



MUTATION
Surveyor®
DNA Variant Analysis Software

Quick Start Guide

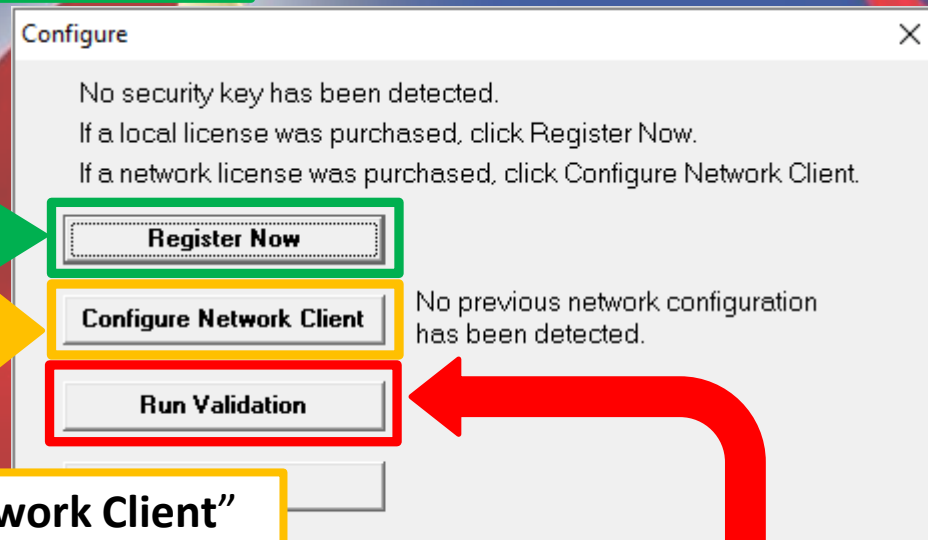
SOFTGENETICS®

Software PowerTools for Genetic Analysis

www.softgenetics.com / info@softgenetics.com

Opening Software for the First Time

Select "**Register Now**" to register your purchased local license



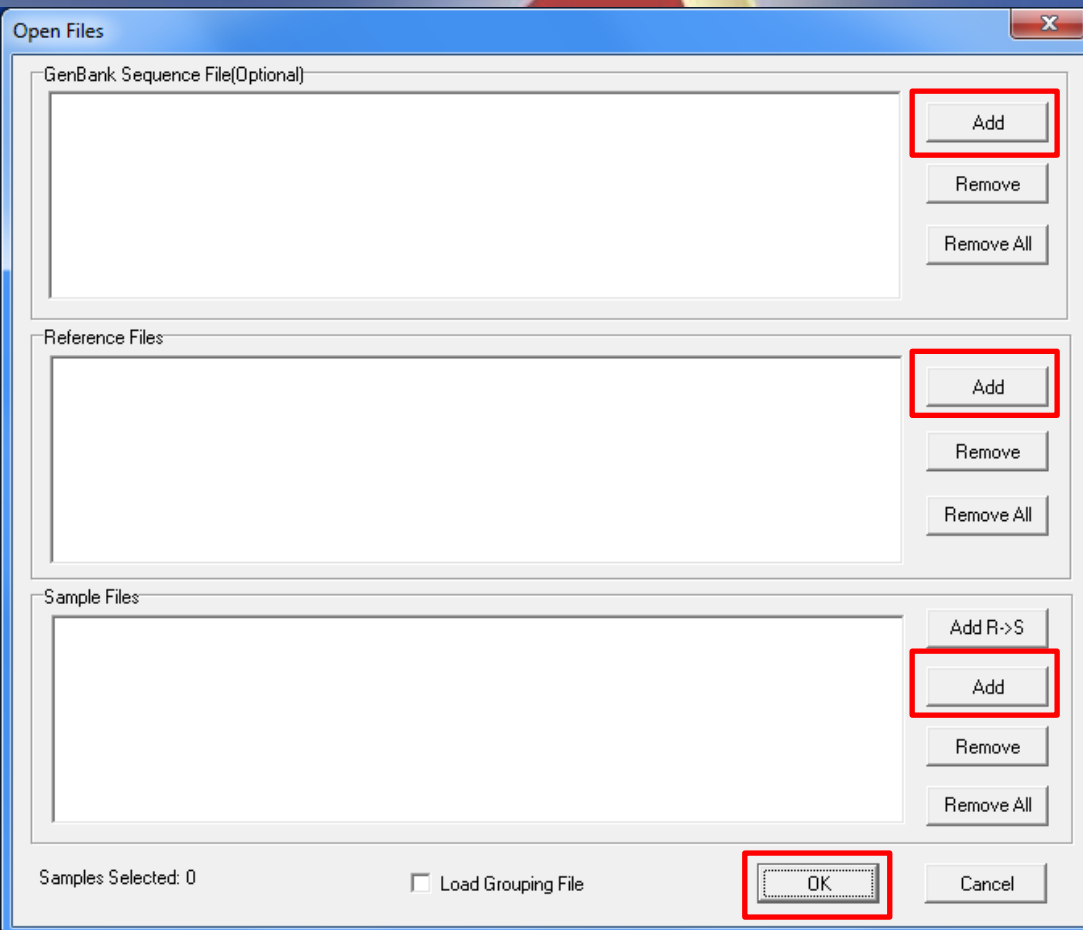
Select "**Configure Network Client**" to connect the software to your registered network license

Select "**Run Validation**" to open software as fully functional trial (trial will expire 35 days from the time this is first selected)

Upload Data



Click **Open Files** icon in the main toolbar



Add **GenBank Files** (GB/GBK/SEQ)
and/or
Reference Trace Files (SCF/AB1/ABI)

NOTE: Both GenBank and Reference files are optional when sequence maps to genes of *Homo sapiens*.

Add **Sample Files** (SCF/AB1/ABI)

Click OK

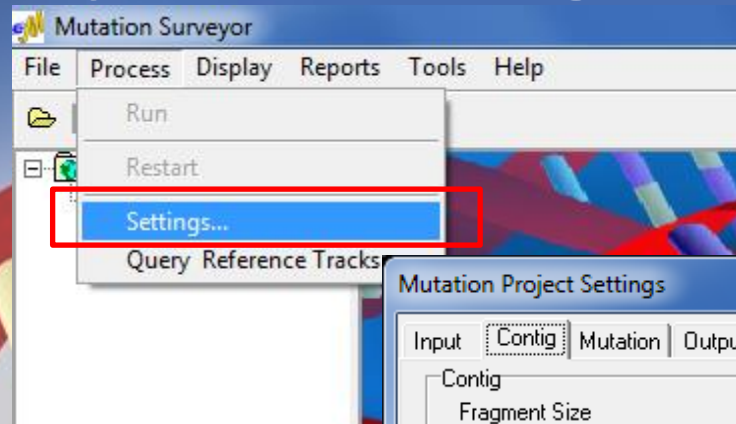
Review Raw Data

The screenshot displays the Mutation Surveyor application interface. On the left is a file browser pane with a tree view under 'Sample File'. A red box highlights three files: 'BRCA1_012345_10A_1_F_0_o', 'BRCA1_012345_10A_1_R_0_s', and 'BRCA1_012345_10A_2_F_0_o'. A blue callout box with white text is overlaid on the browser, stating: 'Double click on files in the **Browser Pane** to view the trace data'. The main area shows two DNA sequence trace plots. The top plot is titled 'BRCA1_012345_10A_1_F_0_o_2007-11-28_22-14-29.scf-->' and shows a sequence from position 75 to 120: 'A G C T G C T T G T G A A T T T T C T G A G A C G G A T G T A A C A A T A C T G A A C A T C A T C A A'. The bottom plot is titled 'BRCA1_012345_10A_1_R_0_s_2007-10-28_23-23-48.scf-->' and shows a sequence from position 75 to 120: 'T A A C T G C T G T T C T C A T G C T G T A A T G A G C T G G C A T G A G T A T T T G T G C C A C A T'. Both plots show signal intensity peaks for each base pair.

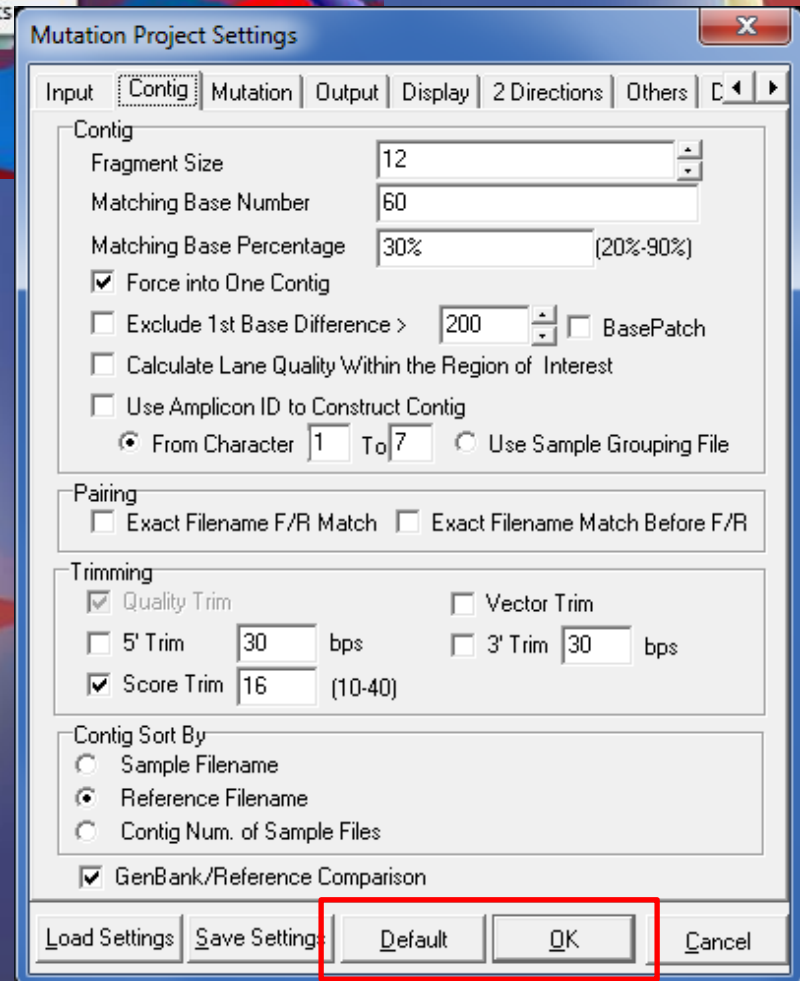
Double click on files in the **Browser Pane** to view the trace data

Analysis Settings

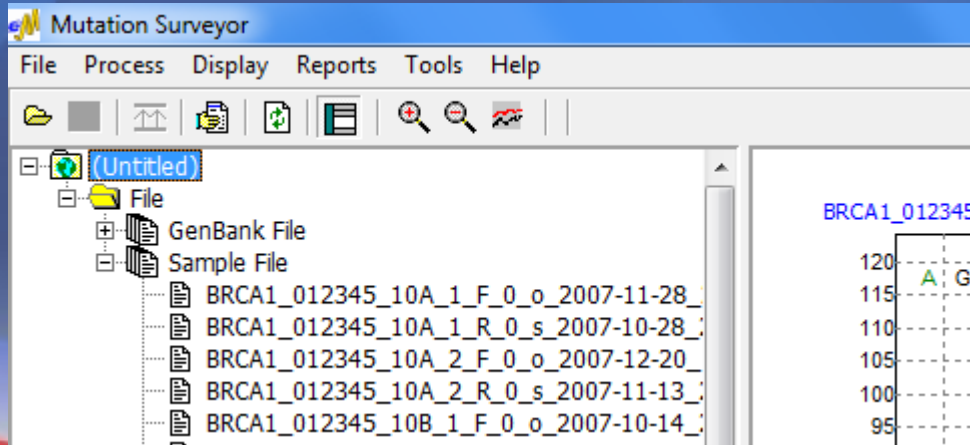
In the main toolbar select
Process → Settings



For first run analysis select
Default and then select **OK**



Analyze Data



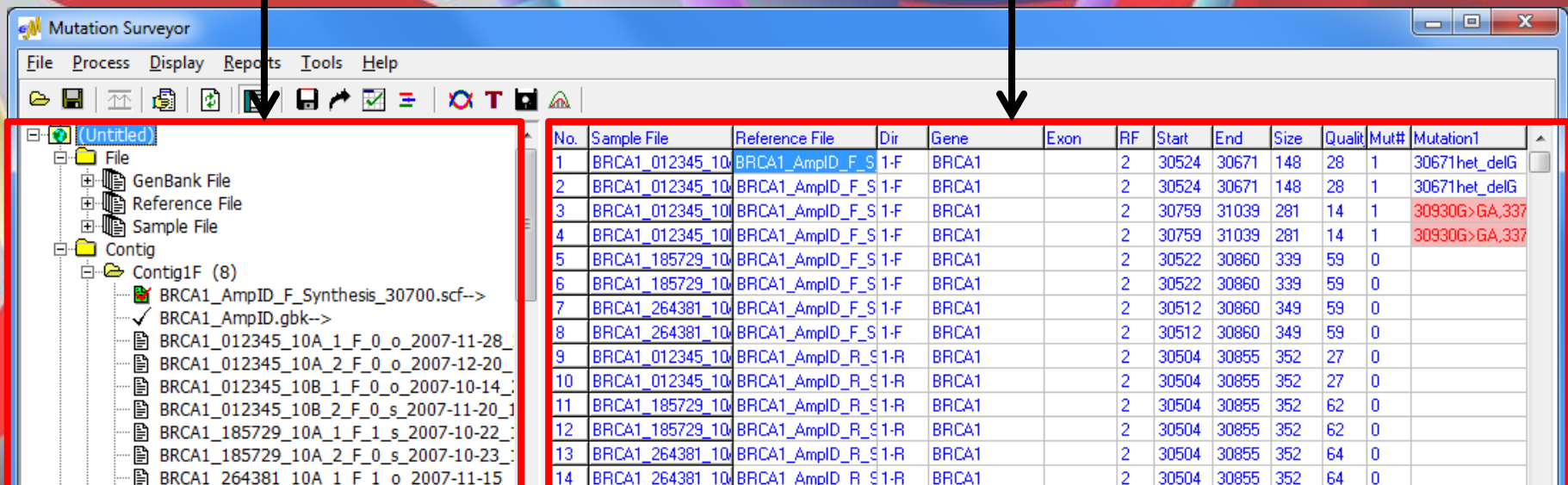
Click **Run** icon in main toolbar to begin analysis

Your samples will be compared to references

The first window to appear is the **Mutation Report**

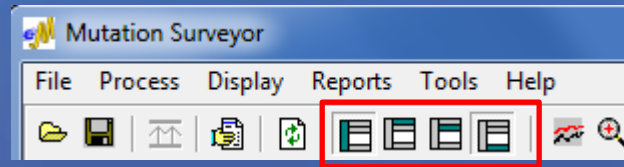
Browser Pane

Mutation Report

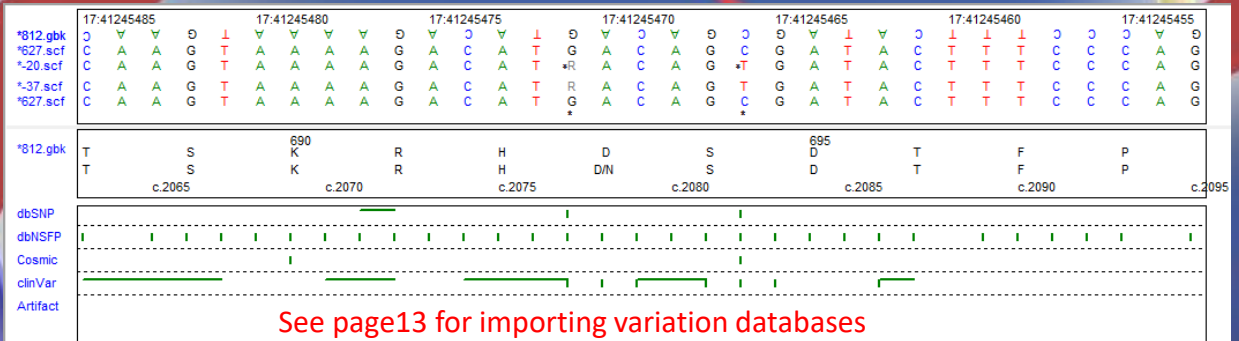


Graphic Analysis Display

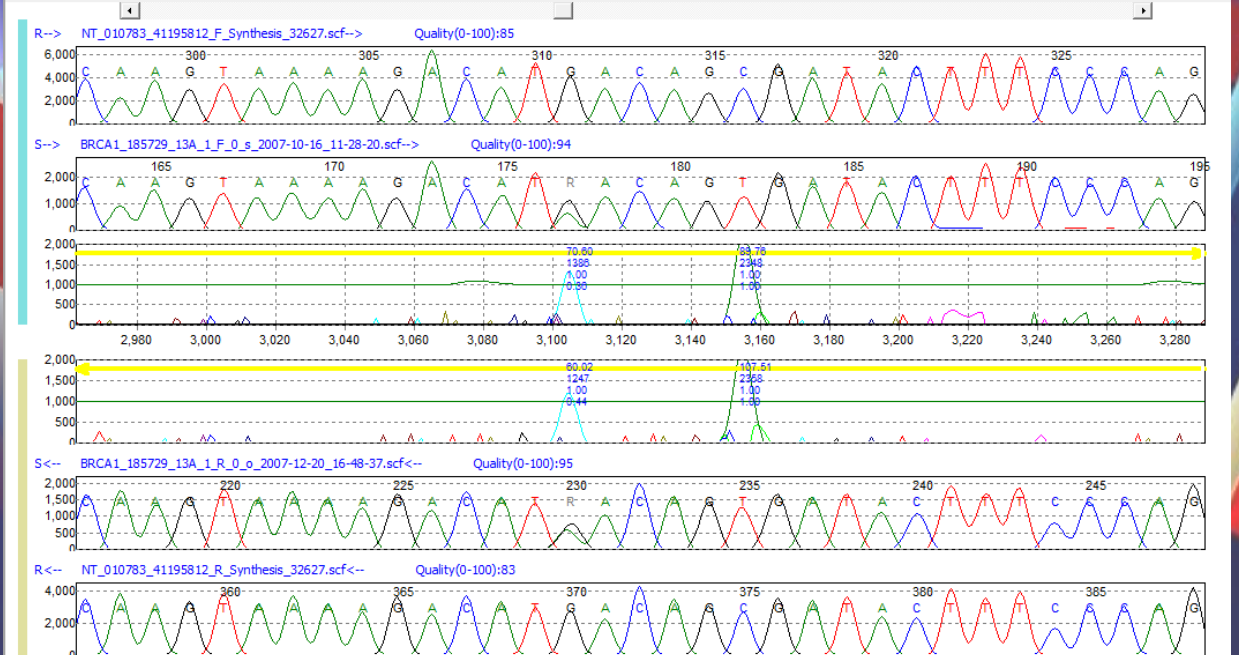
Use icons to show/hide windows



Nucleotide, Amino Acid,
and Database window



Trace windows



Graphic Analysis Display

By double clicking a mutation cell in the **Mutation Report**, the **Graphic Analysis Display** window appears

Forward Reference Trace

Forward Sample Trace

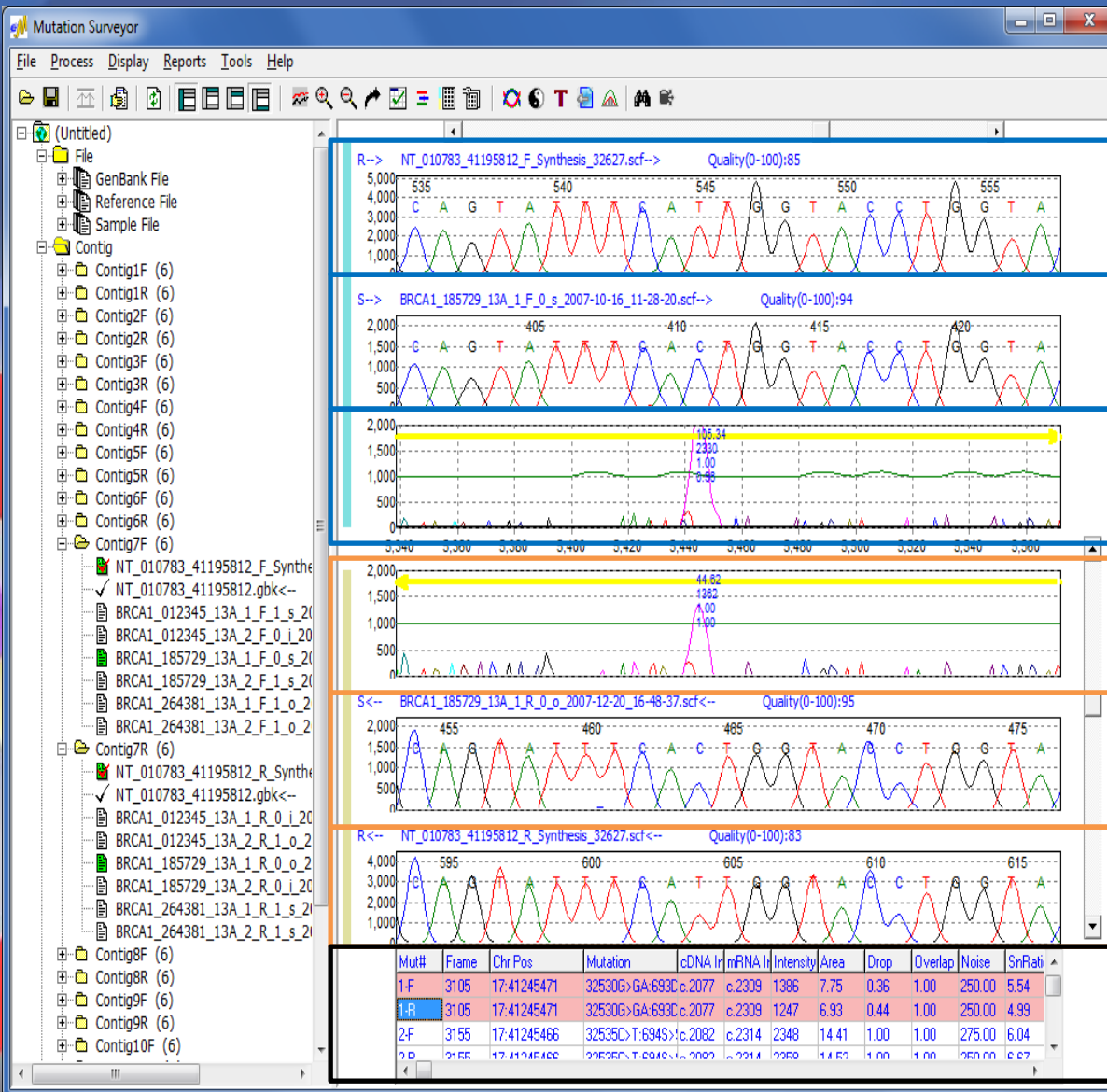
Forward Comparison

Reverse Comparison

Reverse Sample Trace

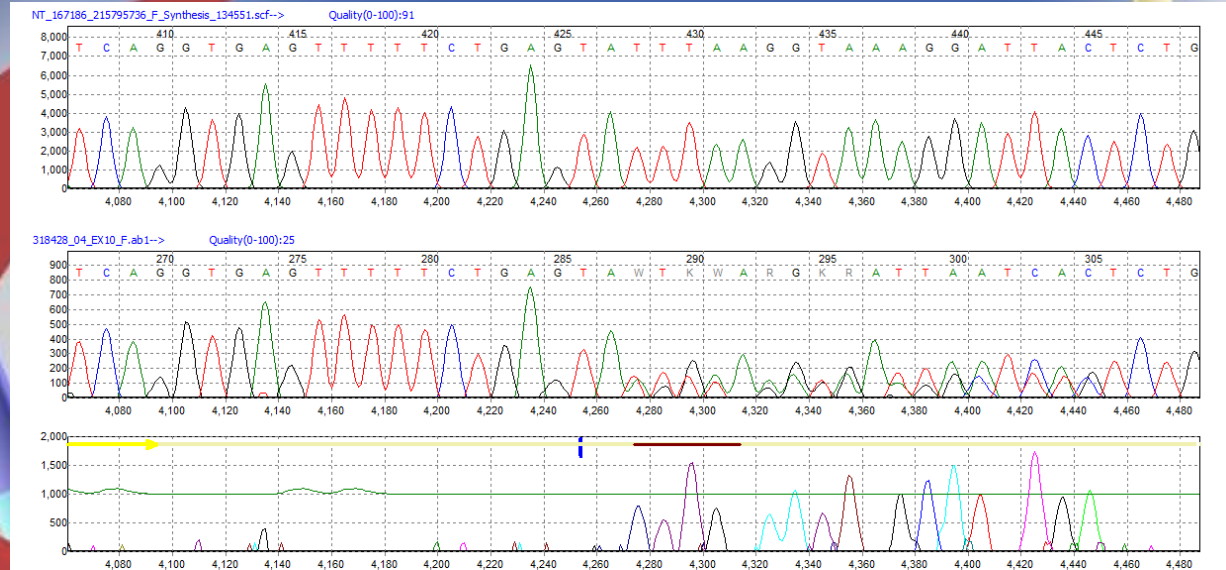
Reverse Reference Trace

Mutation Table



Heterozygous Indel Detection

Brown bar in Mutation Electropherogram indicates that a heterozygous indel is detected



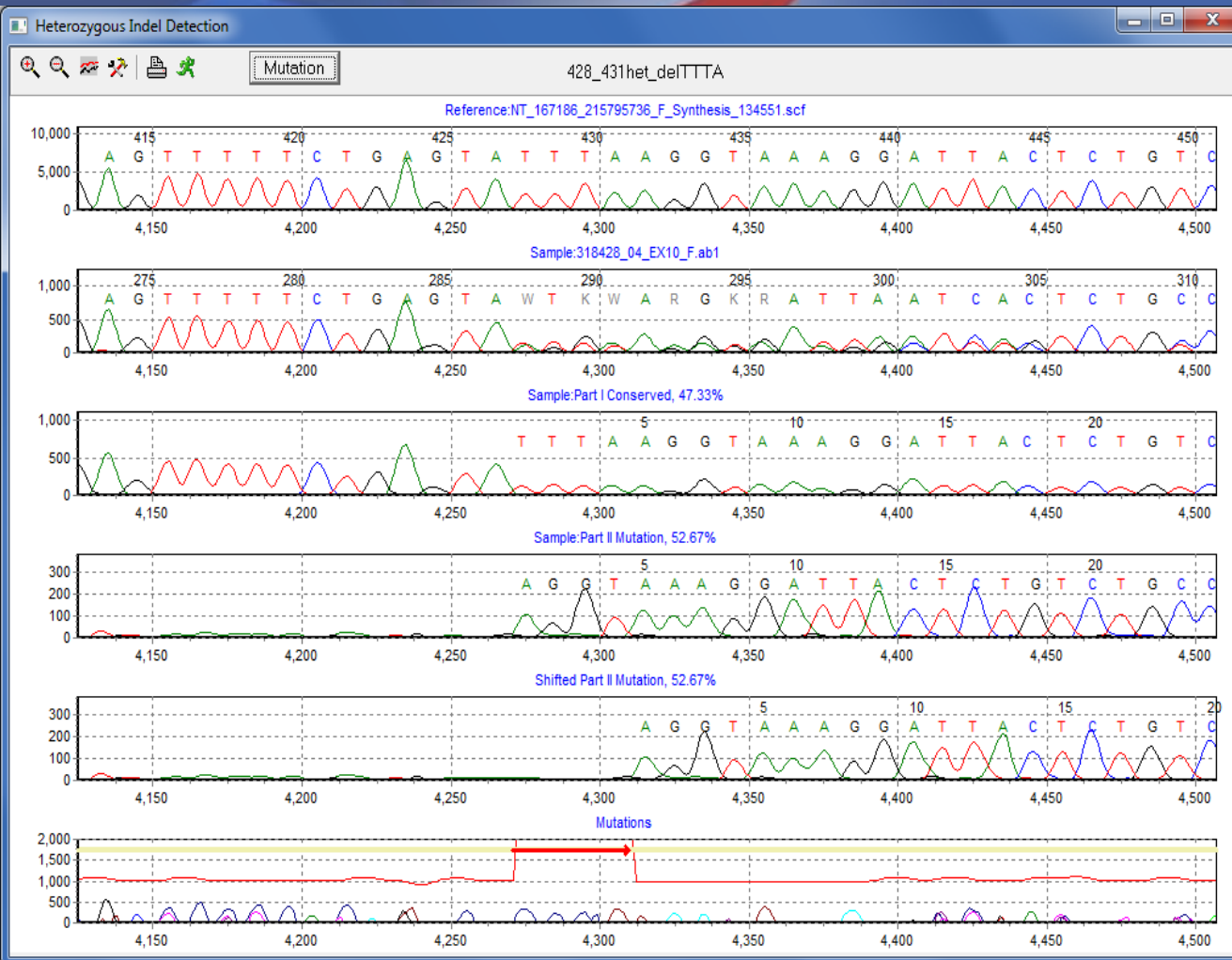
Double click mutation call or select icon to open heterozygous indel deconvolution tool

18	465130_10_EX10_F	NT_167186_215795	1-F	USH2A	11	2	134375	134750	376	38	1	134531_134532het
19	465130_12_EX10_F	NT_167186_215795	1-F	USH2A	11	2	134365	134534	170	0	1	134533_134535het_dupTGC

Mutation Surveyor

File Process Display Reports Tools Help

Heterozygous Indel Deconvolution Tool



Reference Trace

Sample Trace

Reference/Wild Type Allele

Mutant/Novel Allele

Mutant Allele Re-aligned to Reference

Location of Indel

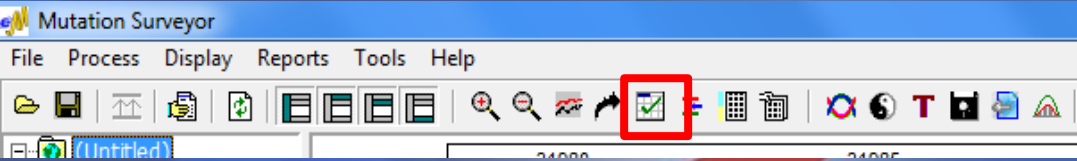
Custom Report Builder



Click the **Custom Report Builder**

Icon in the main toolbar

Default settings group by Contig and pair Forward and Reverse files



Custom Report Settings

Format | Filters | Display | Mutation | Color | Nomenclature | Other

Table Format

- One Trace Per Row
- Generate Forward/Reverse Consensus
- Generate Group Consensus
 - Mutation Position Specific for Column
 - By Group
 - By Table
- One Mutation Per Row
- Transpose
- Report One Nucleotide Position Per Column

Grouping

1st Order: Contig from character 1 to 3

2nd Order: F/R Pairing from character 1 to 3

3rd Order: Trace Position from character 1 to 3

Add Blank Row Between Groups

Group Summary

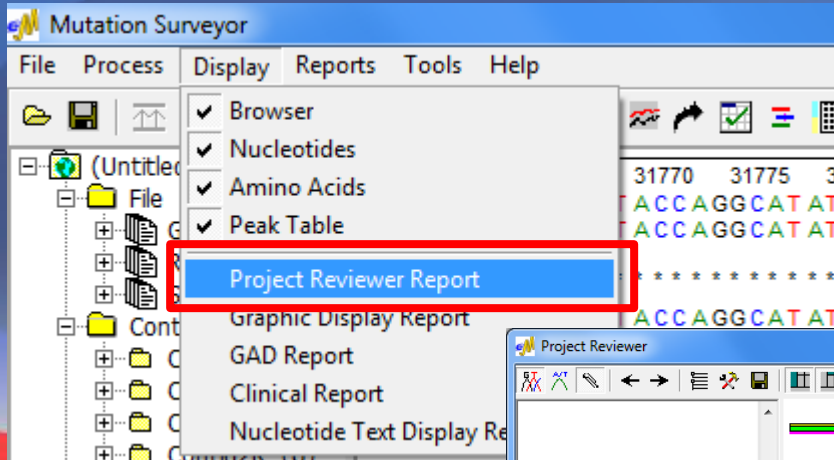
- Do Not Show Group Summary Information
- Show Allele Information
 - Show Allele Frequency
 - Show Advanced Allele Information
- Show Mutation Summary

Default Load

Custom Report

Trace #	Sample Name	Reference N	Gene	RNA Accession	Protein Accession	Exon	Quality (Trace)	Quality (ROI)	Variant1	Variant2	Variant3
1	BRCA1_012345_13A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	53	55			
2	BRCA1_012345_13A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	56	57			
3	BRCA1_012345_13A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	53	55			
4	BRCA1_012345_13A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	56	57			
5	BRCA1_185729_13A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	55	56	31998G>GA, 32003C>T.p.	32232T>C.p.	
6	BRCA1_185729_13A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	55	57	31998G>GA, 32003C>T.p.	32232T>C.p.	
7	BRCA1_185729_13A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	55	56	31998G>GA, 32003C>T.p.	32232T>C.p.	
8	BRCA1_185729_13A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	55	57	31998G>GA, 32003C>T.p.	32232T>C.p.	
9	BRCA1_264381_13A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	56	56	31998G>GA, 32003C>T.p.	32232T>C.p.	
10	BRCA1_264381_13A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	54	57	31998G>GA, 32003C>T.p.	32232T>C.p.	
11	BRCA1_264381_13A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	56	56	31998G>GA, 32003C>T.p.	32232T>C.p.	
12	BRCA1_264381_13A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	54	57	31998G>GA, 32003C>T.p.	32232T>C.p.	
									66.7%	66.7%	66.7%
Trace #	Sample Name	Reference N	Gene	RNA Accession	Protein Accession	Exon	Quality (Trace)	Quality (ROI)	Variant1	Variant2	Variant3
1	BRCA1_012345_14A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	56	58			
2	BRCA1_012345_14A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	55	58			
3	BRCA1_012345_14A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	56	58			
4	BRCA1_012345_14A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	55	58			
5	BRCA1_185729_14A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	55	57		32533C>CT.p.	
6	BRCA1_185729_14A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	54	58		32533C>CT.p.	
7	BRCA1_185729_14A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	55	57		32533C>CT.p.	
8	BRCA1_185729_14A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	54	58		32533C>CT.p.	
9	BRCA1_264381_14A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	54	57	32265delA	32533C>CT.p.	
10	BRCA1_264381_14A_1, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	54	58		32533C>CT.p.	
11	BRCA1_264381_14A_2, BRCA1_Amp	BRCA1	BRCA1	NM_007300.2	NP_009231.1	10	54	57	32265delA	32533C>CT.p.	

Project Reviewer



Click **Display** menu in main toolbar and choose **Project Reviewer Report**

Show all contigs simultaneously, group traces by sample ID, view information in four panes

Project Reviewer

Contig: All

Legend

31405 31410 BRCA1

Q 495 R P L
c.1480 c.1485 c.1490

BRCA1_012345_11A_1_F_1_s_2007
 <- BRCA1_012345_11A_1_R_1_s_2007
 -> BRCA1_012345_11A_2_F_1_o_2007
 <- BRCA1_012345_11A_2_R_1_o_2007
 -> BRCA1_185729_11A_1_F_0_s_2007
 <- BRCA1_185729_11A_1_R_0_o_2007
 -> BRCA1_185729_11A_2_F_0_o_2007
 <- BRCA1_185729_11A_2_R_0_o_2007
 -> BRCA1_264381_11A_1_F_0_l_2007
 <- BRCA1_264381_11A_1_R_0_l_2007
 -> BRCA1_264381_11A_2_F_0_s_2007
 <- BRCA1_264381_11A_2_R_0_s_2007
 -> BRCA1_264381_11B_2_F_0_o_2007
 <- BRCA1_264381_11B_2_R_0_o_2007
 -> BRCA1_264381_11B_1_F_0_s_2007
 <- BRCA1_264381_11B_1_R_1_s_2007
 -> BRCA1_185729_11B_2_F_1_l_2007
 <- BRCA1_185729_11B_2_R_0_s_2007
 -> BRCA1_185729_11B_1_F_1_l_2007
 <- BRCA1_185729_11B_1_R_0_l_2007
 -> BRCA1_012345_11B_2_F_1_s_2007
 <- BRCA1_012345_11B_2_R_1_s_2007

BRCA1_185729_11B_2_F_1_j_2007-12-20_13-47-44.scf--> Quality(0-100):4

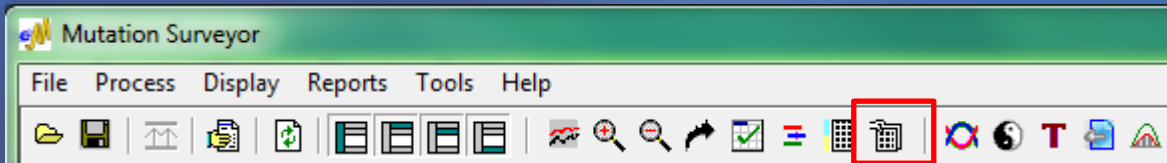
BRCA1_185729_11B_2_R_0_s_2007-10-26_20-48-32.scf<-> Quality(0-100):4u

BRCA1_185729_11B_1_F_1_j_2007-12-10_11-44-56.scf--> Quality(0-100):4

BRCA1_185729_11B_1_R_0_j_2007-12-28_14-53-34.scf<-> Quality(0-100):4o

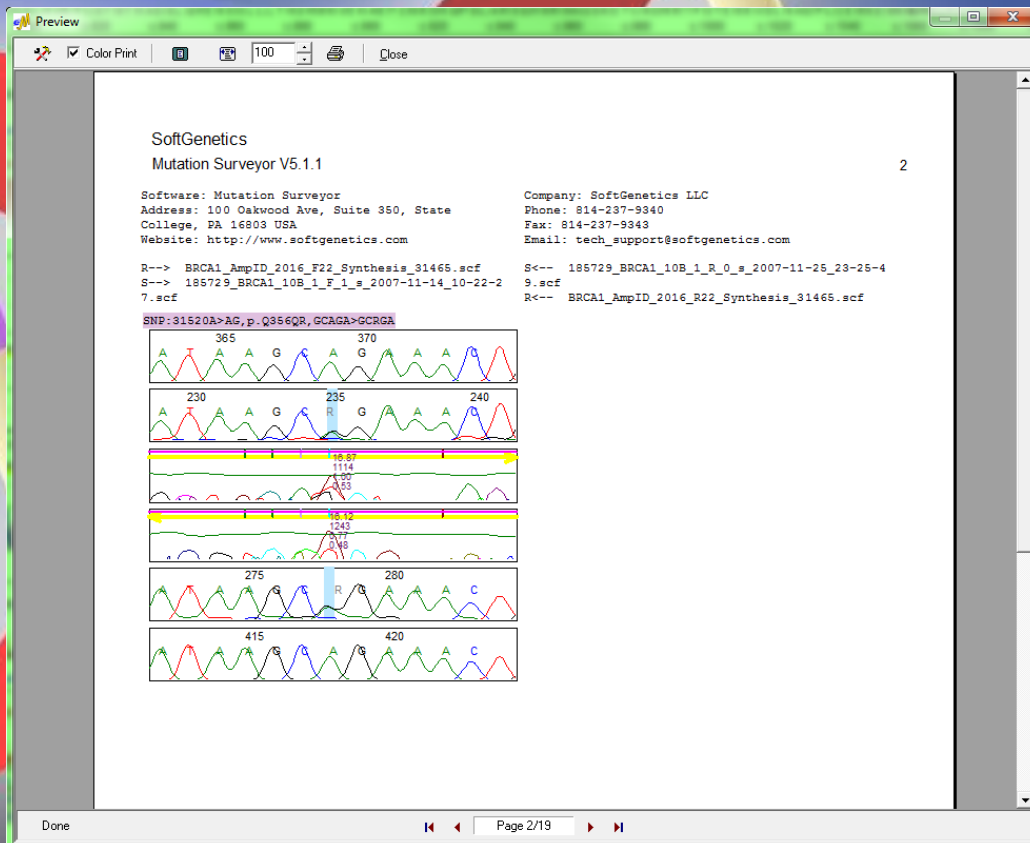
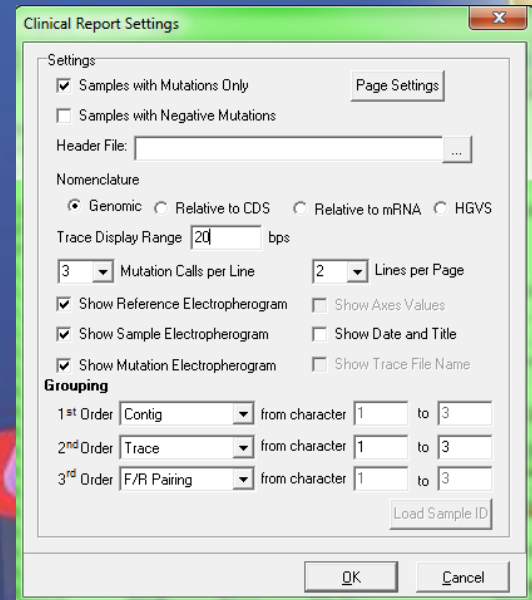
Index	Sample Name	Mutation2	Mutation3	Mutation4	Mutation5	Mutation6	Mutation7	Mutation8
39	BRCA1_264381_11B_1_F_0_s_2007-11-28_15-14-58.scf					c.1486C>CT,p.R496RC,\$26		
40	BRCA1_264381_11B_1_R_1_s_2007-12-20_14-23-25.scf					c.1486C>CT,p.R496RC,\$13		
41	BRCA1_185729_11B_2_F_1_j_2007-12-20_13-47-44.scf					c.1486C>CT,p.R496RC,\$26		
42	BRCA1_185729_11B_2_R_0_s_2007-10-26_20-48-32.scf					c.1486C>CT,p.R496RC,\$13		
43	BRCA1_185729_11B_1_F_1_j_2007-12-10_11-44-56.scf					c.1486C>CT,p.R496RC,\$26		
44	BRCA1_185729_11B_1_R_0_j_2007-12-28_14-53-34.scf					c.1486C>CT,p.R496RC,\$13		
45	BRCA1_012345_11B_2_F_1_s_2007-12-20_19-11-44.scf					c.1486C>CT,p.R496RC,\$12		
46	BRCA1_012345_11B_2_R_1_s_2007-12-27_12-23-24.scf					c.1486C>CT,p.R496RC,\$7		
47	BRCA1_012345_11B_1_F_1_j_2007-11-11_21-23-16.scf					c.1486C>CT,p.R496RC,\$12		
48	BRCA1_012345_11B_1_R_1_j_2007-12-11_12-17-43.scf					c.1486C>CT,p.R496RC,\$7		
49	BRCA1_012345_12A_1_F_1_s_2007-10-28_21-20-36.scf							

Print Clinical Report



Click **Clinical Report** Icon
in main toolbar

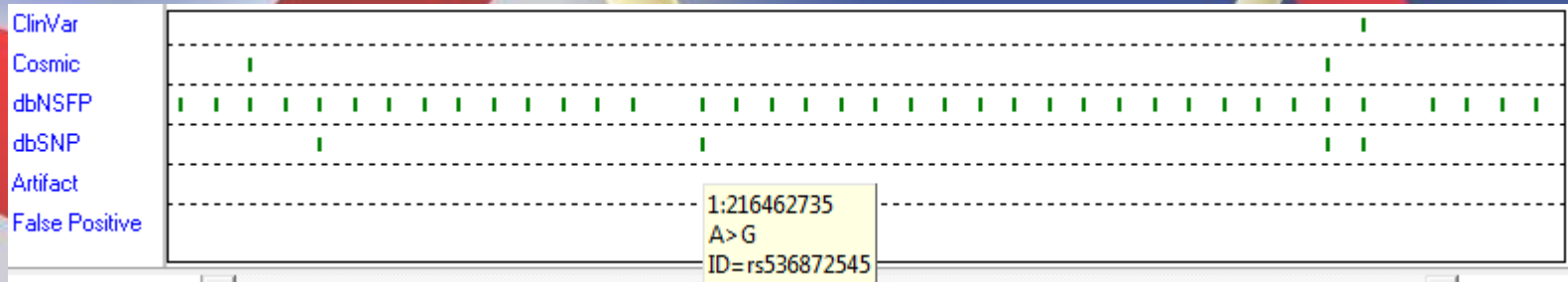
Adjust Display preferences
Add a **Custom Header** file



View and Print a snapshot of
each mutation call

Variation Databases

Variant annotation from external databases can be imported and displayed in the Variation Tracks pane of the Graphical Analysis Display. The Tracks pane will also display the positions of any known deleted mutation calls that have been added to the User Knowledge Database as either a false positive or artifact.



To get started, a whole human genome reference first needs to be imported. Please use the instructions on the following pages to complete this process. Projects will need to include GenBank files with chromosome coordinate information for display of Variation Tracks.

Note: Depending on the size of the files, the import process may require additional computer RAM and disk space and may take several hours to complete. Please contact us for more information at tech_support@softgenetics.com if interested in this feature.

Import Reference

In the main toolbar select
**Tools → Reference & Track
Manager**

Click on **Import Reference** link

The screenshot shows the Mutation Surveyor interface. The 'Tools' menu is open, and 'Reference & Track Manager' is highlighted. The 'SoftGenetics Reference & Track Manager' window is open, showing the 'Import Reference' link highlighted. A 'SoftGenetics Reference Setup Wizard' dialog box is also visible, displaying the following text:

Welcome to the SoftGenetics Reference Setup Wizard

This wizard will guide you through the installation of SoftGenetics Reference.

It is recommended that you close all other applications before starting Setup. This will make it possible to update relevant system files without having to reboot your computer.

Click Next to continue.

Next > Cancel

Follow directions in the **SoftGenetics Reference Setup Wizard** to install a full human genome

Import External Database Annotation

In the main toolbar select **Tools** → **Reference & Track Manager**

Storage Path: C:\Program Files (x86)\SoftGenetics\NextGENE\References

Genome Build: All

Preloaded References

Reference	Genome Build	Annotation DB	Comments
Human_v37p10_dbsnp135	Human_GRCh37	sg_grch37p10	
Human_GRCh38_106_dbSNP141	Human_GRCh38	sg_grch38r106	
Human_v37p13	reference::Human_v37p13	sg_grch37p10	

Build Reference Import Reference

Tracks

Track	Type	Genome Build	Default Query	Last Modified	Location
clinvar\20170801	dbSNP	Human_GRCh37	Yes	08/22/17 16:54:57	Tracks
clinvar\20171029	dbSNP	Human_GRCh37	Yes	2017-11-08 14:32:02	Tracks
dbSNP\b150	dbSNP	Human_GRCh37	Yes	05/09/17 13:37:16	Tracks
UKDB\Artifact	custom	Human_GRCh37	No	2017-12-18 10:55:22	Tracks
UKDB\False Positive	custom	Human_GRCh37	No	2017-12-18 10:55:24	Tracks
ClinVar\20170501	dbSNP	Human_GRCh37	No	05/08/17 08:20:23	Human_v37p10_c
Cosmic\68	Cosmic	Human_GRCh37	Yes	Tuesday, January ...	Human_v37p10_c
dbNSFP\2.9_commercial	dbNSFP	Human_GRCh37	Yes	04/01/15 15:50:47	Human_v37p10_dbsnp135
dbSNP\149	dbSNP	Human_GRCh37	No	01/30/17 18:22:55	Human_v37p10_dbsnp135
ExAC\0.3.1	custom	Human_GRCh37	Yes	06/24/16 13:57:39	Human_v37p10_dbsnp135
ClinVar\142	dbSNP	Human_GRCh38	No	Monday, February ...	Human_GRCh38_106_dbSNP141
dbNSFP\2.5	dbNSFP	Human_GRCh38	No	Thursday, Februar...	Human_GRCh38_106_dbSNP141
dbSNP\142	dbSNP	Human_GRCh38	No	Monday, February ...	Human_GRCh38_106_dbSNP141

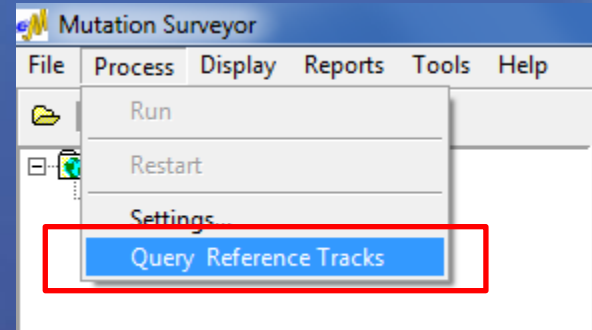
Import Track >

- dbSNP/Clinvar
- COSMIC
- dbNSFP
- dbSCSNV
- Custom Variant Track
- Gene Annotation Track

Click **Import Track** and select the external database to import

Query Database Annotation

In the main toolbar select
Process → **Query Reference
Tracks**



Select databases to query in
Mutation Surveyor projects

