

## SNP Genotyping Service

### Technical Specifications and Requirements

#### I. DNA samples

- Concentration: at least 20 ng per µl. All samples have to be adjusted to identical concentrations.
- Required volume: 30-50 µl. Depending on the number of analysis per sample more material might be required.
- Solvents: DNA samples should be ideally dissolved in water. In cases where buffers (e.g. TE) are required, the buffer concentration must not exceed 0.1x TE.
- DNA samples should be shipped at 4°C (water ice, on -20 °C cool pads) or at -70 °C (dry ice).
- Container: Preferred are 96well plates (PCR grade plates Abgene cat.-nr. AB-0800) Plates must not heat sealed before.
- Sample distribution in plates and over all applicable plates and numbering should be simple and non-ambiguously.  
If “complex sample-IDs” are unavoidable, a sample sheet in “excel format” or tab delimited text format should be provided.
- Multiples of 48 samples are preferred (e.g. 288 instead of 300). Ideally empty wells should be considered to allow negative controls for quality assurance.
- DNA quality (intactness, purity) and quantity (concentration) has to be ensured by customer. Failed samples will be repeated once. If they fail again, no further follow up will be performed. The result will be reported as “technically failed”.

#### II. Submission of sequence information

Sequences information including the SNP definition should be provided in either

- a) tab-delimited text file (.txt) or
- b) in a specially formatted Excel file (.xls or .txt)

Database accession numbers should be given and international SNP nomenclature should be used if applicable. Upon request example files can be provided.

- Assay file definition  
SNPs, which can be analyzed in multiplexed reactions, can be saved in the same file. If any SNP analysis is wished to be performed independently from all other analysis a new assay file has to be provided.

Each file will contain a header line/row of the following layout:

- a) tab-delimited text file  
SNP\_ID 'tab' SEQUENCE
- b) Excel formatted file

All following lines/rows will contain the sequences of interest using the same layout:

- a) tab-delimited text file  
SNP\_ID 'tab' SEQUENCE  
SNP-1 'tab' CCGGTAGT[A/T]TCGAGGATG...
- b) Excel formatted file

| SNP_ID | SEQUENCE                     |
|--------|------------------------------|
| SNP-1  | ...ACGGTAGT[A/T]TCGAGGATG... |

**Do not use** any special characters for SNP naming, like #, ?, \$, ©, å, ã, Ç, Ø, &, etc.

- SNP definition  
The two SNP-bases have to be indicated in brackets separated by a slash. Insertions / deletions up to 40 bases can be treated similar. Whereas the 'missing' bases are indicated by a minus ("-")  
Triallelic SNPs or higher variability are separated by a slash between each base.  
**Do not use** ambiguity code instead!

Examples: [A/G], [A/G/C], [-/A], [G/-], [AGC-/A], [A/G/C/T]

- SNP surrounding sequences  
The defined SNP should be flanked by at least 200 bases to each side.  
Use upper case letters for regions, which are suitable for PCR primer design. Use lower case letters to exclude sequence portions from primer design (e.g. repetitive sequence, like Alu-repeats, pseudogene portions, known highly conserved sequences from different origins).
- Multiple SNPs in one sequence  
Multiple SNPs can be present in one input sequence. However the minimum distance between two SNPs of interest has to be 17 bases.  
If SNPs in a shorter distance are wished to be analyzed they have to be treated as individual input sequences. Then, each sequence has to be named unique and the other SNP should be presented in ambiguity code letters (N, R, Y, etc.).

Example:

| SNP_ID | SEQUENCE                      |
|--------|-------------------------------|
| SNP-1a | ...ACGGTAGT[A/T]TCGAGGNATG... |
| SNP-1b | ...ACGGTAGTNTCGAGG[C/T]ATG... |

- Secondary SNPs not to be analyzed and unknown sequences  
SNPs or other known variation, which are not to be analyzed, as well as unknown sequence portions should be indicated to ensure specificity of assay design. In such cases ambiguity code letters can be used to prevent overlapping primers.

### **III. Assay Design, Assay Evaluation, Failed Assays**

- Assay design will be performed with MassARRAY<sup>®</sup> assay design software specifically developed for the applied MassARRAY<sup>®</sup> platform ensuring best possible success.
- Assay design of human SNP sequences will also acknowledge available information from genbank data base, e.g. duplications target regions.
- In some cases SNPs may rejected from assay design by the software for several reasons. It is recommended not to analyze these SNPs due to an increased risk of unreliable results. SNPs failed the assay design will not be charged.  
Upon request we are happy to replace rejected SNPs with alternative candidates and fit them into the primary assay design. A fee for additional processing of 50.00 EUR will apply to each new SNP.
- Depending on the service volume designed assay will be tested for performance using 15-31 samples, which can be assigned by the customer.
- Instable assays:  
In rare cases, designed assays may fail in the experiment and/or delivering unreliable data. These results will be immediately reported.  
If customer wishes to remove such assays from the study a setup and evaluation fee of 150,- EUR will be charged to each applicable assay. If these assays are kept in the study, then each regular analysis fee will be applied. However, result liability is in the responsibility of the customer.

For any question, please contact:

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