

GENETIC BACKGROUND CHARACTERIZATION

MOST COMPREHENSIVE SNP TESTING IN THE DRUG DISCOVERY INDUSTRY

Introduction

Genetically defined mouse and rat models, including genetically engineered, inbred, congenic, and outbred strains, are powerful tools to model human diseases and conditions. When working with research models, it is extremely important to know the genetic background, as it can have profound influence on phenotype penetrance and expressivity. To assure the reproducibility of experimental results and avoid confounding effects, the genetic background of each model should be carefully characterized before commencing any study or breeding program. Periodic monitoring of the genetic background is also recommended to ensure the genetic integrity of the line.

Why Perform Background Characterization?

- ▶ Assure genetic integrity of the model
- ▶ Verify inbred genetic background
- ▶ Assess genetic diversity of outbred strains
- ▶ Detect and prevent genetic contamination and drift
- ▶ Ensure data reproducibility over time
- ▶ Preserve the genetic background and the mutation(s) of the strain
- ▶ Facilitate marker-assisted breeder selection for speed congenic backcross
- ▶ Quantitative Trait Locus (QTL) mapping

SNP TESTING PROGRAM

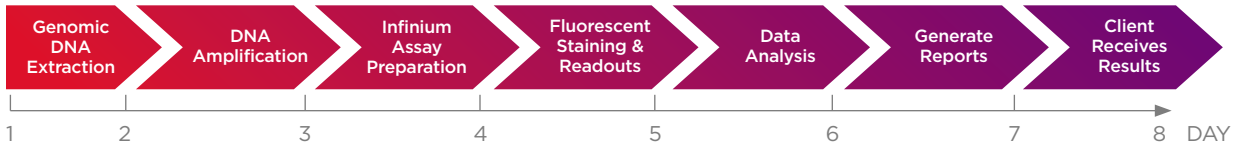
Taconic Biosciences has developed and optimized several custom-designed mouse and rat Single Nucleotide Polymorphism (SNP) panels for characterizing strain background, monitoring genetic quality, or developing congenic lines. Unique and informative SNP markers have been carefully selected for each panel. Taconic offers SNP-based genetic monitoring and genome scanning services using the Illumina Infinium® Beadchips technology and testing platform. The Infinium® platform is a high throughput SNP genotyping system based on direct hybridization of genomic targets to array-bound sequences. Allelic specificity is confirmed by single base extension to incorporate a labelled nucleotide. Upon fluorescent staining, the labelled nucleotide is detected by the Illumina iScan System and subsequently analyzed by the GenomeStudio Software, which has 99% call rates and 99.9% reproducibility.

Advantages

- ▶ Only company to provide a consistent testing schedule for critical data
- ▶ The best panel to provide crucial differentiation among Black 6 substrains
- ▶ Largest commercial panel for rats
- ▶ Only company to provide data analysis and reporting by PhD level geneticists, including an in-depth analysis and recommendation on how to progress program forward

SNP TESTING PROCESS

The Workflow and Timeline



When SNP Testing Is Recommended?

- ▶ When receiving a new GEM line from a collaborator
- ▶ Not sure which C57BL/6 substrain is used
- ▶ Before establishing any new breeding colony
- ▶ Evaluating backcrossing to develop congenic lines
- ▶ Periodic monitoring of a breeding colony
- ▶ After noticing unusual coat color or other strange phenotypes

Deliverables

The client will receive a genetic Background Characterization Report that includes the following:

- ▶ Genetic recommendations and access to PhD level geneticists
- ▶ Data arranged based on chromosome number
- ▶ Samples compared against a specified inbred strain's SNP profile
- ▶ Assessment of the specific genetic background
- ▶ Results presented in terms of % of genetic background content and backcross number equivalent (NE#)

SNP PANEL SELECTION GUIDE

SNP Panel	Application
Mouse GenMon Panel	Mouse genetic background monitoring
Mouse C57BL/6 Substrain Panel	Differentiating B6 Substrain (C57BL/6NTac, C57BL/6JBomTac, and C57BL/6J)
Mouse Genome Scanning Panel	Mouse speed congenics or genetic background characterization
Rat GenMon Panel	Rat genetic background monitoring
Rat Genome Scanning Panel	Rat speed congenics or genetic background characterization

Table 1: SNP panels available at Taconic and their applications.

Mouse C57BL/6 Substrain Panel

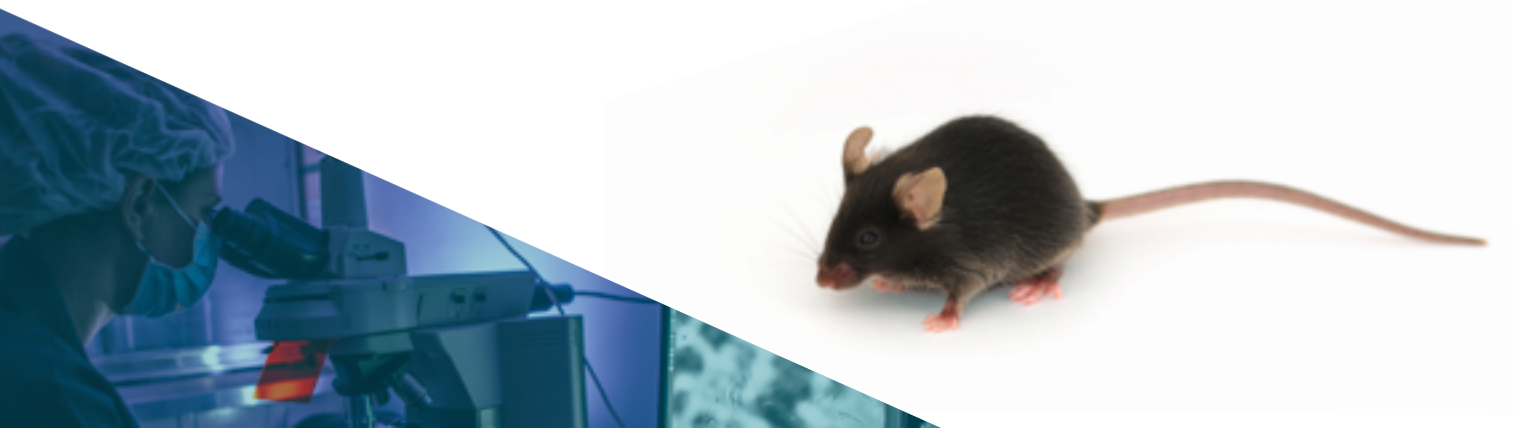
The B6 Substrain Panel consists of 236 SNP markers that allow the differentiation of three different C57BL/6 substrains: C57BL/6NTac, C57BL/6JBomTac, and C57BL/6J. To be able to use this panel, the genetic background of the strain should be 99.9% in the C57BL/6 background. It is recommended to first use the Mouse Genome Scanning Panel to verify the genetic background.

Mouse GenMon Panel

The Mouse Genetic Monitoring Panel has 96 SNP markers that have been optimized to differentiate among mouse strains produced by Taconic. This panel is used for QC verification or routine testing of strains that share the same housing location and SNP profile has previously been characterized. It is not recommended to use this panel for strain background characterization or speed congenics.

B6 Substrain Panel	
Substrains	# Informative Markers
C57BL/6NTac vs C57BL/6J	236
C57BL/6NTac vs C57BL/6JBomTac	129
C57BL/6J vs C57BL/6JBomTac	107

Table 2: SNP marker distribution for the three C57BL/6 substrains.



Mouse Genome Scanning Panel

This is the most comprehensive mouse SNP panel developed by Taconic with 2050 informative markers across the commonly used inbred strains including the biomarkers typically requested by researchers. The average distance between SNP markers per chromosome is 1.0 to 1.3 Mb. This panel can also be used for accelerated backcrossing or speed congenics.

Mouse Genome Scanning Panel														
	C57BL/6NTac	C57BL/6J BomTac	C57BL/6J	BALB/c AnNTac	129S6/SvEvTac	C3H/He NTac	CBA/J	CBSCBG	CB17SC	DBA/1J BomTac	DBA/2 NTac	FVB/NTac	NOD/MrkTac	SJL/JCr NTac
C57BL/6NTac	0	135	246	1148	1154	1230	1196	1123	1141	1145	1172	1094	1079	1058
C57BL/6JBomTac	135	0	111	1245	1252	1325	1291	1216	1238	1242	1269	1189	1176	1157
C57BL/6J	246	111	0	1354	1361	1434	1400	1325	1347	1351	1378	1298	1287	1267
BALB/cAnNTac	1148	1245	1354	0	858	618	680	31	9	829	846	812	777	780
129S6/SvEvTac	1154	1252	1369	858	0	829	843	873	861	859	902	779	798	740
C3H/HeNTac	1230	1325	1434	618	829	0	263	621	615	623	616	782	804	804
CBA/J	1196	1291	1400	680	843	263	0	695	685	559	584	767	798	810
CBSCBG	1123	1216	1325	31	873	621	695	0	22	834	851	823	780	797
CB17SC	1141	1238	1347	9	861	615	685	22	0	835	853	815	780	783
DBA/1JBomTac	1145	1242	1351	829	859	623	559	834	835	0	123	817	805	803
DBA/2NTac	1172	1189	1378	846	902	616	584	851	853	123	0	826	796	812
FVB/NTac	1094	1189	1298	812	779	782	767	823	815	817	826	0	622	484
NOD/MrkTac	1079	1176	1287	777	798	804	798	780	780	805	796	622	0	622
SJL/JCrNTac	1058	1157	1267	780	740	804	810	797	783	803	812	584	622	0

Table 3: SNP marker differences between mouse strains. Number value indicates the number of markers different between the strain in the left column and the strain in the top row.

2 to 10 Informative Markers: Basic Strain Identification
 11 to 95 Informative Markers: Strain Identification
 96 to 400 Informative Markers: Accelerated Backcrossing
 Over 400 Markers: High Resolution QTL

Biomarkers Tested	Allele Discrimination	Comments
Ptprc	Variant a or b	Protein Tyrosine Phosphatase, receptor type C, Variant a, also known as CD45.1, Ly5<a> Variant b, also known as CD45.2, Ly5
Tyrp1	b mutation or WT	Tyrosinase-related protein 1 Variant b, point mutation
Lepr	db mutation or WT	Leptin receptor rdb, point mutation (diabetes)
Pde6b	rd1 mutation or WT	Phosphodiesterase 6B, CGMP, rod receptor, beta polypeptide rd1, spontaneous point mutation (retinal degeneration 1)
Lep	ob mutation or WT	Leptin ob, spontaneous point mutation (obese)
Tyr	c mutation or WT	Tyrosinase c, point mutation (albino)
Foxn1	nu mutation or WT	Forkhead box N1 nu, spontaneous bp deletion (nude)
Nnt	C57BL/6J mutation or WT	Nicotinamide nucleotide Transhydrogenase C57BL/6J, spontaneous 17 Kbp deletion
Prkdc	scid mutation or WT	Protein kinase DNA activated, catalytic polypeptide scid, spontaneous point mutation

Table 4: Common biomarkers included in the Mouse Genome Scanning Panel.

Mouse 2050 SNP Panel		
Chromosome #	#Markers	Average Distance in Mbp
1	169	1.1
2	140	1.1
3	127	1.1
4	109	1.3
5	130	1.1
6	130	1
7	127	1.1
8	98	1.1
9	95	1.1
10	94	1.2
11	93	1.1
12	89	1.1
13	86	1.2
14	88	1.2
15	83	1.1
16	80	1.1
17	78	1.1
18	68	1.2
19	50	1
X	115	1.2
Y	1	N/A

Table 5: Number of SNP markers per chromosome and average distance between SNP markers per chromosome.



Up To 30% Of Genetically Engineered Models In The Research Community Have Genetic Contamination.*



Rat GenMon Panel

The Rat Genetic Monitoring Panel consists of 96 SNP markers and has been optimized to differentiate among rat strains produced by Taconic. This panel is used for QC verification or routine testing of strains that share the same housing location and that their SNP profile has previously been characterized. It is not recommended to use this panel for strain background characterization or for speed congenics.

Rat Genome Scanning Panel

The Rat Genome Scanning Panel has been developed by Taconic with 759 informative SNP markers across the most commonly used inbred rat strains. This panel is also suitable for accelerated backcrossing or speed congenics.

	DA/MolTac	Lew/MolTac	GK/MolTac	F344/NTac	NTac:SHR
DA/MolTac	0	329	293	302	293
Lew/MolTac	329	0	311	401	331
GK/MolTac	293	311	0	375	232
F344/NTac	302	401	375	0	377
NTac:SHR	293	331	232	377	0

Table 6: SNP marker differences between rat strains. Number value indicates the number of markers different between the strain in the left column and the strain in the top row.

Rat SNP Panel		
Chromosome #	# Markers	Average Distance in Mbp
1	74	3.8
2	64	4.1
3	35	4.8
4	43	4.2
5	36	4.8
6	41	3.2
7	41	3.3
8	38	2.7
9	27	5
10	30	3.4
11	39	2.2
12	27	1.2
13	42	2.2
14	33	3.5
15	30	3.8
16	44	2
17	19	5.2
18	28	2.7
19	22	2.8
20	16	3.6
X	30	5.3

Table 7: Number of SNP markers per chromosome and average distance between SNP markers per chromosome.

Taconic PhD level geneticists can assist you in identifying the right panel for your research. Additional strains can be tested based on request. Please contact us via GenMon@taconic.com for a free consultation.

* Results based on 1450 SNP panel findings for genetically modified mice tested by Taconic between 2014-2016.

LEARN MORE AT: TACONIC.COM/GENETICS

US: 1-888-822-6642 | EU: +45 70 23 04 05 | INFO@TACONIC.COM