AMPLICON

Sequence Analysis

of Sanger Sequence and Ion Torrent PGM™ Sequencer Data

MUTATION Surveyor®
DNA Variant Analysis Software

NextGENe®
Next Generation Sequencing Software for Biologists

SOFTGENETICS®
Software PowerTools for Genetics Analysis

Amplicon Sequence Analysis?

SoftGenetics is the obvious choice…

Mutation Surveyor® for Sanger Sequence Analysis.

Patented “anti-correlation” technology provides 99.9% accuracy with sensitivity greater than 5% in the detection of SNPs, Indels and Mosaic variants from Sanger Sequencing traces generated by all major capillary electrophoresis platforms.

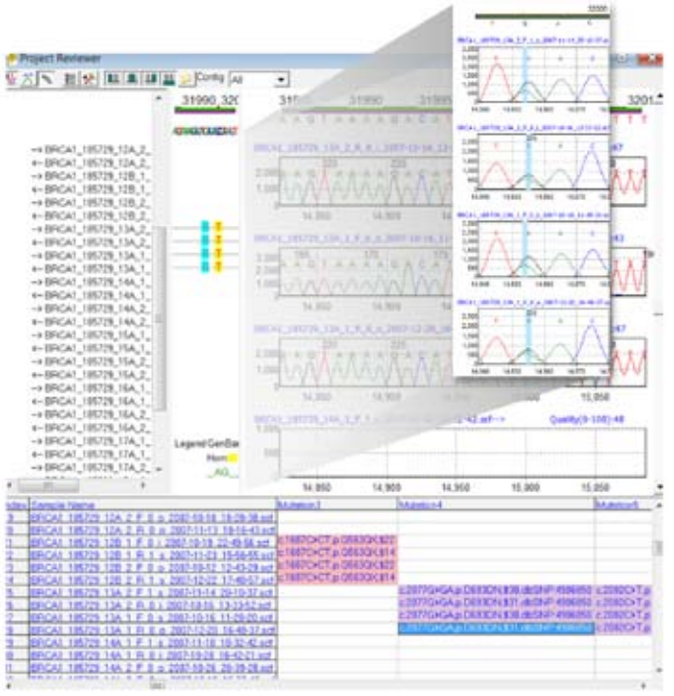
NextGENe®software for Ion Torrent PGM™ Sequence Analysis

Free standing Windows® based software for the rapid and accurate analysis of Ion Torrent PGM data. NextGENe utilizes point and click operation: requires no scripting, automatically sets analysis parameters for semiconductor sequencing data and provides results and analysis details in a highly graphical, user friendly interactive windows format.

Softgenetics’ Mutation Surveyor software’s patented “anti-correlation” technology and simplicity of use has made it the leading DNA Variant Analysis software tool of Sanger sequencing reads worldwide. Our NextGENe software applies the same formula of unique technologies in an easy-to-use Windows® computing environment to the analysis of Next Generation Amplicon sequencing data.

NextGENe’s proven technologies perform read quality assessment, automatically set analysis parameters based upon the data set and sequencing platform, perform alignment detecting SNPs and Indels with high accuracy and sensitivity, all in a biologist friendly, no scripting required interface.

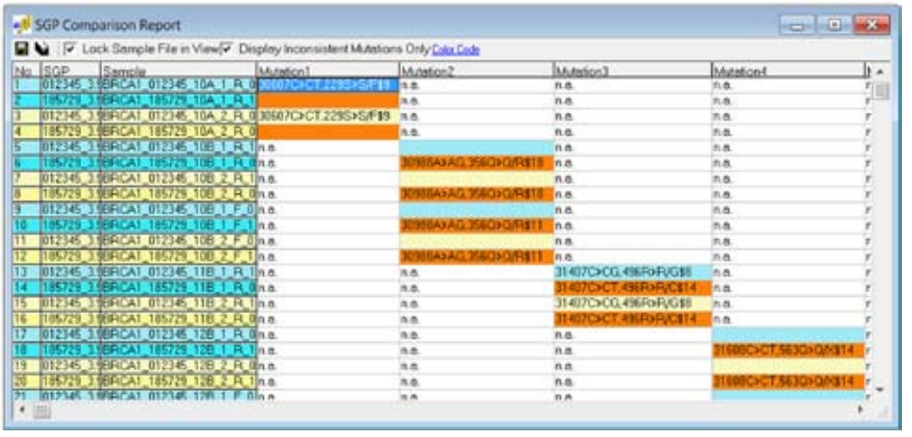
Mutation Surveyor



NextGENe

Like Mutation Surveyor (left), NextGENe (right) analysis results are presented in a highly informative graphical and user friendly interface; logically presenting important biological information regarding the analysis and found variants.

Both NextGENe and Mutation Surveyor software programs include many tools to assist the biologist or clinician in interpreting, comparing, and researching results, all designed to enhance the analysis confidence in an efficient manner.



Mutation Surveyor's color-coded Comparison Report provides quick review of shared and negative variants between multiple patient sequences.

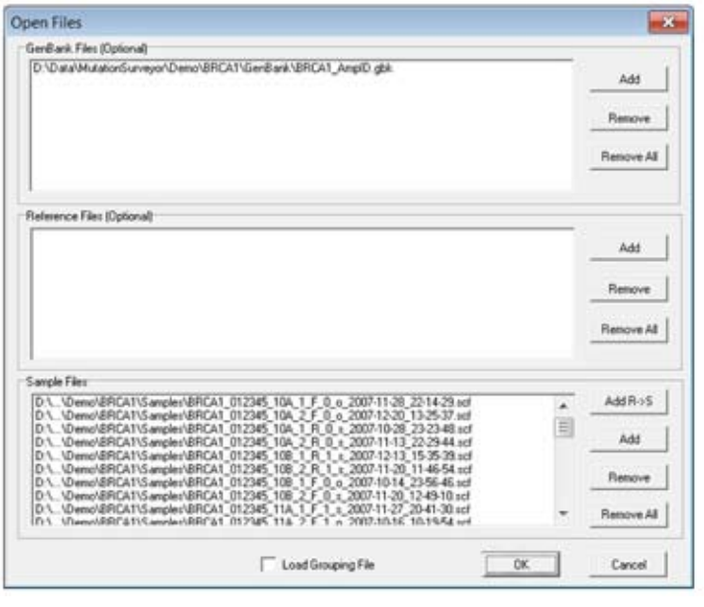
NextGENe's Rare Variant Analysis Tool compares familiy members or other groups indicated shared or negative mutations by patient, additional filtering by 1000 genomes frequency, and Polyphen score dramatically eases identification of rare variants.

Biologist-Friendly Analysis Set-up

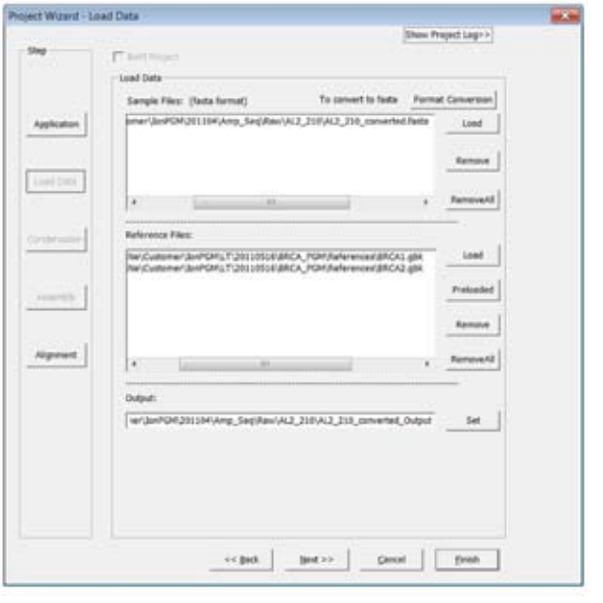
NextGENe and Mutation Surveyor were developed for use by the biologist and medical research clinician. Analysis set-up is simple and straightforward, no need for complicated scripting or informatics support. Simply follow the point and click Project Wizard to begin your analysis.

NextGENe contains unique technologies that examine your data, make necessary adjustments, and begin processing. Both programs have an automation feature which will process multiple projects sequentially. If you are using multiplexed Ion Torrent PGM data, the software automatically de-multiplexes and then will perform individual analyses on all of the sample sets.

Mutation Surveyor



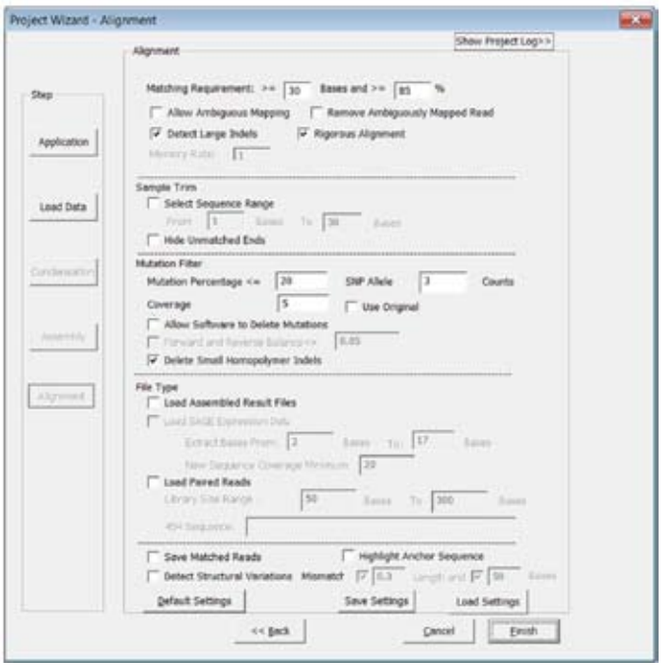
NextGENe



Adding Input data to be processed and selecting annotated references is simple and quick in both programs. Use the above dialogue box to select sequencing files, annotated reference and analysis output location. Both programs begin processing or further parameter optimizing can be accomplished within the advanced project wizard.

Mutation Surveyor Project Wizard

NextGENe Project Wizard

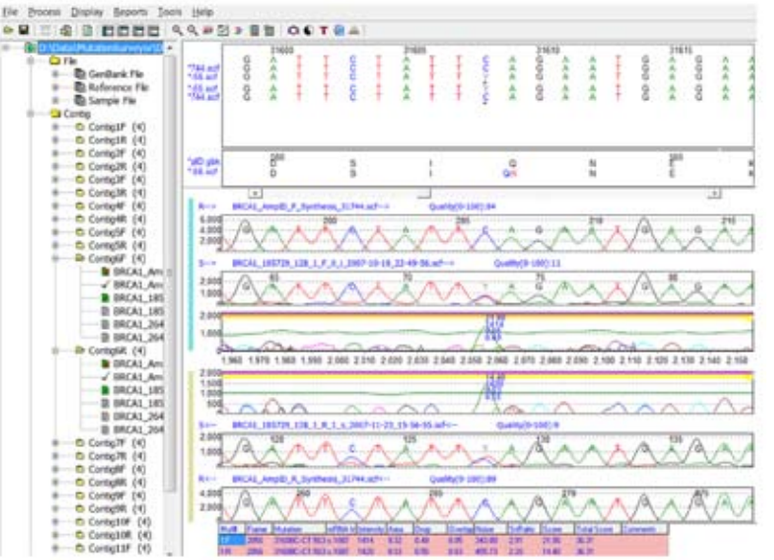


NextGENe's and Mutation Surveyor settings dialogue provide for complete control of the analysis. Advanced Users may wish to increase sensitivity, adjust to minimize false positives or, in the case of NextGENe, adjust the alignment stringency to detect large and small Indels. Default settings are applicable for most Amplicon data sets.

Usable/Informative Analysis Presentation

Analysis results in NextGENe and Mutation Surveyor are presented in highly graphical, succinct, interactive viewers. Graphical views are linked to customizable reports, which include gene, amino acid, links to dbSNP and other information such as depth of coverage in NextGENe, coding regions, mRNA, and regions of interest.

NextGENe Viewer provides graphical review of found variants (blue or purple background), annotation, including amino acid sequence, CDS, and depth of coverage as well as indicating variants which fell below user defined settings(grey highlights).

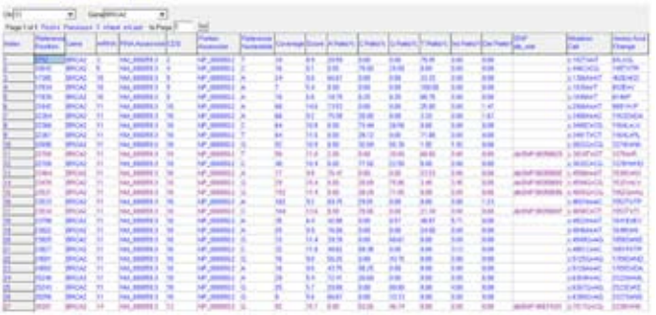


Mutation Surveyor presents found variants in the two central "mutation electropherograms" allowing immediate review of all found variations including SNPs, Indels and Mosaicism. Amino Acid Sequence, AA change as well as annotation are provided.

Comprehensive Reporting

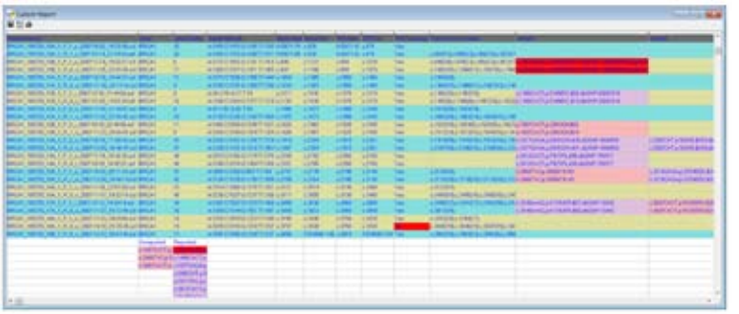
Analysis results are offered and reported with several filtering options to speed the review process. Edits to the analysis are permanently recorded in the project record. Each program offers specialized filtering options and are exportable in several formats for linking to LIMS systems or further investigation.

NextGENE's Comprehensive Mutation Report



Included in NextGENe's reporting capabilities, all selectable by the user, are mutation confidence score, read balance, coverage, RNA Accession, mRNA, Amino Acid as well as Amino Acid changes, allele ratios and mutation calls. Previously reported variants and their dbSNP number are reported in purple for easy identification, novel findings are reported in blue. Previously reported variants include a direct link to the dbSNP database.

Mutation Surveyor Variant Analysis Report



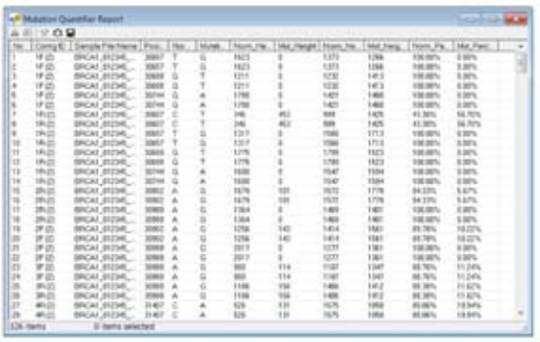
Mutation Surveyor analysis reporting allows customizable reporting of variants by pathogenicity, reported or novel variants and other customer selectable formats.

Unique Tools Enhance Analysis

NextGENe's Multiple Sample Comparison Tool

To facilitate discovery and/or clinical applications, NextGENe includes a comparison tool which allows up to 10 separate analyses to be compared to one another for similarities and differences. All views are linked for easy identification and review.

Mutation Surveyor's Low Frequency Quantification and Methylation Tool



Mutation Surveyor includes a unique quantification tool to measure percent change of mosiac, somatic and heteroplasmy variants and also methylation, based upon the intensity change in relationship to neighboring bases of the same wave length.

NextGENe's Expression Report

Index	Contig	Chr	Chr Position St	Chr Position End	Gene	CDS	Length	Max Counts	Average Cou	Read Counts	Forward Read	RPKM
1	NG_007572	1	962168	962228	NRAS, +	2	61	11543	11193.00	11543	6361	2067660.4913
2	NG_023302	15	726002	726061	IDH2, +	4	60	9859	9533.70	9859	5549	1790234.4247
3	NG_007572	1	959957	960013	NRAS, +	1	57	9105	8643.50	9105	5002	1740337.1151
4	NG_007726	7	353007	353146	EGFR, +	20	140	12579	6984.10	12597	5959	980318.9130
5	NG_007524	12	837077	837160	KRAS, +	2	84	5715	5583.20	5715	3003	741250.9047
6	NG_007673	7	171394	171454	BRAF, +	15	71	5626	5515.90	5626	2774	863315.7555
7	NG_007666	11	691734	691801	HRAS, +	2	68	4090	4706.20	4090	3013	763468.5764
8	NG_007726	7	346432	346561	EGFR, +	19	130	6230	4326.10	6280	4801	526313.5840
9	NG_023319	2	709056	709145	IDH1, +	2	90	3886	3747.50	3886	1462	470423.0297
10	NG_007524	12	838779	838863	KRAS, +	3	85	3231	3141.90	3231	942	414139.1809
11	NG_007726	7	363491	363618	EGFR, +	21	128	3553	2520.40	3553	2567	302422.1006
12	NG_007524	12	819069	819137	KRAS, +	1	69	2541	2467.50	2541	1354	401221.1904
13	NG_007458	4	805936	806033	KIT, +	17	98	2205	2114.00	2205	966	245138.0345
14	NG_007726	7	345376	345766	EGFR, +	18	391	7677	1743.60	7677	2443	213915.9024
15	NG_007666	11	691317	691397	HRAS, +	1	81	1541	1508.50	1541	1166	207274.5350
16	NG_023302	15	726098	726173	IDH2, +	4	76	1536	1477.40	1536	921	220194.2162

NextGENe provides analysis information such as depth of coverage, similar to intensity value of Sanger Sequencing, normalized count values, as well as read directionality.

Acknowledgement
We wish to thank Halo Genomics AB for furnishing the Ion PGM BRCA data from their Selector BRCA Panel Kit.

For further information or to request a 30 day trial of NextGENe or Mutation Surveyor software please visit **www.softgenetics.com** or email **info@softgenetics.com**

Minimum Hardware Configuration:
PC: 32-bit Windows® XP OS, Dual Core Processor with 3GB RAM
MAC: Dual Core Processor, 32-bit Windows® XP OS, Boot Camp or VM Fusion, 3GB RAM

Optimal Hardware Configuration:
PC: 64-bit Windows® 7 OS, Quad Core Processor, 6GB RAM
MAC: Quad Core Processor, 64-bit Windows® 7 OS, Boot Camp or VM Fusion, 6GB RAM

SOFTGENETICS®
Software PowerTools for Genetics Analysis

SoftGenetics
Oakwood Centre
100 Oakwood Avenue
Suite 350
State College PA 16803 USA
info@softgenetics.com
www.softgenetics.com