AMPLICON
Sequence Analysis
of Sanger Sequence and Ion Torrent PGM™ Sequencer Data
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.

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.

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.

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.
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

Mutation Surveyor Variant Analysis 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.

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 mosaic, somatic and heteroplasmic variants and also methylation, based upon the intensity change in relationship to neighboring bases of the same wave length.

NextGENe's Expression Report

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