Product information

GVG Genetic Monitoring

Genetic monitoring of laboratory mice using highly informative STR marker panels

The unclear or misclassified genetic background of laboratory rodents or a lack of strain awareness causes a number of difficulties in performing or reproducing scientific experiments. Until now, fine definition and genetic differentiation between strains and substrains of inbred mice have been a challenge.

GVG Genetic Monitoring (GVG GM) has developed a new method for the highly accurate genotyping of mice. The technology is based on the use of specific genetic markers well known from forensic genetics. In a nutshell, we have adapted the analysis of genetic fingerprinting data for use with laboratory animals.

GVG GM's genetic monitoring platform is based on chromosomal panels of highly informative STR markers (STR – short tandem repeats, microsatellites) with tetranucleotide repeating units. Compared to SNPs (single nucleotide polymorphisms), the estimated mutation rate is up to 10,000 times higher. This favours the occurrence of new alleles, allowing differentiation between closely related substrains of laboratory mice and even between single individuals of an identical inbred strain. GVG GM's genotyping platform can be applied to any mouse strain or substrain.

Applications

- Genetic background check of inbred animals
- Backcrossing from a mixed genetic background to a defined substrain
- Speed congenics between any strains or between different substrains of same inbred strain
- Unknown localization of transgenic marker: identification of the chromosome and its approximate localization
- Elimination of "off-target" sites in CRISPR/Cas9-generated animals
- Verification of correct Y-chromosome in transgenic lines
- Parentage verification

Our service: Fast results, customer-friendly presentation of analysis data

- Results within 10 working days
- Analysis data in customer-friendly tabular form and as an easy-to interpret karyogram (see example overleaf)

We'd be delighted to explain to you the details of our method, work with you to plan your project, and put forward an attractive proposal. Just get in touch with us!

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

What are short tandem repeats (STRs)?

STRs are regions of genomic DNA with tandemly arranged repeating units of 2–6 nucleotides, e.g. (CA)n, (TAA)n, (GATA)n. Example: 7 GATA repeating units / Allele 7 (shown in blue)

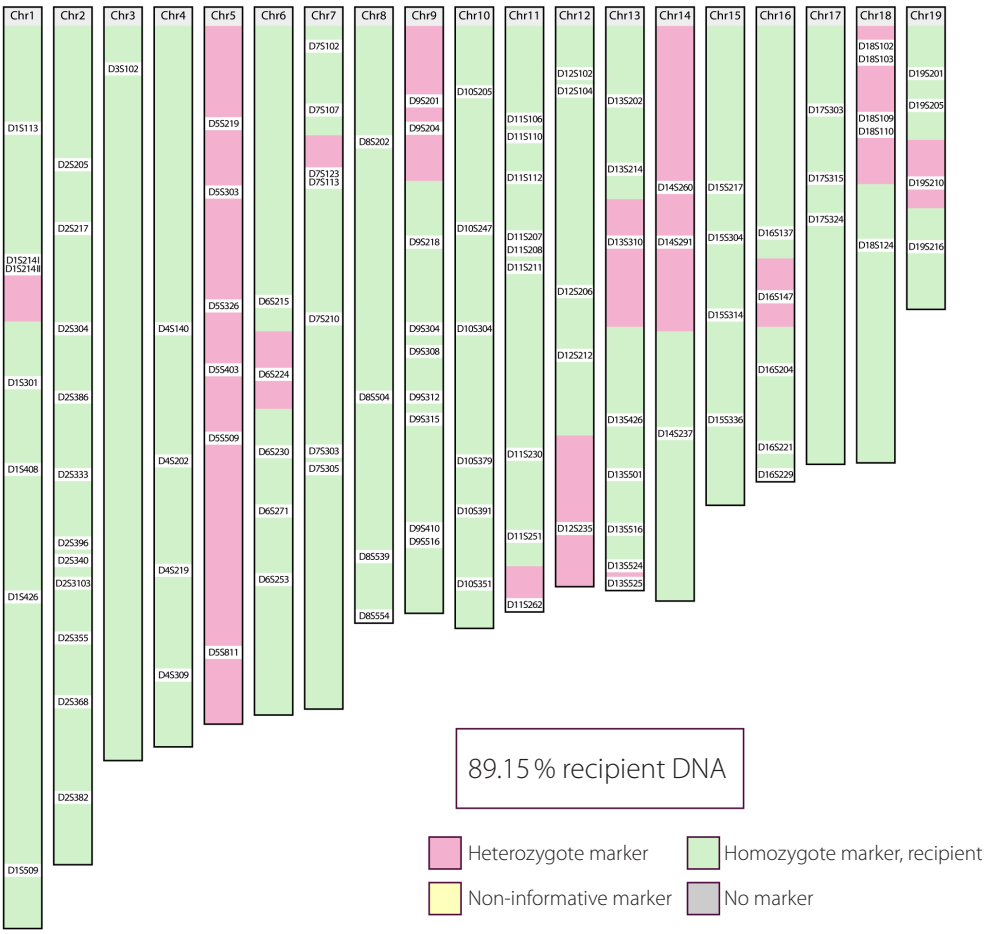
1 2 3 4 5 6 7

TCCCAAGCTCTTCCTCTTCAGA **GATA GATA GATA GATA GATA GATA GATA** TATTGACAG**TACAGATGCACAC**

Using flanking primer sequences (underlined), the STR locus can be PCR-amplified. PCR products are separated on a DNA analyzer and the size of alleles is determined. Different alleles vary from each other in size by increments of four nucleotides.

Example: Speed congenics

Karyogram of the N3 generation (89.15 % recipient DNA): A self-explanatory genomic overview of the localization of remaining heterozygous DNA regions. The positions of all the informative STR loci used to distinguish between donor and recipient DNA are included.



GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

Application note

GVG Genetic Monitoring

Speed congenics using highly informative STR marker panels

Using multiplex PCR methods and technologies developed by GVG Genetic Monitoring we offer a new approach for speed congenics projects which is more flexible and more efficient for both targeted breeding and the transfer of transgenic characteristics to different mouse lines.

Method

Speed congenics (also MASP, marker assisted selection protocol) is used for the accelerated creation of genetically modified mouse lines (knockout, knockin or transgene) by sequential backcrossing of modified gene from donor to recipient. Offspring at each generation are genotyped using an STR marker panel (STR – short tandem repeat, microsatellite) of evenly distributed loci along the chromosomes. The individual with the highest level of recipient genomic DNA is identified and used to breed the next generation.

Defined STR loci with high mutation rates can be applied to distinguish not only between different strains, but also between substrains of the identical inbred strain. This allows speed congenics technology to be applied to new fields such as the monitoring of backcrossing between substrains of the same inbred strain or from a mixed genetic background to a pure substrain background.

Standard marker panel with 246 STR loci

For speed congenics projects GVG GM has developed a standard marker panel with 246 STR loci that is extremely flexible and can be applied to any combinations of inbred mouse strains or substrains.

- More than 200 STR loci with differing alleles between different inbred strains
- More than 120 STR loci with differing alleles between any substrain of C57BL/6J and C57BL/6N

Our service: Fast results, customer-friendly presentation of analysis data

- Results within 10 working days
- Analysis data in customer-friendly tabular form and as an easy-to interpret karyogram (see examples overleaf)

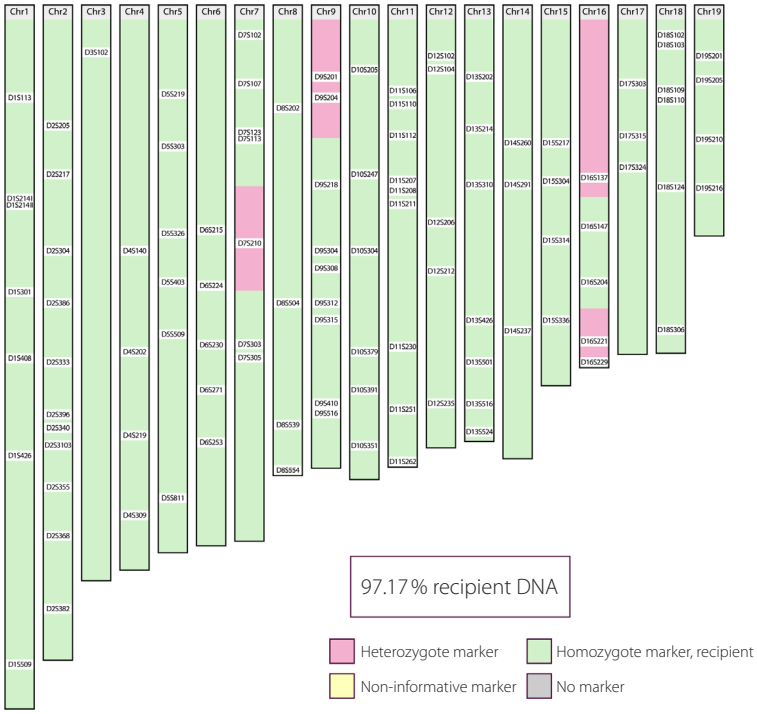
We'd be delighted to explain to you the details of our method, work with you to plan your speed congenics project, and put forward an attractive proposal. Just get in touch with us!

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

Example 1: Karyogram of the N2 generation

Backcrossing from a mixed C57/BL6 & 129 genetic background to C57BL/6JHsd.

Example 2: Karyogram of the N4 generation



Backcrossing from a mixed C57/BL6 & 129 genetic background to C57BL/6JHsd.

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

Application note

GVG Genetic Monitoring

Genetic background check of inbred animals using highly informative STR marker panels

The unclear or misclassified genetic background of laboratory rodents or a lack of strain awareness causes a number of difficulties in performing or reproducing scientific experiments. Until now, fine definition and genetic differentiation between strains and substrains of inbred mice have been a challenge.

GVG Genetic Monitoring (GVG GM) has developed a new method for the highly accurate genotyping of mice. The technology is based on the use of specific genetic markers well known from forensic genetics. In a nutshell, we have adapted the analysis of genetic fingerprinting data for use with laboratory animals.

A set of highly informative STR (short tandem repeat) markers covering the 19 autosomes as well as X and Y chromosomes have been identified. Inbred strains and substrains can be distinguished with a large number of informative markers, and mixed genetic profiles can be easily detected.

Method

- Genotyping with GVG GM's standard marker panel of 246 STR loci, comparison with strain- or substrain-specific consensus alleles
- Determination of Y-chromosome STR-haplotype
- Checking for the presence of C57BL/6-specific mutations

Our service: Fast results, customer-friendly presentation of analysis data

- Genotyping results within 10 working days
- Analysis data in customer-friendly tabular form (see example overleaf)

We'd be delighted to explain to you the details of our method, work with you to plan your project, and put forward an attractive proposal. Just get in touch with us!

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

Example: Characterization of C57BL/6-derived inbred mice

Service 1: Genotyping of key markers

Combination of C57BL/6-specific markers with Y-chromosomal STR haplotype allows the fast assignment of inbred mice to strains and substrains. The mixed genetic background of BL6/J and BL/6N can be detected easily. Service 1 provides no further information about other chromosomal regions (see Service 2).

Crb1rd8		DIP686		DIP1606			*Snca1*		*Nnt*		C57BL/6
Chr 1/139,2		Chr 6/86,4		Chr 16/6,1			Chr 6/60,7		Chr 13/119,3		Chrom. No/MBp
wt	*mut*	wt	mut	wt	mut-A	mut-B	wt	mut	wt	mut	
	+										all C57BL/6N
	+	+				+	+		+		NTac, NRj
	+	+			+		+		+		NHsd, NCrl, NTjl
			+								all C57BL/6J
+			+		+		+			+	JCrl, JRj, JTjl
+			+		+			+	+		JOlaHsd
+			+		+		+		+		JRccHsd
+		+		+			+		+		no C57BL/6

Identification of C57BL/6 strain- and substrain-specific markers

	DYS101	DYS102	DYS201	DYS204	DYS301	DYS601
JCrl	21-24	24	28	20	16	14-16
JRj	21-23	24	28	20	16	14-16
JOlaHsd	21-24	23	28	20	16	14-16
JRccHsd	21-24	24	28	21	15	15-16
JBomTac	21-24	24	27	20	16	0-0
NCrl	21-24	22	28	21	16	14-16
NHsd	21-24	23	28	21	16	14-16
NTac	24-24	23	28	21	17	14-16
NRj	21-24	23	28	21	17	14-15

Y-chromosomal STR loci. Each substrain has its characteristic haplotype (combination of alleles)

Service 2: Genotyping of key markers and full STR genotyping (246 markers)

Combination of key markers with full STR genotyping allows the reliable assessment of the complete genetic background. Mixed profiles are identified and the mixture ratio can be calculated.

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

Application note

GVG Genetic Monitoring

Elimination of "off-target" sites in CRISPR/Cas9-generated animals using highly informative STR marker panels

CRISPR/Cas9 system is a convenient genome editing tool. However, due to its tendency to cut DNA not only in the target region but also in sequences similar to the target, "off-target" effects occur. The localization and subsequent removal of potential "off-targets" in transgenic mice is labour-intensive and time-consuming. By combining CRISPR/Cas9 technology with speed congenics using GVG GM's standard STR panel "off-targets" can be eliminated without prior knowledge of their localization.

Principle

Expample for a generation of a C57BL/6NCrl mouse with a CRISPR/Cas9 mutation

Step 1

- Generation of the desired CRISPR/Cas9 mutation in one of the C57BL/6J substrains
- Potential "off-targets" would have a "BL/6J" genetic background.

Step 2

- Backcrossing to substrain C57BL/6NCrl by speed congenics, positive selection for CRISPR/Cas9 mutation
- Since all genomic parts of "BL/6J" are replaced by C57BL/6NCrl, all "off-targets" are effectively removed from the genome.

Standard marker panel with 246 STR loci

GVG GM has developed a standard marker panel with 246 STR loci that is extremely flexible and can be applied to any combinations of inbred mouse strains or substrains.

- About 50 % can distinguish between different substrains of C57BL/6J and C57BL/6N
- About 85 % can distinguish between different inbred strains

Our service: Fast results, customer-friendly presentation of analysis data

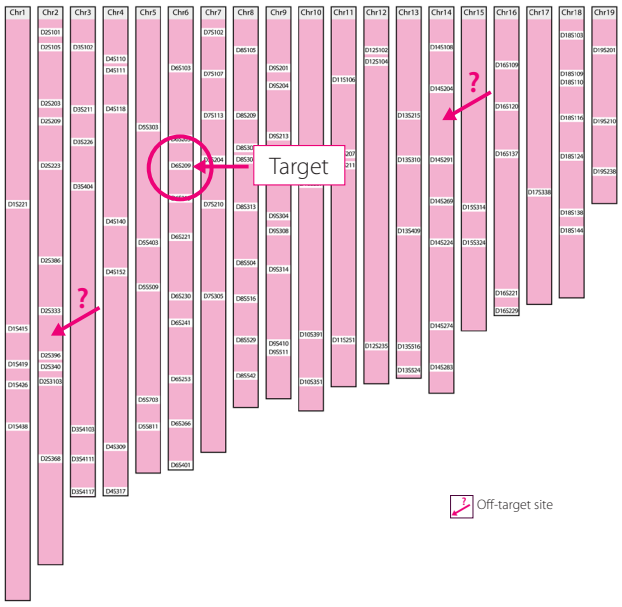
- Genotyping results within 10 working days
- Analysis data in customer-friendly tabular form and as an easy-to interpret karyogram (see examples overleaf)

We'd be delighted to explain to you the details of our method, work with you to plan your project, and put forward an attractive proposal. Just get in touch with us!

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

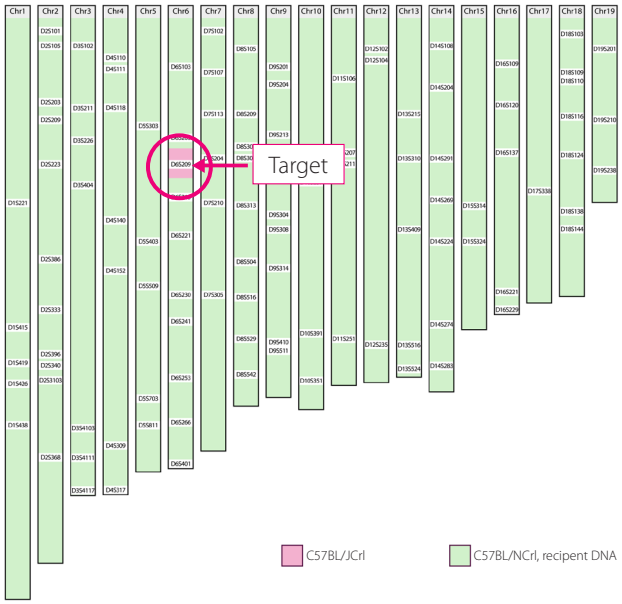
Ex.: Generation of a C57BL/6NCrl mouse with a CRISPR/Cas9 mutation

Figure 1: Karyogram of substrain C57BL/6JCrl



CRISPR/Cas9-mutated mouse of substrain C57BL/6JCrl with target region on chromosome 6 and two additional "off-target" regions on chromosomes 2 and 14

Figure 2: Karyogram of N5 generation, genetic background C57BL/6NCrl



CRISPR/Cas9-mutated mouse after five backcrossing generations. Thanks to the replacement of "BL/6J" genetic background by recipient DNA of substrain C57BL/6NCrl, all "off-target" sites have been eliminated.

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

Application note

GVG Genetic Monitoring

Unknown insertion site of transgenic marker: Identification of the chromosome and approximate position on chromosome

Depending on the method used to generate transgenic animals, the genomic position of the transgenic locus might be unknown. Identifying the integration site is labour-intensive and time-consuming. Using the GVG GM genotyping platform, the chromosome with the transgenic marker can be detected easily and the appropriate position on the chromosome can be postulated.

Method

- Complete STR genotyping of animals of one generation with the GVG GMstandard STR panel (usually 10–15 target-positive animals of the N2 or N3 generation)
- Identification of informative STR loci
- Comparative analysis of each chromosome
- Identification of genomic regions lacking crossing over
- Identification of individuals best suited for further breeding

Standard marker panel with 246 STR loci

GVG GM has developed a standard marker panel with 246 STR loci that is extremely flexible and can be applied to any combinations of inbred mouse strains or substrains.

- About 50 % can distinguish between different substrains
- About 85 % can distinguish between different inbred strains

Our service: Fast results, customer-friendly presentation of analysis data

- Genotyping results within 10 working days
- Analysis data in customer-friendly tabular form (see examples overleaf)

We'd be delighted to explain to you the details of our method, work with you to plan your project, and put forward an attractive proposal. Just get in touch with us!

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

Example

Tabular data of genotyping results for chromosomes 9 and 10 in nine animals. STR markers are arranged according to their chromosomal position beginning from the centromere (on top) to the distal part. All chromosomes were examined for the presence of green-marked genomic regions (successfully backcrossed parts of genomic DNA due to crossing-over events). Red-marked STR loci are still heterozygous, consisting of donor and recipient DNA.

				animal	1	2	3	4	5	6	7	8	9
1	D9S201	Chr9	14.69		14 / 14	13 / 14	13 / 14	13 / 14	13 / 13	13 / 14	13 / 14	13 / 14	14 / 14
2	D9S204	Chr9	21.45		18 / 18	15 / 18	15 / 18	15 / 18	15 / 18	18 / 18	15 / 18	15 / 18	18 / 18
3	D9S211	Chr9	37.19		19 / 19	19 / 19	18 / 19	18 / 19	18 / 19	18 / 18	18 / 19	18 / 19	18 / 18
4	D9S213	Chr9	37.53		18.2 / 23	18.2 / 23	18.2 / 23	18.2 / 23	18.2 / 23	23 / 23	18.2 / 23	23 / 23	23 / 23
5	D9S218	Chr9	46.44		18 / 19	18 / 19	18 / 19	18 / 19	18 / 19	18 / 18	18 / 19	18 / 18	18 / 18
6	D9S219	Chr9	47.17		22.2 / 25.2	22.2 / 25.2	22.2 / 25.2	22.2 / 25.2	22.2 / 25.2	25.2 / 25.2	22.2 / 25.2	25.2 / 25.2	25.2 / 26.2
7	D9S304	Chr9	64.81		12 / 21	12 / 21	12 / 21	12 / 21	12 / 21	12 / 12	12 / 21	12 / 12	12 / 21
8	D9S308	Chr9	69.95		17.3 / 21	17.3 / 21	17.3 / 21	17.3 / 20	17.3 / 21	21 / 21	21 / 21	21 / 21	17.3 / 21
9	D9S312	Chr9	80.21		15 / 19	19 / 19	15 / 19	15 / 19	15 / 19	19 / 19	19 / 19	19 / 19	15 / 19
10	D9S323	Chr9	100.58		19 / 24	24 / 24	19 / 24	19 / 24	19 / 24	18 / 24	24 / 24	19 / 24	19 / 24
11	D9S410	Chr9	107.86		13 / 14	13 / 13	13 / 14	13 / 14	13 / 14	13 / 14	13 / 13	13 / 14	13 / 14
12	D9S516	Chr9	108.96		14 / 18	15 / 18	14 / 18	14 / 18	14 / 18	14 / 18	18 / 18	14 / 18	14 / 18
13	D9S511	Chr9	108.96		20 / 21	20 / 20	20 / 21	20 / 21	20 / 21	20 / 21	20 / 20	21 / 21	20 / 21

All chromosomes without target:
Each STR-locus is marked green at least one time. (green fields of No 1, 2 and 6 or 7, 8 and 9 do cover complete chrom. 9)

1	D10S205	Chr10	12.91		20 / 20	3 / 20	20 / 20	20 / 20	3 / 20	3 / 20	3 / 20	3 / 20	19 / 20
2	D10S213	Chr10	28.11		20 / 21	15 / 20	20 / 21	20 / 21	15 / 20	15 / 20	15 / 20	15 / 20	20 / 20
3	D10S247	Chr10	42.91		13 / 13	13 / 14	13 / 13	13 / 13	13 / 14	13 / 14	13 / 14	13 / 14	13 / 14
4	D10S218	Chr10	43.39		23.2 / 23.2	23.2 / 24.2	23.2 / 23.2	23.2 / 23.2	23.2 / 23.2	23.2 / 23.2	23.2 / 23.2	23.2 / 24.2	23.2 / 24.2
5	D10S257	Chr10	53.78		43 / 43	42 / 43	43 / 43	43 / 43	43 / 43	43 / 43	43 / 43	42 / 43	42 / 43
6	D10S304	Chr10	65.19		23.2 / 24.2	23.2 / 24.2	23.2 / 23.2	23.2 / 23.2	23.2 / 24.2	23.2 / 24.2	23.2 / 24.2	23.2 / 24.2	23.2 / 24.2
7	D10S391	Chr10	104.79		34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2
8	D10S344	Chr10	115.16		21 / 22	21 / 22	21 / 22	21 / 22	21 / 22	21 / 22	21 / 22	21 / 22	21 / 22
9	D10S351	Chr10	121.31		15 / 20	15 / 20	15 / 20	15 / 20	15 / 20	15 / 20	15 / 20	15 / 20	15 / 20

Chromosome with target:
Red fields for all animals. Target-position is the distal part of chrom. 10

1	D10S205	Chr10	12.91		20 / 20	3 / 20	20 / 20	20 / 20	3 / 20	3 / 20	3 / 20	3 / 20	19 / 20
2	D10S213	Chr10	28.11		20 / 21	15 / 20	20 / 21	20 / 21	15 / 20	15 / 20	15 / 20	15 / 20	20 / 20
3	D10S247	Chr10	42.91		13 / 13	13 / 14	13 / 13	13 / 13	13 / 14	13 / 14	13 / 14	13 / 14	13 / 14
4	D10S218	Chr10	43.39		23.2 / 23.2	23.2 / 24.2	23.2 / 23.2	23.2 / 23.2	23.2 / 23.2	23.2 / 23.2	23.2 / 23.2	23.2 / 24.2	23.2 / 24.2
5	D10S257	Chr10	53.78		43 / 43	42 / 43	43 / 43	43 / 43	43 / 43	43 / 43	43 / 43	42 / 43	42 / 43
6	D10S304	Chr10	65.19		23.2 / 24.2	23.2 / 24.2	23.2 / 23.2	23.2 / 23.2	23.2 / 24.2	23.2 / 24.2	23.2 / 24.2	23.2 / 24.2	23.2 / 24.2
7	D10S391	Chr10	104.79		34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2	34.3 / 38.2
8	D10S344	Chr10	115.16		21 / 22	21 / 22	21 / 22	21 / 22	21 / 22	21 / 22	21 / 22	21 / 22	21 / 22
9	D10S351	Chr10	121.31		15 / 20	15 / 20	15 / 20	15 / 20	15 / 20	15 / 20	15 / 20	15 / 20	15 / 20

Nos. 3 and 4 with the closest point of crossing over are recommended for further breeding

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

Application note

GVG Genetic Monitoring

Genotyping of the Y-chromosome of inbred animals using highly informative STR marker panel

The unclear or misclassified genetic background of laboratory rodents or a lack of strain awareness causes a number of difficulties in performing or reproducing scientific experiments.

The Y-chromosome of mice plays a crucial role in sex determination, gender equilibrium and male fertility. In addition it acts as mediator of physiological traits like behavior, metabolism, susceptibility to infections, autoimmune and heart dieases or weight and size of adult animals. A father's "wrong" Y-chromosome can result in clear differences even in phenotypes of daughters (Nelson et al, 2010).* Due-to errors in the breeding strategy (recipient male not crossed in), a considerable percentage of male transgenic animals harbors a Y-chromosome which differs from the targeted genetic background.

GVG GM has identified a series of highly informative, strain- and substrain-specific STR-markers (Short Tandem Repeats, microsatellites) covering the Y-chromosome. These markers can be used for validation of strains, their substrains and parentage lines to ensure consistency with genetic background of reference strains.

Method

- Genotyping by GVG GM's Y-chromosomal panel of STR-loci
- Determination of Y-chromosome STR-haplotype and comparison with haplotypes of different strains or substrains
- Checking for the presence of C57BL/6-specific mutations

Our service: Fast results, customer-friendly presentation of analysis data

- Genotyping results within 10 working days
- Analysis data in customer-friendly tabular form (see example overleaf)

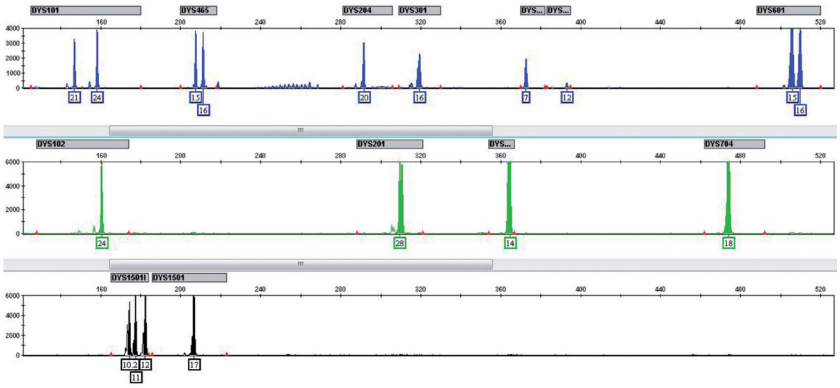
We'd be delighted to explain to you the details of our method, work with you to plan your project, and put forward an attractive proposal. Just get in touch with us!

* Nelson et al. 2010. Transgenerational genetic effects of the paternal Y chromosome on daughter´s phenotype. Epigenomics. 2(4) 513–521.

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de

Example: Characterization of C57BL/6-derived inbred mice

Step 1: Genotyping of Y-chromosomal STR-markers



Y-chromosomal STR-genotyping: Circles characterize strain-specific loci, others are used to differentiate between substrains.

	DYS101	DYS102	DYS201	DYS204	DYS301	DYS601
JCrl	21-24	24	28	20	16	14-16
JRj	21-23	24	28	20	16	14-16
JOlaHsd	21-24	23	28	20	16	14-16
JRccHsd	21-24	24	28	21	15	15-16
JBomTac	21-24	24	27	20	16	0-0
NCrl	21-24	22	28	21	16	14-16
NHsd	21-24	23	28	21	16	14-16
NTac	24-24	23	28	21	17	14-16
NRj	21-24	23	28	21	17	14-15

Each substrain has its characteristic combination of alleles (Y-STR haplotype). Two alleles: PCR-primers target two different sites.

Step 2: Genotyping of C57BL/6-specific markers

The combination of C57BL/6-specific markers with Y-chromosomal STR haplotype allows fast assignment of inbred mice to strains and substrains. Mixed genetic background of BL6/J and BL/6N as well as of non-BL/6 can be detected easily.

Crb 1rd8		DIP 686		DIP1606			*Snca 1*		*Nnt*		Substrain
Chr 1 / 139,2		Chr 6 / 86,4		Chr 16 / 6,1			Chr 6 / 60,7		Chr 13 / 119,3		Chrom. No / MBp
wt	mut	wt	mut	wt	mut-A	mut-B	wt	mut	wt	mut	
											C57BL/6N
	+	+				+	+		+		NTac, NRj
	+	+			+		+		+		NHsd, NCrl, NTjl
											C57BL/6J
+			+		+		+			+	JCrl, JRj, JTjl
+			+		+			+	+		JOlaHsd
+			+		+		+		+		JBomTac, JRccHsd
+		+		+			+		+		no C57BL/6

Identification of C57BL/6 strain- and substrain-specific markers (deletion-insertion-polymorphism – DIP)

GVG Genetic Monitoring GmbH
Deutscher Platz 5 I 04103 Leipzig I Germany I Tel: +49 (0) 341 3929 8690 I info@gvg-gm.de I www.gvg-gm.de