

# Evaluation of Four Automated Mutation Detection Programs for Clinical Re-sequencing

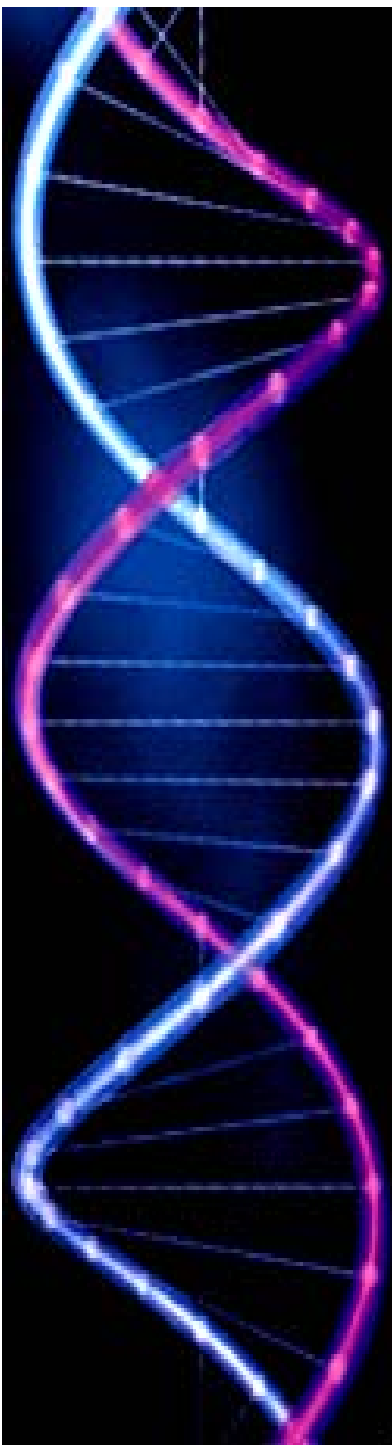
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Stollery Children's Hospital



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**Objective:** To evaluate all commercially available automated mutation detection software for use in the diagnostic testing of large genes.

## Study Workflow

15 Qualitative Traits

Ability to flag 114 variants

Ability to name 114 variants

Sensitivity & specificity

Results from 125 BRCA patients



## Method of Variant Detection

### Mutation Surveyor v3.25

-Uses anti-correlation method (compares a reference electropherogram to a test – indicates discordance)

### Variant v1.0/Seqscape v2.6

-uses base caller only

### Seqpilot v3.2.1.2

-variant detection done using base caller however, all peak morphology including noise are stored and used for statistical comparisons of later peaks

# Qualitative Aspects

	Variant Reporter	Seqscape	Seqpilot	Mutation Surveyor
Ease of template creation	X	X	√	√
Ability to lock template	X	√	X	X
Ease of sequence assembly	√	√	√	X
Able to auto-import directly from CE	X	√	√	√
Ability to create ROI	√	√	√	√
Audit trail capability	X	√	√	X
Visualization of 2X coverage of ROI	X	√	√	X
Measurement for seq. quality	√	√	X	√
Record variant type	X	√	√	√
Attach variant information (ie. PDF)	X	X	√	X
Program can run on a server	X	X	√	√
Variants named using HGVS*	W	W	√	√
Able to de-convolve frameshifts	X	X	√	√
Automatic variant detection on de-convolved fragments	X	X	X	X
Overall user friendliness^	3	2	1	4

\* W indicates workaround possible

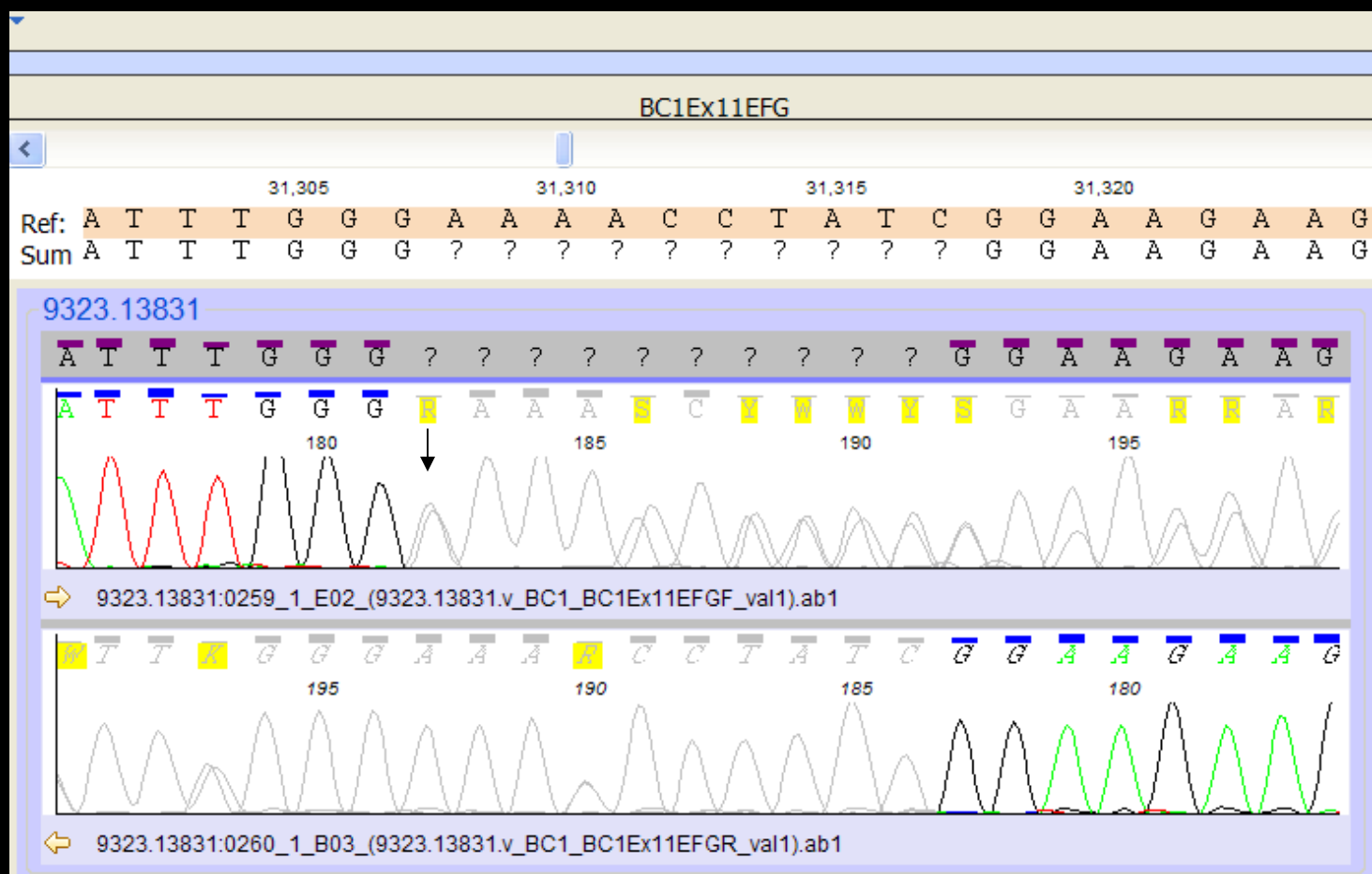
^ 1 being the most user friendly

## Ability to 'Flag' 114 Variants

	Point Mutation (76)	Frameshift (36)	Complex Change (2)
Variant Reporter	99% (75)	85% (31)	100%(2)
Seqscape	99% (75)	94% (34)	100% (2)
Seqpilot	100% (76)	97% (35)	100% (2)
Mutation Surveyor	100% (76)	100% (36)	100% (2)

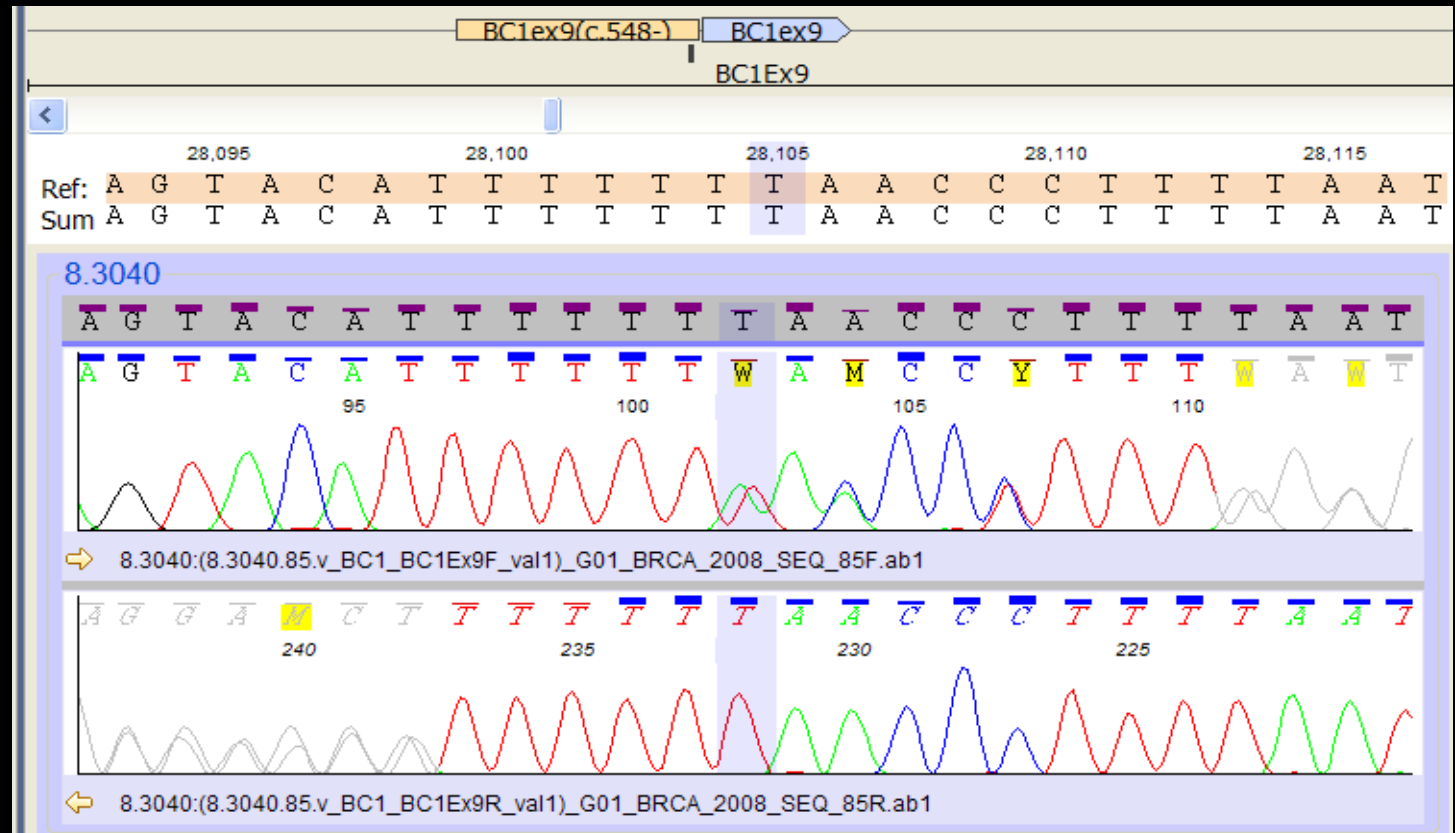
- variants must be listed in 'variant' table (or 'possible heterozygous indel mutation' table for seqscape)
- complex changes included: BRCA2 c.10095delCins11 & MSH6 c.866GC>AA
- Note: one frameshift mutation not detected by seqpilot, variant reporter and seqpilot was present only on one allele

## Variant Reporter – Examples of missed point mutation & indel



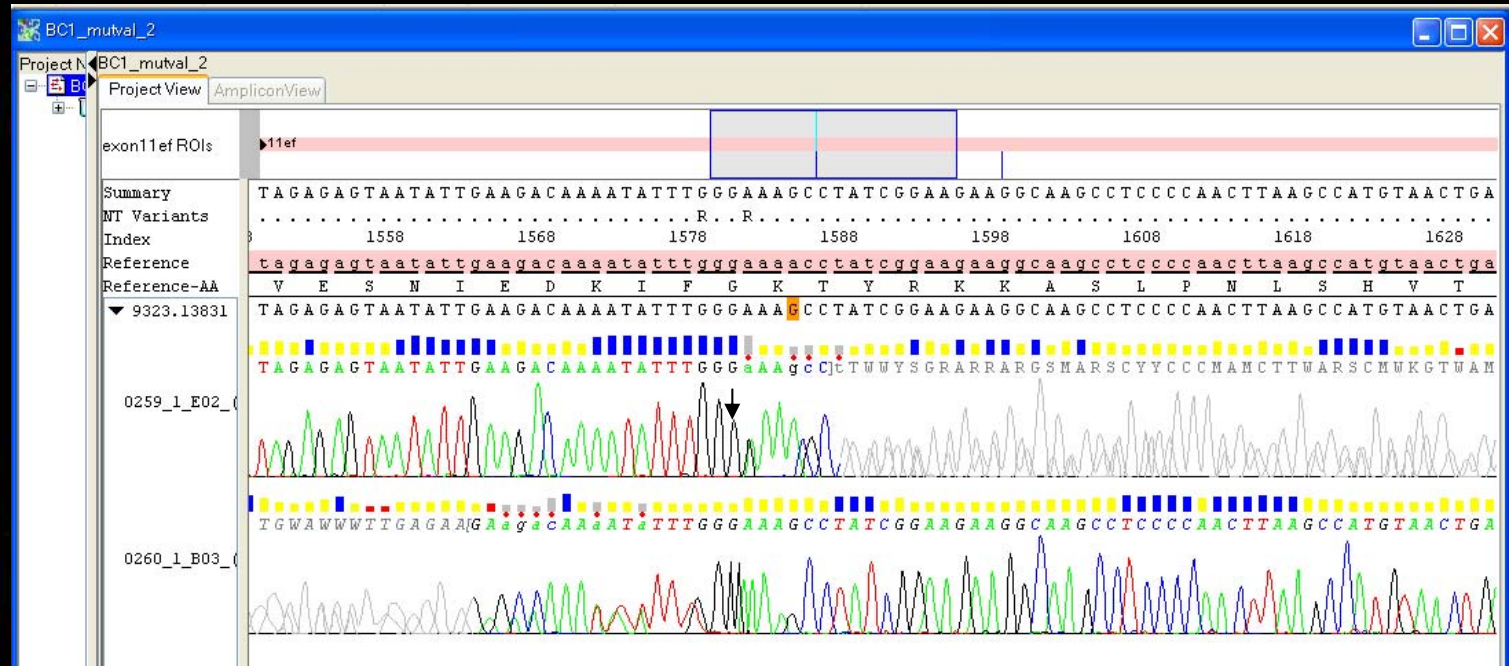
- BRCA1 c.1390\_1391insG (1/2) and c.1387A>G (1/2) or c.1386dupG & c.1390 A>G
- VR trims off the “low quality” sequence, leaves a gap, but does not document it in the variant table, or a “position of interest”.
- This variant is completely missed.

# Variant Reporter – Examples of missed indels



- BRCA1 c.548-58delT (1/2)
- VR needs filters removed in order to see this mutation, and it does not call it even if both sequences are assembled.
- It does show some of the mixed bases under the “position of interest” tab, but nothing is listed in the variant table

# Seqscape – Missed base substitution



BRCA1 Variant:

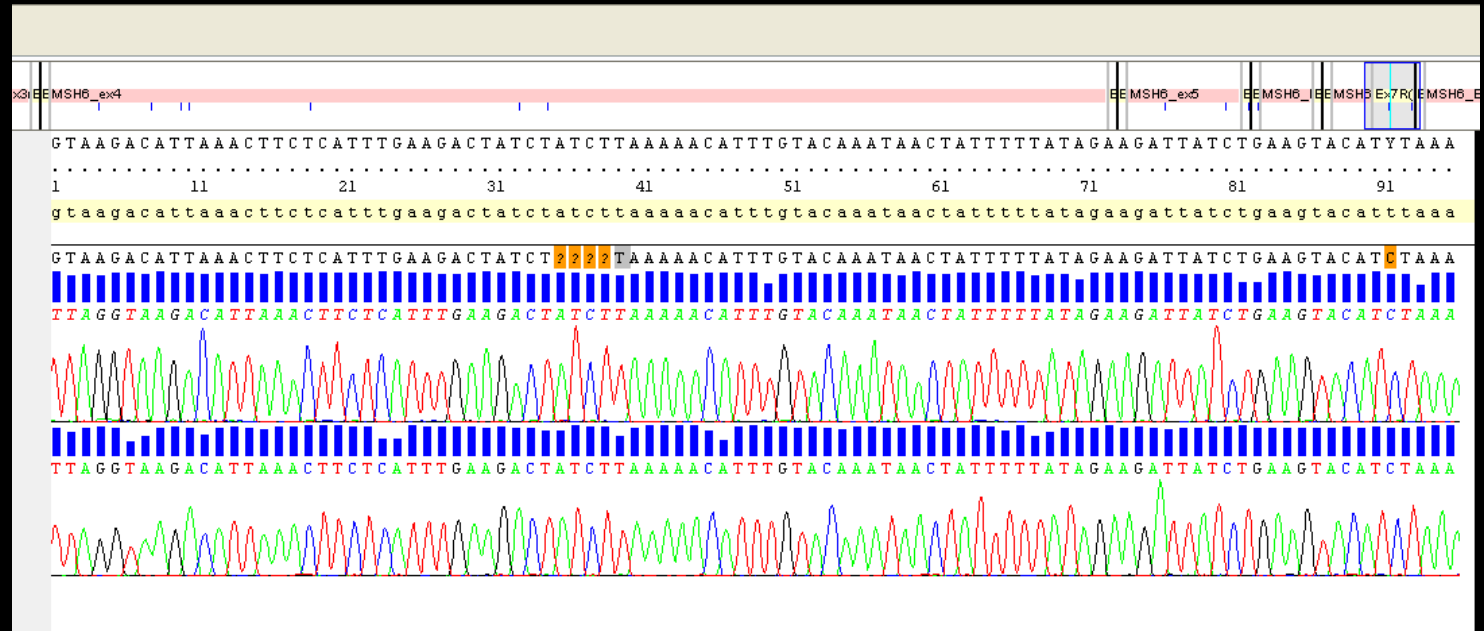
c.1386dupG & c.1390 A>G

or

c.1387 A>G & c.1391insG

- seqscape picked up indel but missed base substitution

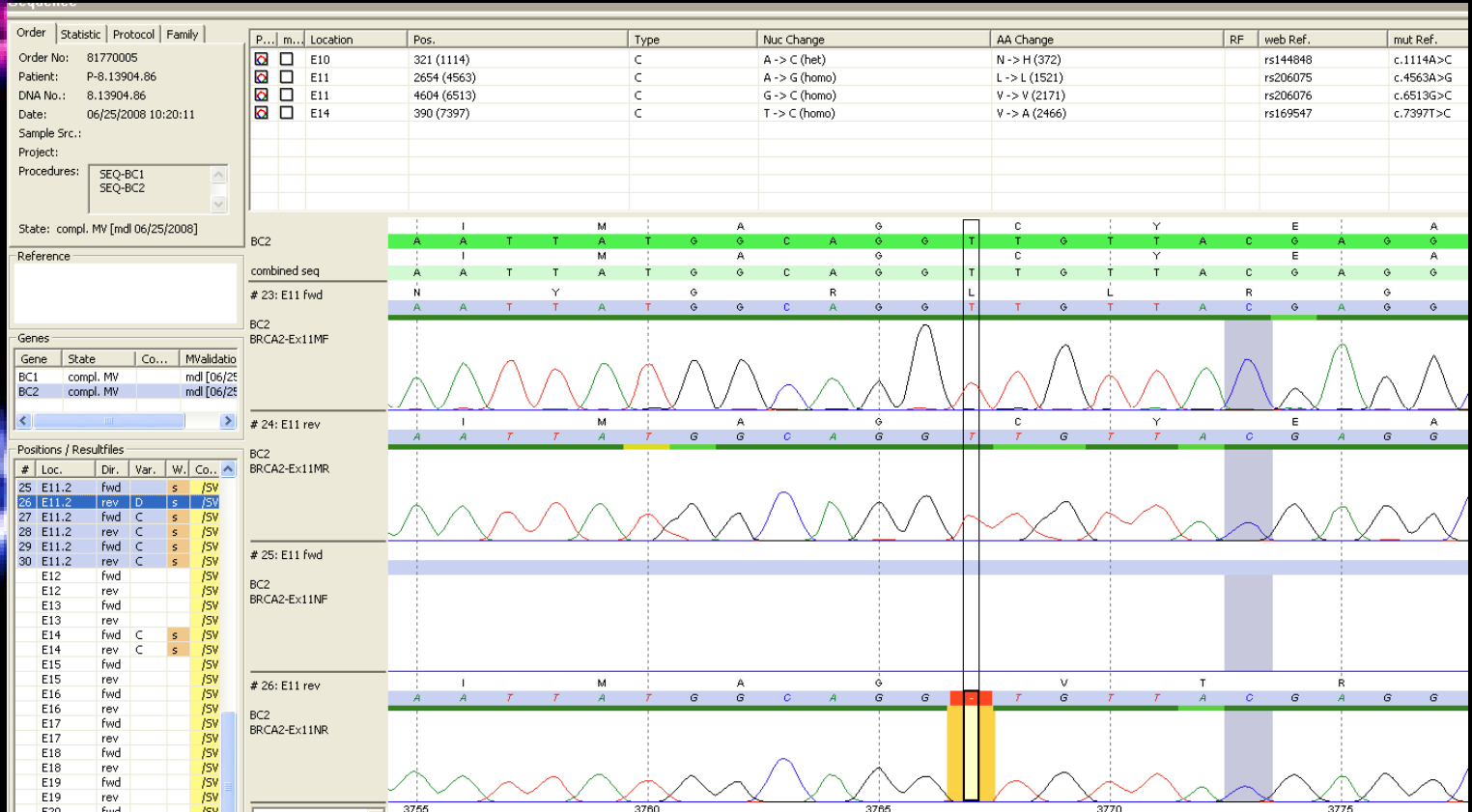
# Seqscape – Missed indel



MSH6 homozygous c.3646+35\_38delATCT

- not listed in either indel table or in mutation report; is manually searchable as a 'discrepancy'

# Seqpilot – Missed base substitution



- This variant was due to an error in one primer and therefore was present on only one allele; although not found in the variant table, it is flagged with a 'D' in the position/resultfiles window (left)

## Ability to Name (HGVS) 114 Variants

	<b>HGVS</b>	<b>Point Mutation</b>	<b>Frameshift</b>	<b>Complex Change</b>
<b>Variant Reporter</b>	<b>cDNA</b>	<b>100%</b>	<b>63%</b>	<b>0%</b>
	<b>protein</b>	<b>100%</b>	<b>0%</b>	<b>0%</b>
<b>Seqscape</b>	<b>cDNA</b>	<b>100%</b>	<b>56%</b>	<b>0%</b>
	<b>protein</b>	<b>100%</b>	<b>0%</b>	<b>0%</b>
<b>Seqpilot</b>	<b>cDNA</b>	<b>86%*</b>	<b>46%</b>	<b>0%</b>
	<b>protein</b>	<b>100%</b>	<b>0%</b>	<b>0%</b>
<b>Mutation Surveyor</b>	<b>cDNA</b>	<b>100%</b>	<b>91%</b>	<b>0%</b>
	<b>protein</b>	<b>100%</b>	<b>3.6%</b>	<b>0%</b>

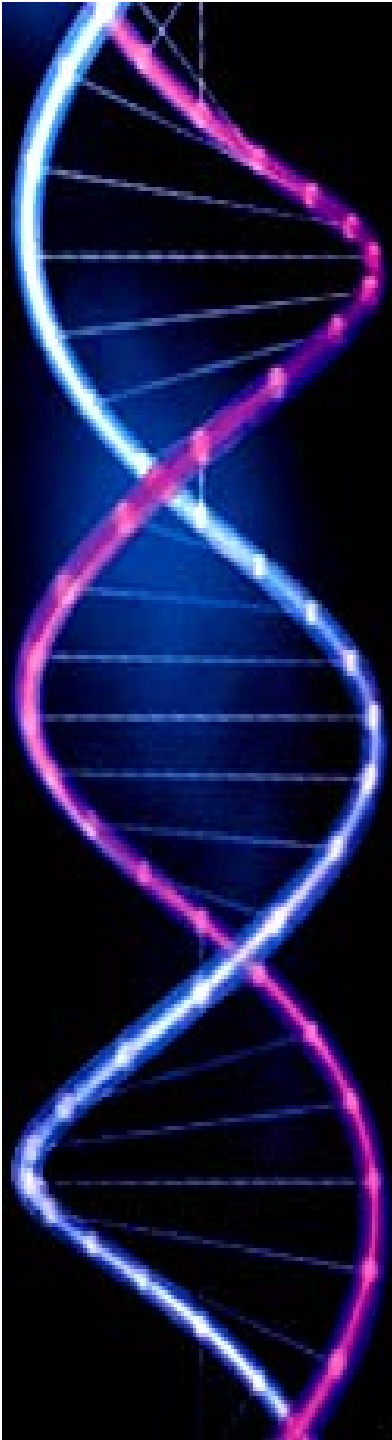
- \*Seqpilot point mutation calling incorrect for intronic mutations: “IVS” instead of referring to cDNA position



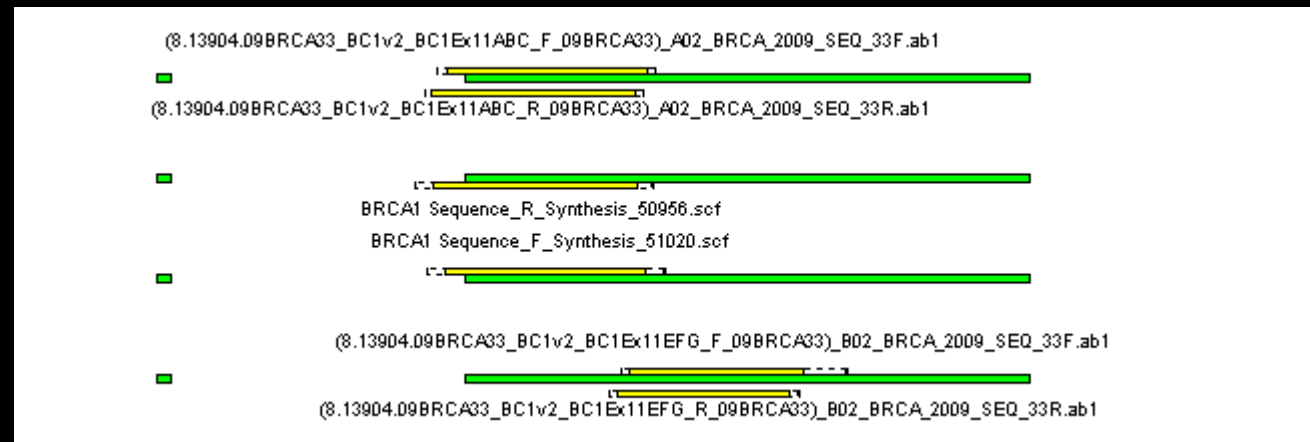
## Sensitivity and Specificity

	<b>Sensitivity</b>	<b>Specificity</b>
<b>Seqscape</b>	<b>100%</b>	<b>36%</b>
<b>Seqpilot (without Stats)</b>	<b>100%</b>	<b>59%</b>
<b>Seqpilot (with Stats)*</b>	<b>100%</b>	<b>73%</b>
<b>Mutation Surveyor</b>	<b>100%</b>	<b>76%</b>

\* Statistics included 80 analyzed patients



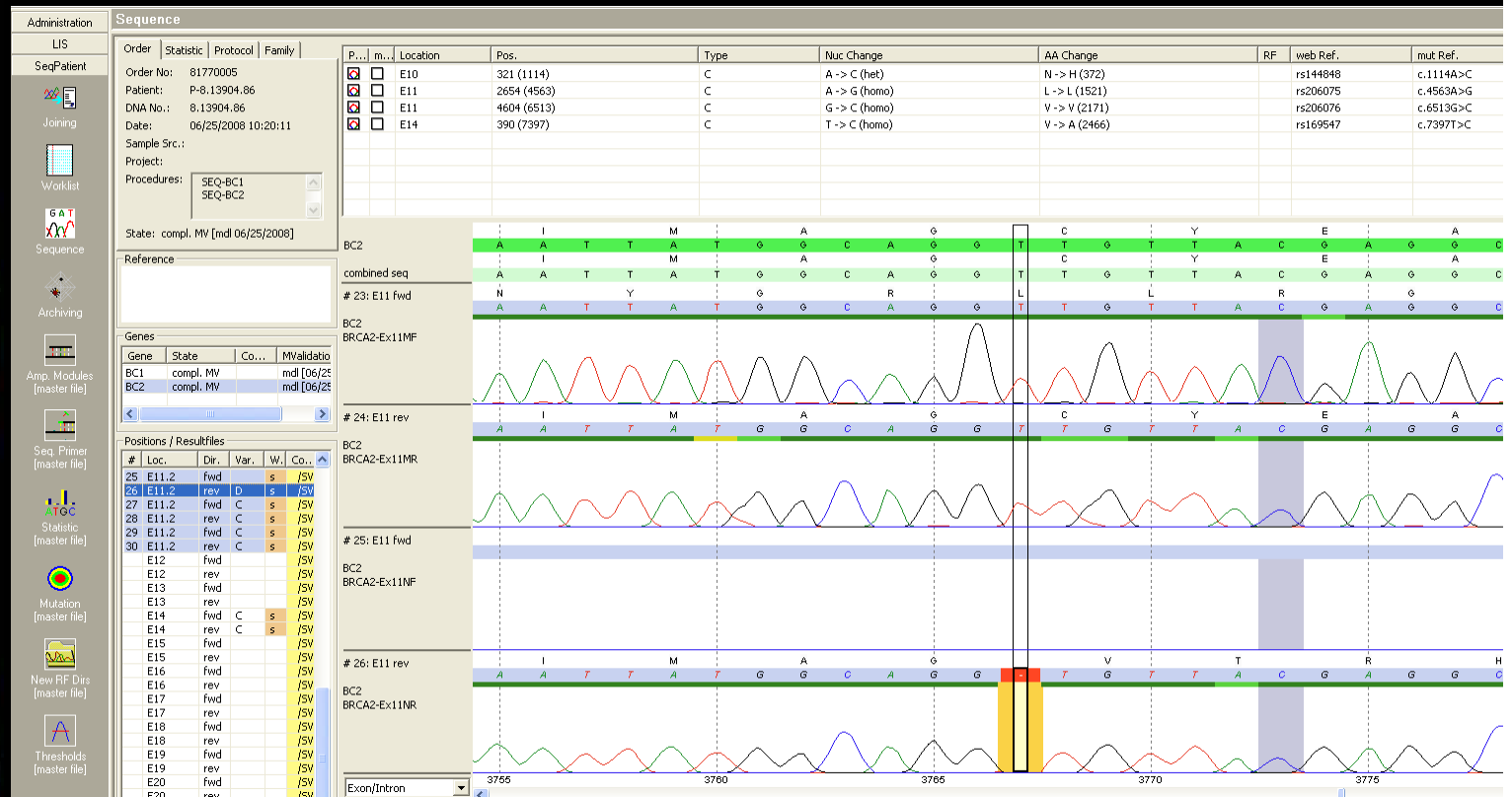
- decision not to use mutation surveyor for further validation studies
- mutation surveyor assembled BRCA1, but not BRCA2 into correct overlapping contigs therefore patients would have to be analyzed by amplicon (our workflow is patient centered not exon/amplicon centered)
- we found it difficult to determine graphically whether there was bi-directional overlap between contigs (however it can be done numerically)



- staff did not find this software to be user friendly (ie. familial mutations involve searching one cDNA position; difficult to determine with mutation surveyor)

# Final Phase of Evaluation

- 125 BRCA patients were analyzed in duplicate with seqscape and seqpilot: over 2 million bp sequenced in two directions



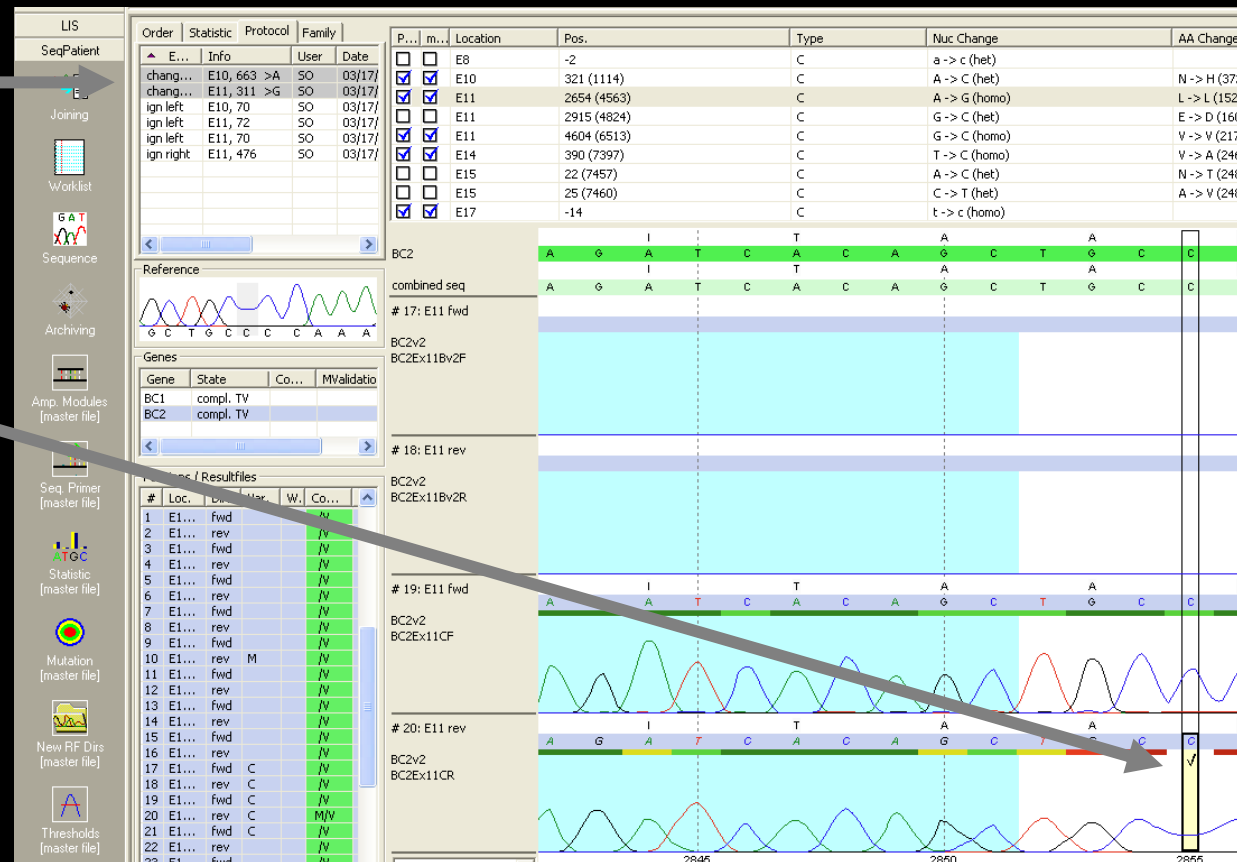
- BRCA2 primer error not found in variant table (noted as a 'D' in the positions/results files table); we questioned the potential for the software to miss homozygous deletions if spacing is adjusted differently on both strands



# Final Decision

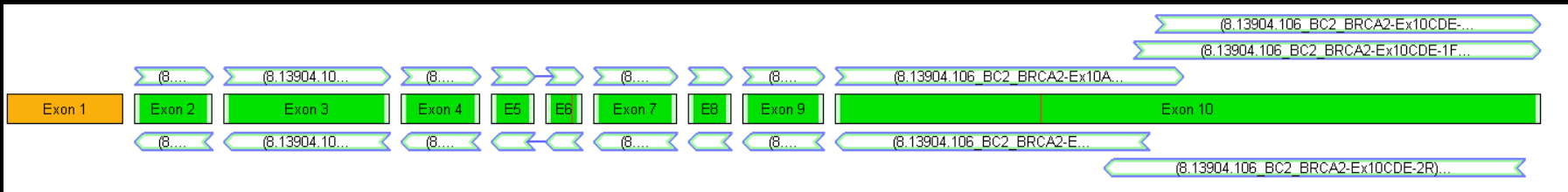
- final decision was to go with seqpilot – high sensitivity and specificity
- total time for either a technologist to analyze a BRCA1/2 patient or director to sign-off = 15 minutes
- has an audit trail important for clinical QA/QC:

Changes to base calls made by technologist are recorded and can be evaluated by lab director (using a single click to locate the position)



# Final Decision

- fairly easy to determine if bi-directional coverage was achieved



- available as a server version
- able to hyper-link data on variants in mutation database

**Mutation**

Info: E11, 3546 (5455), C, C -> T, P -> S (1819) Frequency: 0/2 of 159  
homo/het of total

Nuc Name: c.5455C>T AA Name: p.Pro1819Ser  show Color

Info Intern: Show  exist Info Extern: Show  exist Color:

Disease No:  Show Effect: UCV in BIC 24/02/09 SB Default Color:

Weblinks:

Link	Comment
Z:\BioInformatics\SeqPilot_MutDB\BRCA2\SIFT_BRCA2_P1819S.pdf	
Z:\BioInformatics\SeqPilot_MutDB\BRCA2\BRCA2_Polyphen_P1819S.pdf	
Z:\BioInformatics\SeqPilot_MutDB\BRCA2\AGVGD_BRCA2_P1819S.pdf	
http://research.nhgri.nih.gov/projects/bic/Member/cgi-bin/bic_query_result.cgi?table=brca2_exons&nt=5683&base_ch...	
Z:\BioInformatics\Lab_Papers\BRCA2\Spearman2008.pdf	classifies variant a

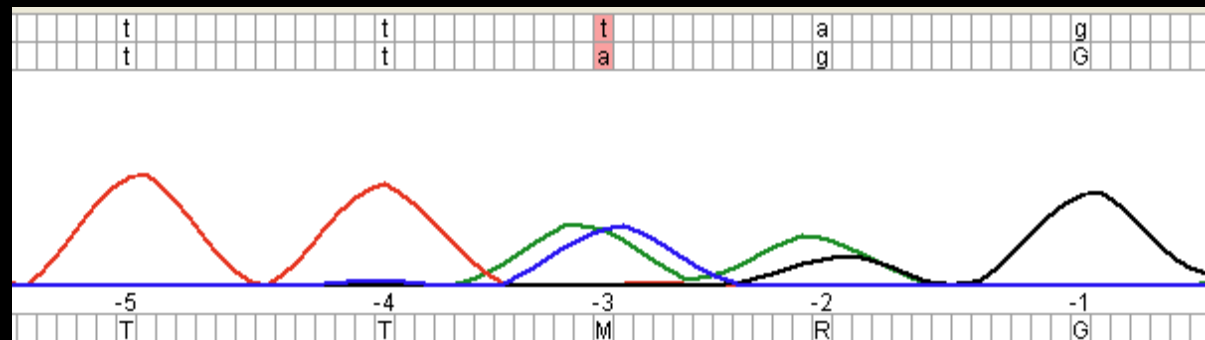
Add Remove

## Final Decision

- able to deconvolve heterozygous indels; also provides a semi-automated method for sequence evaluation of each allele after deconvolution

Red 'flag' indicates that the allele call does not match the electropherogram

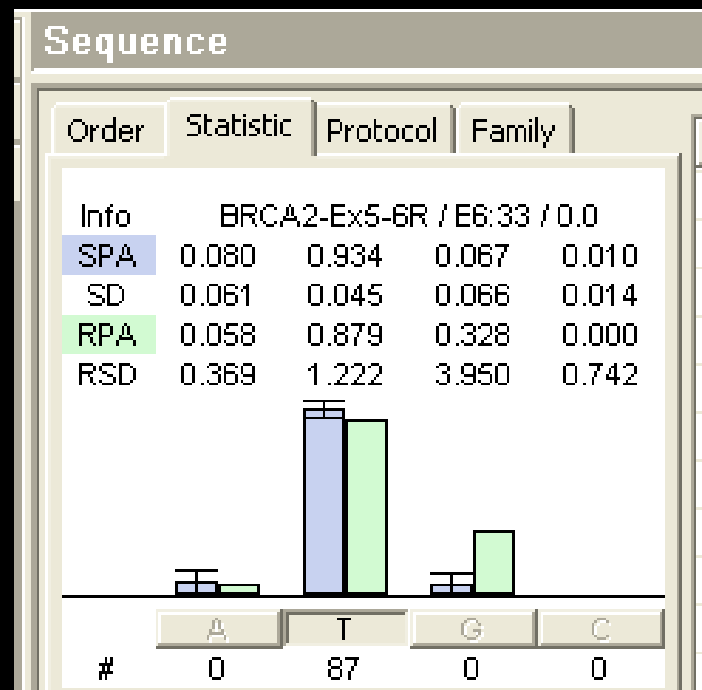
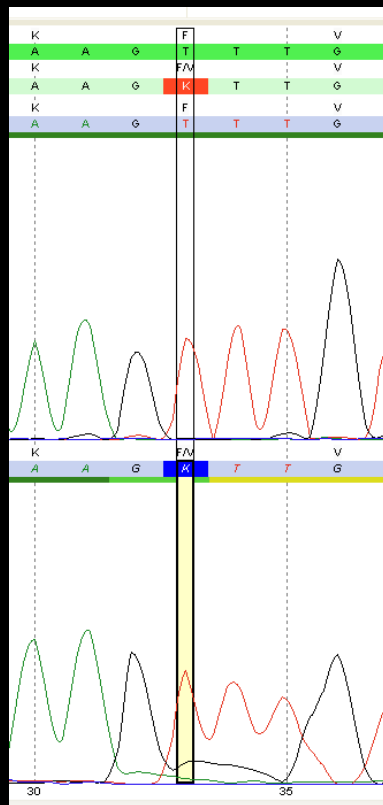
Expected call for each allele



- able to mark primer locations; important for large 'multi-amplicon' exons whereby the presence of SNPs might result in a null allele in a neighbouring amplicon

## Final Decision

- does not require a similar quality reference trace: will use peak statistics to evaluate similarity of current peak to previously analyzed peaks



Bars indicate peak area; blue is average of all previous peaks and includes std dev.; green is current peak area



## Acknowledgements

- **My entire lab and notably the individuals who performed most of the evaluation:**

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**Christine Walker**

**Mark Hicks**

- **JSI (Seqpilot), Softgenetics (Mutation Surveyor), ABI (Applied Biosystems)**
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- **Dr. Jillian Parboosingh – Associate Director Molecular Diagnostic Lab, Calgary, Alberta**