In-house breeding of inbred strains and gene drift - detection and monitoring by STR genotyping

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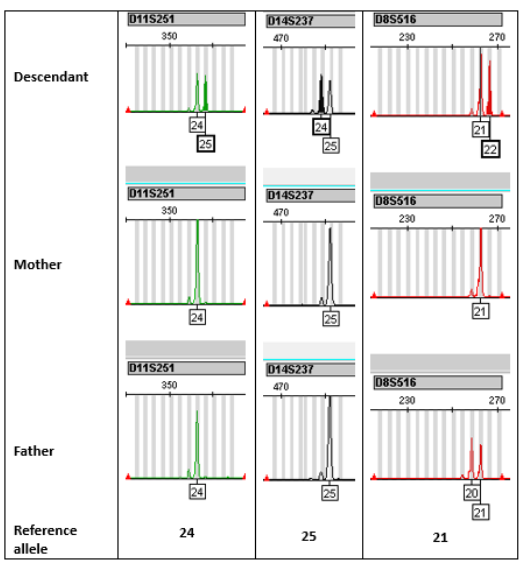
The spontaneous occurence of new mutations is the source of biological evolution and cannot be prevented even under ideal breeding conditions. Whether and how quickly a new mutation becomes established in the population depends crucially on the population size. The smaller the population, the greater the probability that a mutation will become established and subsequently even completely displace the original allele variant. This process is also known as gene drift. In many research institutions, commercially acquired mouse lines are bred in-house for many generations. One is aware of the danger of gene drift, but has no way of evaluating the specific situation without extensive complete sequencing.

GVG Genetic Monitoring GmbH has a genotyping set with a large number of STR markers (microsatellites): These can be used representatively for all types of new mutations for genetic monitoring of gene drift. A comparison of the STR profile of in-house breeding with that of commercially available source animals provides information about the degree of drift that has occurred and can help to make a decision about the need to refresh the mouse line by backcrossing with original animals.

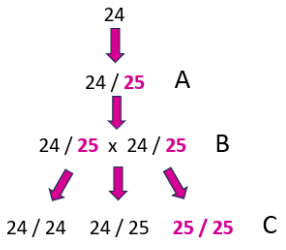
Furthermore, they allow a statement on the quality of in-house breeding per se and allow to uncover a mixed genetic background, e.g. due to undetected breeding issues, and to clearly distinguish them from gene drift.

Direct, visual detection of new mutations (STR markers) and determination of the reference STR genotype of a breeding.



From original animals of a breeder or an in-house breeding, 3-5 individuals are completely genotyped. For each STR marker, the most frequently detected allele is determined. This allele is defined as the reference allele of the respective marker. Of all alleles, it has the highest probability of establishing itself as a homozygous allele in the next generations. The complete set of reference alleles of all STR markers results in the reference genotype.

Gene drift



A new mutation is a one-step event (A - allele 25) and must first be inherited by further animals. Only by mating two heterozygous offspring (B) the initial allele 24 can be completely replaced by allele 25 (probability 25%).

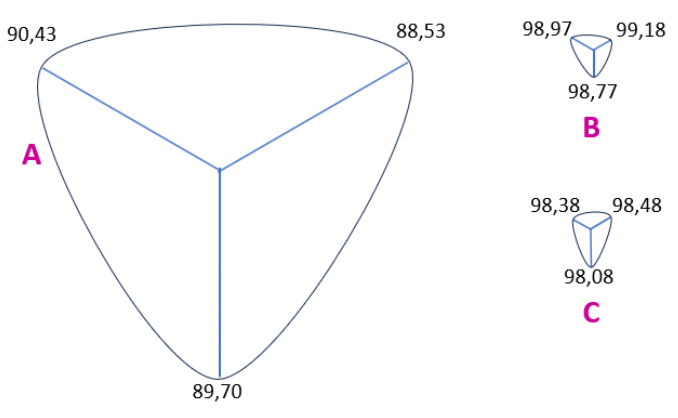
Summary:

- The gene drift of an in-house breeding can be measured and visually displayed
- The quality of a breeding can be estimated
- Breeding errors can be detected quickly and reliably

- A genotyping set containing the approximately 50 most suitable STR markers for determining gene drift is under validation and will allow rapid and cost-effective analysis of in-house breeding.

Evaluation of the quality of a breeding in the animal house and the evidence of breeding issues or incomplete backcrossing.

The genetic variance of single individuals compared to the reference genotype (=100%) is a quality feature for the assessment of breeding in the animal house and can be presented comparatively as the size of the area of the dispersion body. Values with an agreement above 97% are to be assessed as very good. Original animals from the breeder also differ with respect to STR profile and show values in the range of 97-99% (C57BL/6) with respect to the reference genotype. Original animals from BALB/c vary more (only about 90%).



A - An unusually high number of heterozygous STR markers and resulting values below 90% indicate a mixed genetic background or a recent breeding issue.

B und C - Two examples of exemplary breeding based on the values of three individual animals: Low variation with respect to the reference genotype in original animals of C57BL/6J (B) and C57BL/6JBomTac (C).

Calculation method: The detected genotype is evaluated numerically for each STR marker as follows: 1 - homozygous, identical to reference allele, 0.75 - heterozygous, 0 - homozygous, but different from reference allele. The sum of the calculated values over all STR markers is divided by the number of markers. Note: For heterozygotes, 0.5 was not taken because the step from heterozygous to homozygous (new allele) requires further breeding over several subsequent generations (see Fig. for gene drift).

Detection of the degree of genetic drift in in-house breedings

The current gene drift status of a mouse line is determined by matching the in-house reference genotype (B and C) with that of the original strain (A). The deviation from the reference genotype (A) is 4.2% for B, and 10.0% for C.
Numbers in magenta: The values for the individual animals when matched with the reference genotype A (Charles River) also document the drifting away of the breeding.

Comparison of 3 different breedings of C57BL/6J:
A – Charles River original animals
B – in-house breeding with beginning gene drift
C – commercial supplier of C57BL/6J (Poland) with significant gene drift