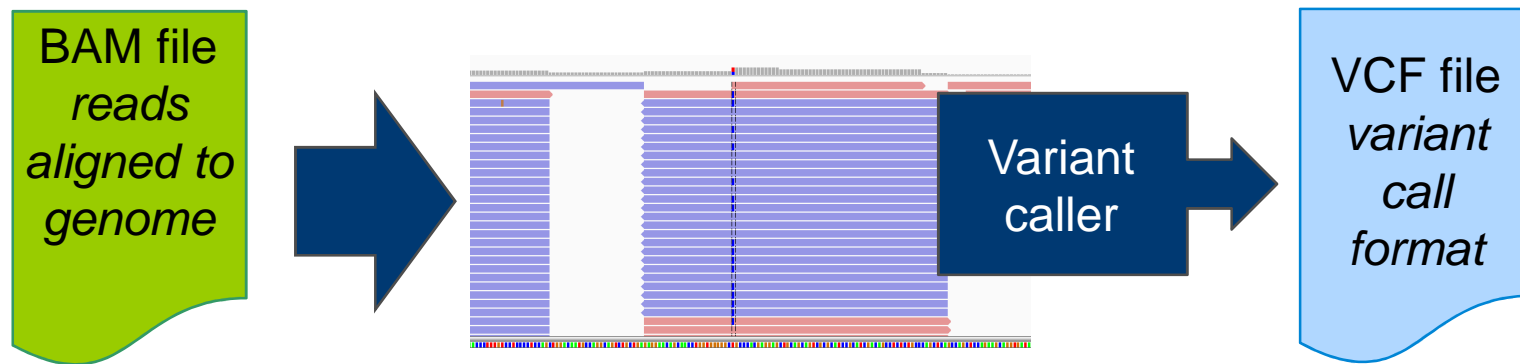
Stages of NGS Analysis –
Primary analysis, base calling
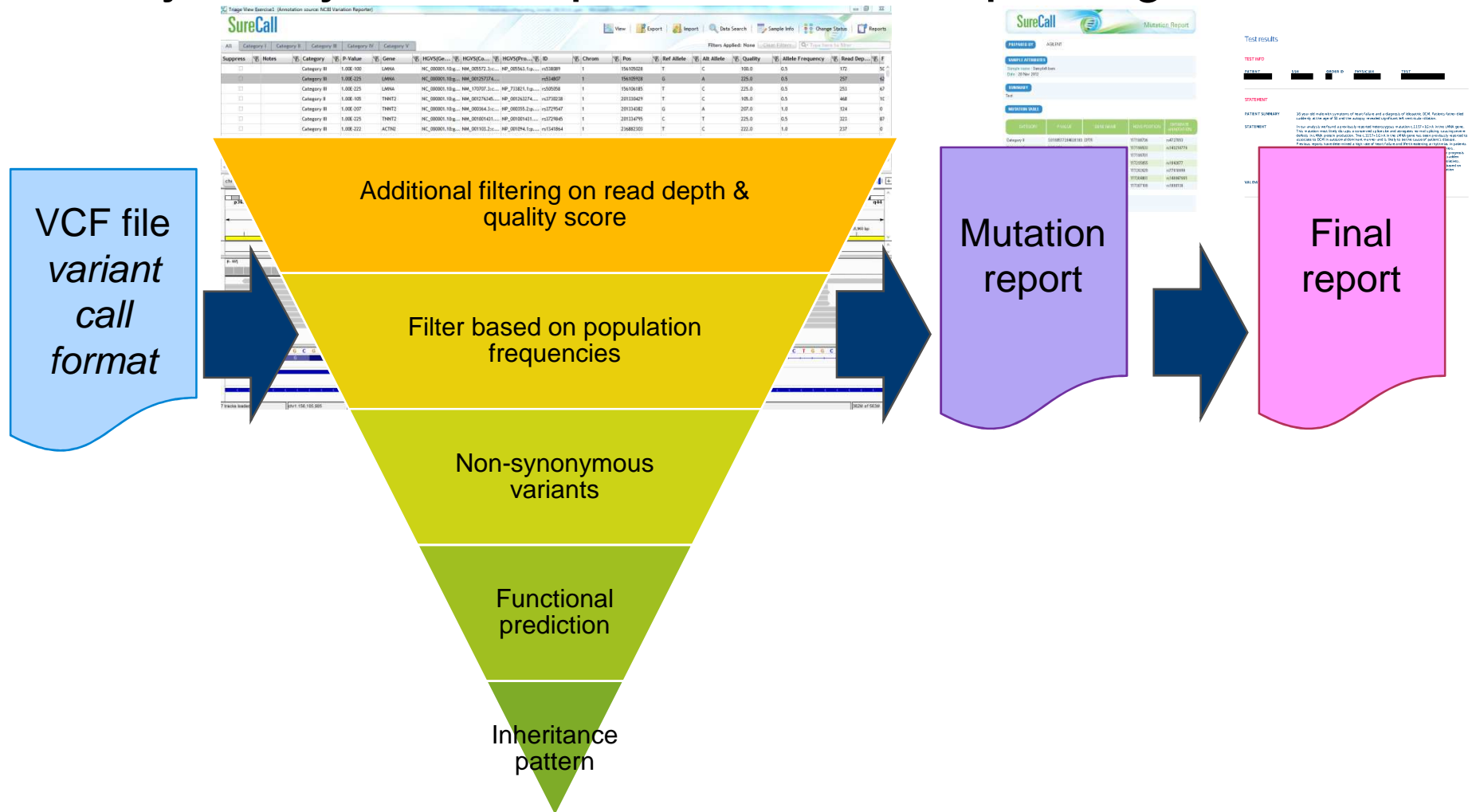
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Stages of NGS Analysis – Secondary analysis part 1, read alignment

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

```
┌─────────────┐           ┌──────────────┐           ┌─────────────┐
│  BAM file   │           │              │           │  VCF file   │
│   reads     │  ──────▶  │   Variant    │  ──────▶  │   variant   │
│ aligned to  │           │   caller     │           │    call     │
│   genome    │           │              │           │   format    │
└─────────────┘           └──────────────┘           └─────────────┘
```

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Stages of NGS Analysis –
Tertiary analysis, interpretation and reporting

VCF file *variant call format*

Additional filtering on read depth & quality score

Filter based on population frequencies

Non-synonymous variants

Functional prediction

Inheritance pattern

Mutation report

Final report

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SureCall – from alignment to mutation report
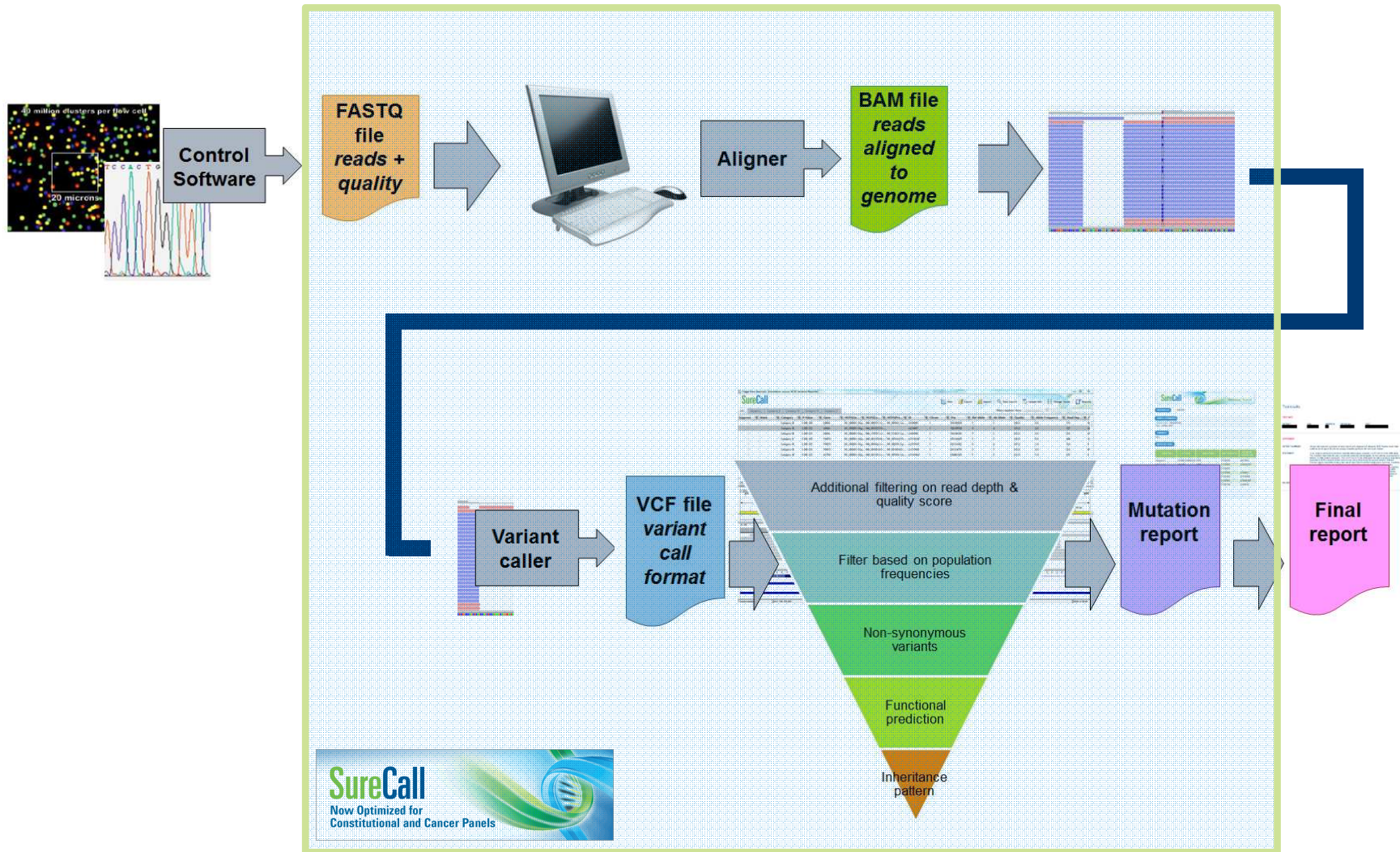
Agilent SureCall Software
Resolves data analysis bottleneck

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

SureCall makes NGS data analysis easy, fast, cheap

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Choose one of the three analysis types available in SureCall

Single Sample Analysis

Pair Analysis

Trio Analysis

	Description	Result
Single Sample Analysis	• For individual samples	• SNPs and indels
Pair Analysis	• To determine copy number changes (use a normal reference). • To determine somatic mutations in tumor-normal samples	• SNPs and indels • CNVs • Somatic mutations
Trio Analysis	• For trios, typically mother, father and child	• SNPs and indels • De novo mutations

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

```
☐ 📁 Single Sample
    📄 Default HaloPlex Method
    📄 Default SureSelect Method
    📄 Default SureSelect QXT Method
☐ 📁 Pair
    📄 Default HaloPlex Tumor Normal Method
    📄 Default SureSelect Tumor Normal Method
    📄 Default SureSelect QXT Tumor Normal Method
    📄 Default HaloPlex Copy Number Method
    📄 Default SureSelect Copy Number Method
    📄 Default SureSelect QXT Copy Number Method
☐ 📁 Trio
    📄 Default HaloPlex Trio Method
    📄 Default SureSelect Trio Method
    📄 Default SureSelect QXT Trio Method
```

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

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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

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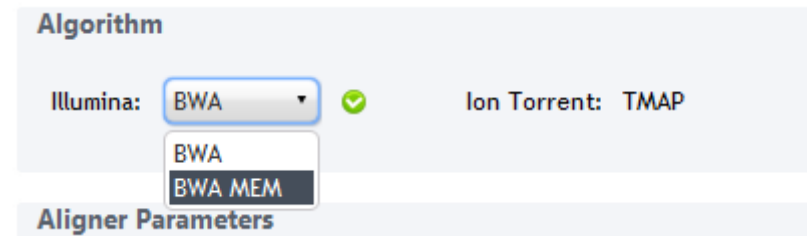# Five examples when to use your own settings

Example 1: Only analyze a subset of regions from the original design
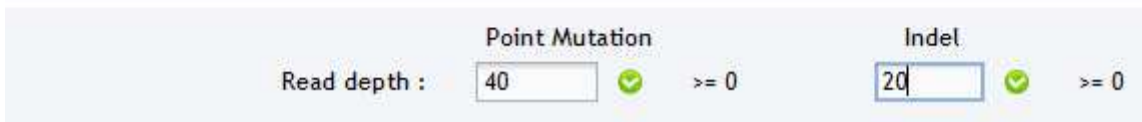
Example 2: Filter mutations on read depth

Example 3: Choose a different aligner

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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: ▼ !

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Example 5: Change category names and supporting evidence

Follow the simple 3-step Analysis Workflow

1. Import Samples

2. Describe Samples

3. Run Analysis

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

mutation table

genome viewer

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Use the Genome Viewer to review the variants
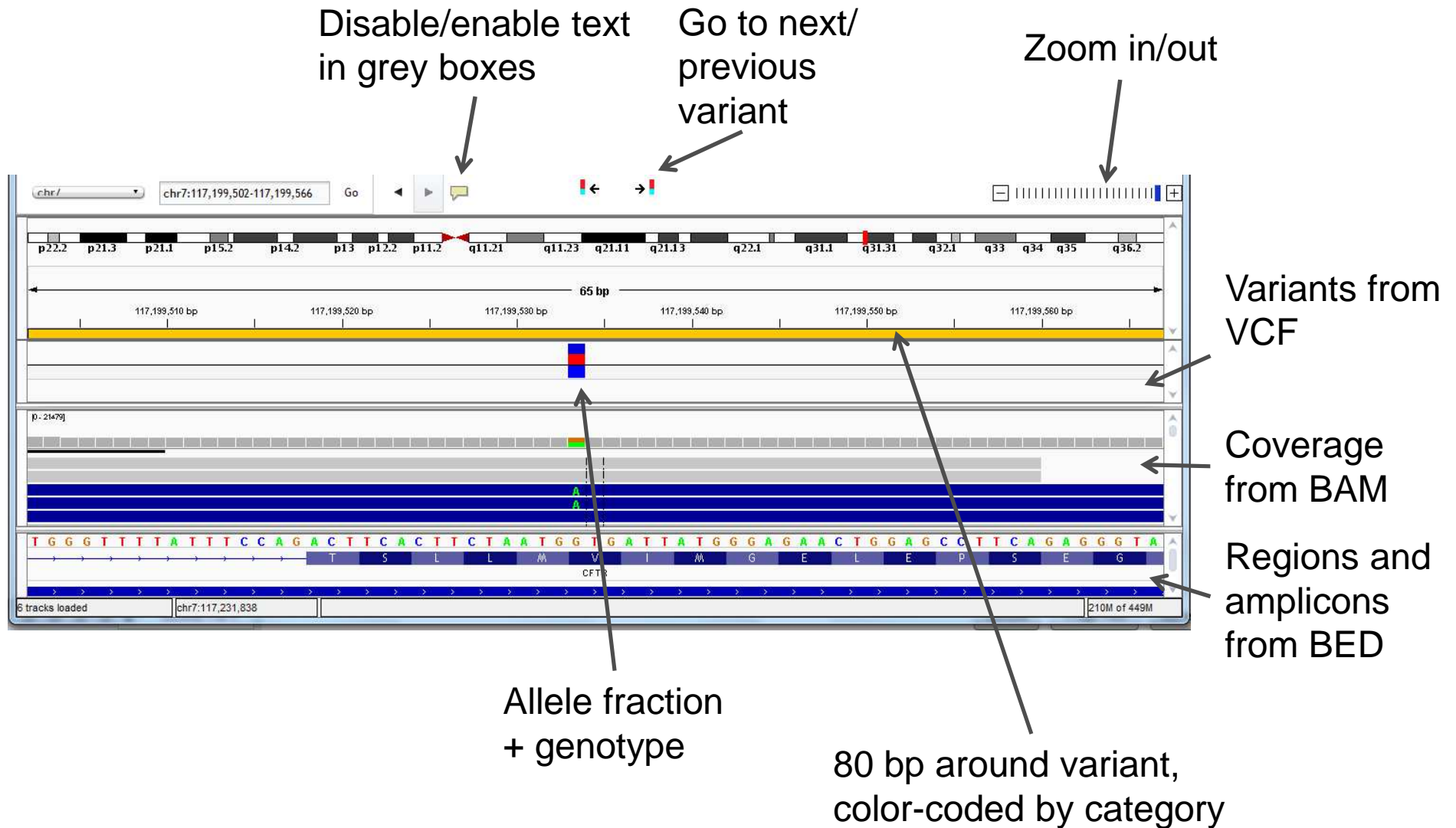
Display regions and exons without sufficient read depth

Analyzable target region
Amplicons
Exons Below Threshold

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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

Further filtering on read depth & quality score

Filter based on population frequencies

Non-synonymous variants

Functional prediction

Inheritance pattern
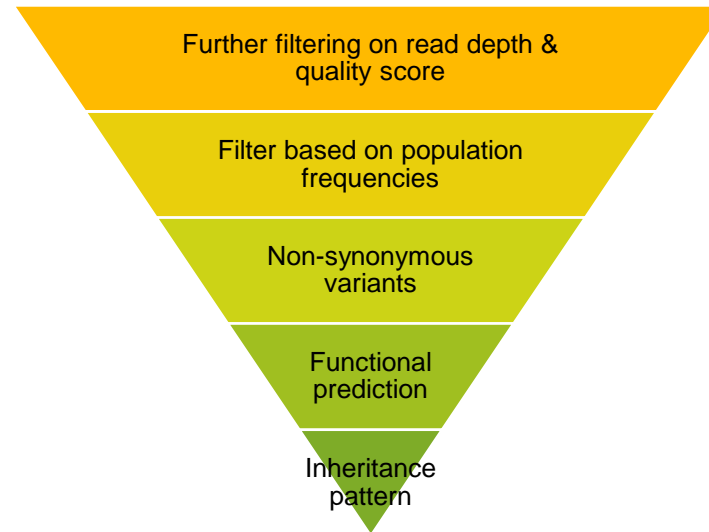
1 Filters Applied: None   Clear Filters   Q~ myh

Result	6		5	4									3
Suppress	🌂	Notes	🌂	Chrom	🌂	Category	🌂	Pos	🌂	ID	🌂	Ref Allele	🌂	Alt Allele	🌂	Quality	🌂	Allele Fre...	🌂	HGVS(Genomic)	🌂	HGVS(Coding)	🌂

Suppress	Notes	Chrom	Category	Pos	ID	Ref Allele	Alt Allele	Quality	Allele Fre...	HGVS(Genomic)	HGVS(Coding)
☐		14	Category IV	23857097	rs74039310		T	255.0	0.415		NR_047545.1:n.749T>C
☐		14	Category III	23888494	rs45501694		C	255.0	0.545	NC_000014.8:g.2388...	NR_047545.1:n.749T>C
☐		14	Category III	23899027	rs735711	C	T	255.0	0.425	NC_000014.8:g.2389...	NR_047544.1:n.1502T>C

2

NM_001257374.1:c.525T>C
NM_005572.3:c.861T>C
NM_170707.3:c.861T>C
NM_170708.3:c.861T>C

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Perform 'known variant' analysis – NEW

- Known variant list selected during analysis set up
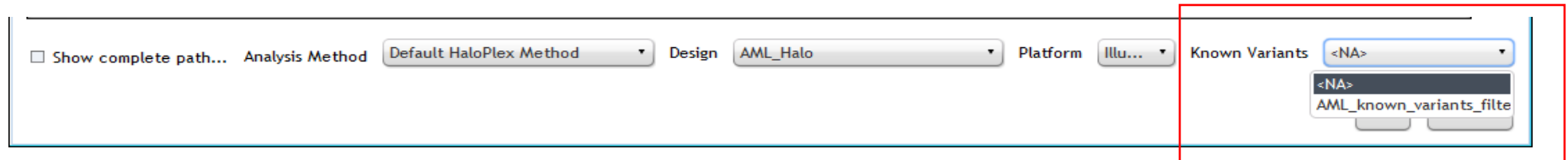
- Known variants found in sample will be highlighted in results table

Suppress	Notes	Category	P-Value	Gene	HGVS(Ge...	HGVS(Co...	HGVS(Pro...	ID	Chrom	Pos	Ref Allele	Alt Allele	Quality
☐		Category III	1.00E-255	NRAS	NC_000001.10:g...	NM_002524.4:c...		rs969273	1	115256669	G	A	255.0
☐		Category III	1.00E-255	TET2	NC_000004.11:g...	NM_001127208....		rs2647243	4	106196092	C	T	255.0
☐		Category II	1.00E-255	TET2	NC_000004.11:g...	NM_001127208....	NP_001120680....	rs2454206	4	106196951	A	G	255.0
☐		Category III	1.00E-255	NPM1		NM_199185			5	170837513	CTT	TT	255.0
☐		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

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

Data Search
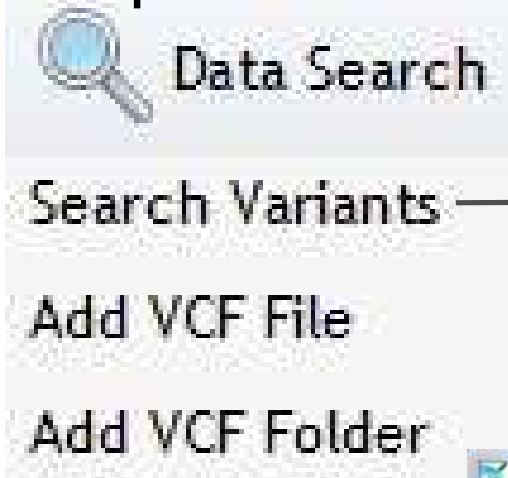
Search Variants →

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

Add VCF File

Add VCF Folder

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

Triage View BobSample26IDH2 (Annotation source: NCBI Variation Reporter)

SureCall

| Result | Search Results | ✕ |

Sample ID		Sample Name
BobSample27IDH2_12Jan2014_15_33_27_816		

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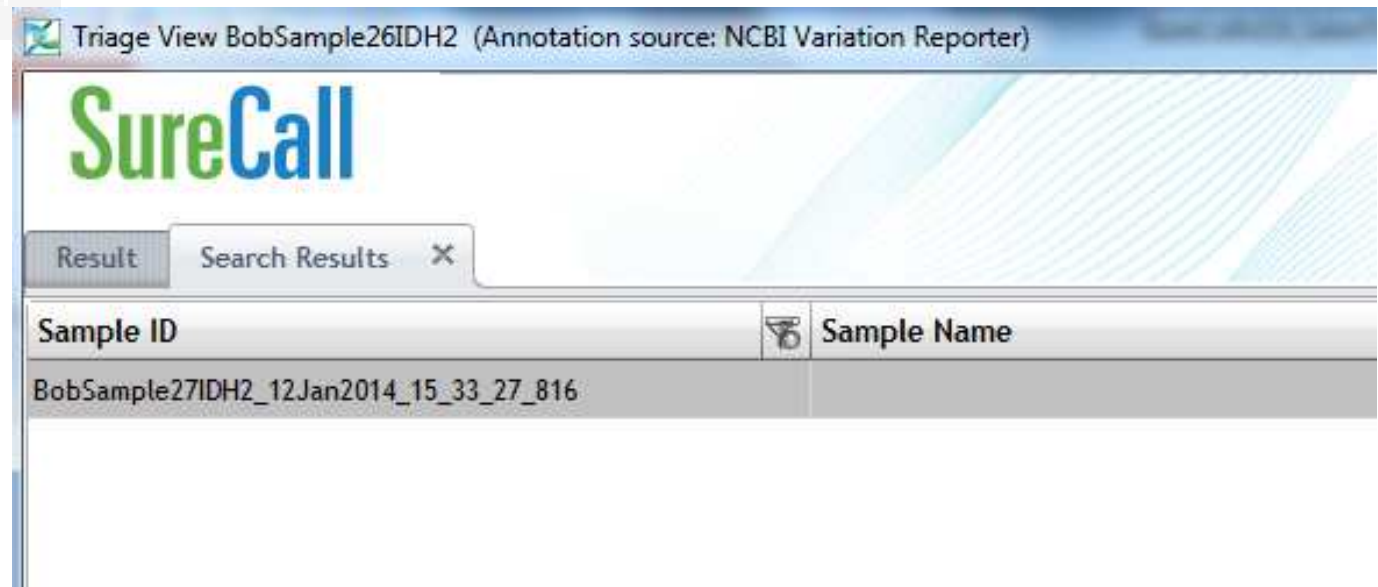
Generate Reports

Variant report & Known variant report

QC report

Make your own report

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Other important features

- Check out, check in, sign off of samples

Check out	• In order to review you will need to check out the sample from the database. Once you checked out the sample, no other user in the system will be able to make changes to this sample. Other users can still view the sample, but cannot make any changes until you checked the sample back into the database.
Check in	• When you want to stop reviewing but have not completed the process you can check the sample back into the database. This will save all changes you made and will allow you at a later point to check out the sample again, or let other reviewers review the sample.
Sign Off	• Once you have completed the review of the sample you click Sign Off. This will lock the sample into its final reviewed state and after this point no more changes can be made. Clicking Sign Off also triggers generation of the final reports.

- Audit trail

Audit Trail

2012-11-01 22:17:03 : AGILENT\adewitte has checked out the record
2012-11-01 22:17:05 : AGILENT\adewitte has suppressed the CHROM7:117120049

- User roles: technician, scientist, administrator

Role	Capabilities

Technician	• Run analysis workflows
	• Add sample information
	• Monitor workflow jobs
	• Triage samples
	  ◦ Check in/out samples
	  ◦ Add notes
	  ◦ Suppress mutations
	  ◦ Change category assigned to mutations
	  ◦ Compare mutations across samples

Scientist	Technician tasks, plus: • Configure analysis methods • Configure categorizations • Sign-off results and generate reports • Unlock results
Administrator	Complete system access, including all Technician and Scientist tasks, plus: • Add users and roles • Change database connection settings for client systems

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Demo…

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