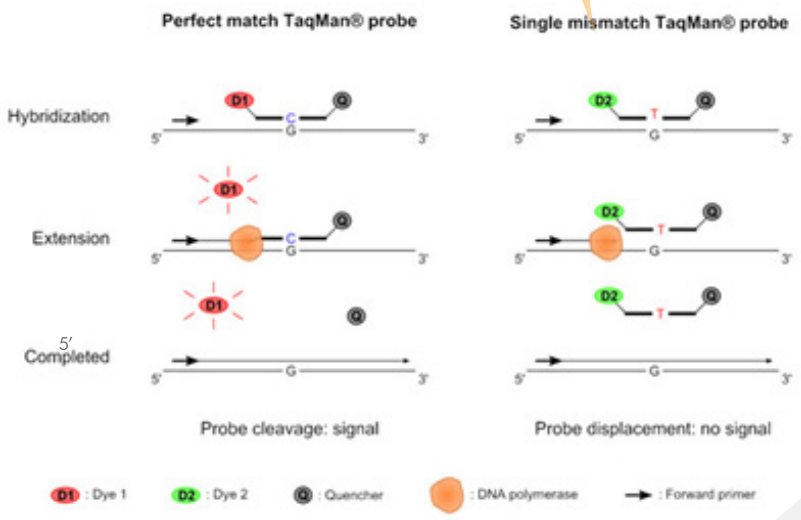
TAQMAN-BASED SNP GENOTYPING

What is Taqman-based SNP genotyping?

The TaqMan® SNP genotyping technology utilizes the 5′ nuclease activity of Taq polymerase to generate a fluorescent signal during PCR. For each SNP, the assay uses two TaqMan probes that differ in sequence only at the SNP site, with one probe complementary to the wild-type allele and the other to the variant allele. The technique utilizes the FRET technology whereby a 5′ reporter dye and a 3′ quencher dye are covalently linked to the wild-type and variant allele probes.

When the probes are intact, fluorescence is suppressed because the quencher dyes are in the proximity of the reporter dyes. In the PCR annealing step, the TaqMan probes hybridize to the targeted SNP site. During PCR extension, the reporter and quencher dyes are released due to the 5′ nuclease activity of the Taq polymerase, resulting in an increased characteristic fluorescence of the reporter dye. Exonuclease activity only happens on the perfectly hybridized probes, since a probe containing a mismatched base will not be recognized by the Taq polymerase.



At the end of the PCR reaction, the fluorescent signal for the two reporter dyes is measured. The ratio of the signals will be indicative for the genotype of the sample.

In most assays, the fluorescent signals of the two reporter dyes are normalized using the signal of a third dye (e.g. ROX), of which the intensity is proportional to the template DNA concentration and the extent of the PCR reaction. Typically, the reporter dye signals are visualized in a plot. A number of related genotyping systems utilize the reporter-quencher technology (e.g. Invader®, Molecular Beacons®, Scorpion® and other probe technologies) and can be analyzed and visualized in the same way as TaqMan probes using the SNP genotyping plugin.

SNP genotyping in BioNumerics

The BioNumerics SNP calling plugin provides a fully automated platform for reliable SNP calling and genotyping.
The plugin is very flexible and allows different workflows for TaqMan SNP genotyping data analysis:

(1) Perform an auto-calling in other software and import the calls and their corresponding confidence values in BioNumerics;
(2) Perform an auto-calling in BioNumerics during import;
(3) Import data as "No call" and perform an auto-calling in the SNP calling window.

YOUR ADVANTAGES

WHY USE BioNumerics FOR YOUR SNP GENOTYPING?

- ✓ Various supported file formats
- ✓ Automatic SNP calling
- ✓ Detailed overview
- ✓ Impressive set of analysis tools

TaqMan SNP genotyping in BioNumerics®

Various supported file formats

- Applied Biosystems 7900HT Fast Real-Time PCR System
- Douglas Scientific Array Tape system
- Tecan Safire or Tecan Infinite microplate readers
- BMG Labtech 384-well microplate readers
- Fluidigm Dynamic Array

Multiple files can be batch imported with optional SNP calling.

Detailed overview

Single or multiple SNP files can be opened. An individual SNP can be selected to show in the plot window. SNP calls can also be plotted per file or for selected files. On the plot, SNP calls can be selected using the lasso selection tool.

By default, SNP calls are shown in changeable colors according to the defined zygosity calls, the NTC calls and the 'No calls'. The statistical confidence of the calls can also be used as a basis for plot colors. In a third view, colors are based on the SNP files. SNP calls can be sorted and filtered by their confidence, ROX signal or other parameters. The plot can be displayed with Cartesian coordinates, polar coordinates and contrast coordinates.

Selected SNP calls can be changed by the user. BioNumerics displays whether the call was assigned manually or automatically. Tab-delimited files with calls and confidence information can be exported for any selection of samples in the database.

NOTE:
The SNP calling plugin is license-based. The minimum configuration for installation of the plugin includes the "Character Data" module. Please contact us for more information.

Automatic SNP calling

The automatic SNP calling algorithm is the most crucial element of the plugin and is determinative for the efficient and successful SNP genotyping in a high-throughput setting.

SNP calling in BioNumerics is achieved by an iterative seed-based partitioning algorithm. The auto-call settings are divided in two tabs: one will show the general settings and thresholds for SNP assignment while the other illustrates the advanced settings for the partitioning algorithm.

While the default partitioning settings are ideal for 99% of the cases, they can be optimized for any particular data source. The algorithm allows SNPs to be called successfully in polyploid genomes as well (theoretically up to heptaploidy).

Impressive set of analysis tools

BioNumerics offers numerous advanced tools for data mining, clustering, screening, identification and statistical analysis.

This combined with the powerful databasing capacities make BioNumerics the perfect solution for long term genotyping projects on an (inter-)lab basis.

Most importantly, BioNumerics offers equally powerful tools for various other genotyping techniques, including AFLP band scoring, MLPA analysis, (whole) genome sequencing and SNP analysis, etc.

Results from different techniques can be combined in various ways to obtain more conclusive analyses.

CONVINCED?
INTRIGUED?
TRY IT FOR YOURSELF!

Request a free **BioNumerics** trial license.

Scan the QR code

visit www.bionumerics.com for more information

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