Genetic Background Detection Report

Report No.	TR-MT-20230506-001
Genotyping Strain	B-NDG mice
Genotyping Department	Biocytogen Jiangsu Co., Ltd. Haimen Testing Center
Report Date	2023.05.06

Genetic Background Detection Report

1 Experimental purpose

Annual genetic background monitoring of B-NDG mice of Biocytogen Jiangsu Co., Ltd.

2 Experiments and methods

2.1 Experimental method

SSLP detection method

2.2 Primer selection

2-4 markers are selected for each chromosome according reference 1 and 2, a total of 60 loci, the average genetic distance is 25.06 cM. Referring to MGI data, although the fragments of these 60 loci are different, compared with C57BL/6, the difference trend is the same, so that they are believed in reliability.

2.3 Animal information

C57BL/6N and NOD-*scid* mice were performed as controls to verify the NOD background of B-NDG mice. Laboratory animal vendors, animal numbers, and animal week ages are detailed in Table 1.

Table 1: Animal information

Strain	Number		Provider	Week age
	♂	♀		
C57BL/6N	0	1	Biocytogen Jiangsu Co., Ltd.	7
NOD-*scid*	0	1	Biocytogen Jiangsu Co., Ltd.	7
B-NDG	3	3	Biocytogen Jiangsu Co., Ltd.	7

2.4 Genomic DNA extraction and PCR amplification of mouse tail

Refer to Appendix 1 for the extraction method of mouse tail genomic DNA.

See Table 2 and Table 3 for the PCR reaction system and procedure.

2.5 Analysis of capillary electrophoresis results

Although the results can be judged by 4% gel electrophoresis in reference 2, according to our experimental experience, Gel electrophoresis is very difficult to clearly distinguish bands about 200bp. In this experiment, the capillary electrophoresis method of reference 1 is used for detection.

Table 2: PCR Reaction of KOD-FX DNA Polymerase(Total volume:20 μL)

Reaction component	Volume (μL)	Final concentration
ddH_2O	2.4	—
2×FX buffer	10	1×
2 mM dNTPs	4	400 μM each
10 μM Primer-F（FAM）	0.6	0.3 μM
10 μM Primer-R	0.6	0.3 μM
1.0 U/μL FX DNA Polymerase	0.4	0.02 U/μL
100-200 ng/μL Template DNA	2	10-20 g/μL

Table 3: KOD-FX DNA Polymerase PCR Procedure

Step	Temp	Time	Cycles
1	94℃	2 min	1
2	98℃	10 sec	30
3	62℃	30 sec	
4	68℃	30 sec	
5	68℃	10 min	1
6	4℃	hold	1

3 Result analysis and conclusion

According to the feedback results of capillary electrophoresis, it is found that the size of SSLP fragment in B-NDG mice is the same as that in NOD-*scid* mice and there is significant difference with C57BL/6N mice (mean difference 13.23bp) (Table4), and the overall fragment difference is basically the same as that in the reference/MGI.

Conclusion: B-NDG mice have the same background as NOD-*scid* mice derived from Charles River, it was significantly different from the C57BL/6N mice derived from Biocytogen.

Table 4. SSLP PCR Product Sizes in the B-NDG, NOD-*scid* and C57BL/6N Strains

Marker	Position (cM)	B-NDG	NOD-*scid*	C57BL/6N	B-NDG/ NOD-*scid*[1]	B-NDG/ C57BL/6N[2]
D1Mit303	34.8	113	113	123	0	10
D1Mit132	43.1	160	160	141	0	19
D1Mit150	81.08	120	120	131	0	11
D2Mit42	54.85	125	125	140	0	15
D2Mit311	83.1	114	114	127	0	13
D2Mit346	91.8	100	100	94	0	6
D3Mit189	43.89	156	156	131	0	25
D3Mit85	72.9	216	216	210	0	6
D3Mit89	86.1	210	210	216	0	6
D4Mit308	57.66	117	117	81	0	36
D4Mit203	63.26	111	111	104	0	7
D4Mit256	82.7	129	129	133	0	4
D5Mit146	1	123	123	119	0	4
D5Mit158	55.99	323	323	307	0	16
D5Mit161	65.34	156	156	118	0	38
D6Mit296	2.25	109	109	99	0	10
D6Mit100	41.03	96	96	83	0	13
D6Mit304	75	103	103	112	0	9
D7Mit267	11	180	180	194	0	14
D7Mit220	55.69	115	115	129	0	14
D7Mit189	72.4	115	115	132	0	17
D8Mit155	1	158	158	162	0	4
D8Mit80	43.06	119	119	105	0	14
D8Mit88	58	124	124	112	0	12
D9Mit83	6	127	127	131	0	4
D9Mit97	29	156	156	146	0	10
D9Mit52	72	171	171	169	0	2
D10Mit2	16	134	134	128	0	6
D10Mit230	45.28	137	137	110	0	27
D10Mit266	62	80	80	88	0	8
D11mit151	15.29	151	151	134	0	17
D11mit298	42.76	216	216	192	0	24
D11Mit48	77	124	124	130	0	6
D11mit303	82.9	103	104	106	1	3
D12Mit12	8.49	166	166	139	0	27
D12mit2	18.94	146	146	132	0	14
D12Mit133	56	97	97	112	0	15
D13Mit51	11.94	141	141	139	0	2
D13mit191	45.05	120	120	114	0	6
D13mit78	67.21	202	202	224	0	22

D14mit126	11.94	127	127	133	0	6
D14Mit225	42.5	96	96	113	0	17
D14mit95	57.2	163	163	120	0	43
D15mit154	16.82	144	144	151	0	7
D15mit92	32.19	139	139	141	0	2
D15Mit42	59.2	180	180	184	0	4
D16Mit129	3.4	165	165	178	0	13
D16Mit140	40.3	157	157	140	0	17
D16Mit106	71.5	134	134	145	0	11
D17mit164	2.11	90	90	126	0	36
D17Mit68	23.55	168	168	129	0	39
D17Mit93	44.5	142	142	155	0	13
D18Mit12	17	131	131	118	0	13
D18Mit91	29	137	137	139	0	2
D18Mit187	47	108	108	112	0	4
D19mit45	16.14	134	134	138	0	4
D19mit1	50.32	143	143	121	0	22
DXMit55	1.4	128	128	137	0	9
DXMit48	25.51	98	98	105	0	7
DXMit179	53.17	113	113	122	0	9
average	42.96	139.33	139.35	136.73	0.02	13.23

1. Represents the difference in the size of SSLP fragments between B-NDG mice and NOD-*scid* mice

2. Represents the difference in the size of SSLP fragment size between B-NDG mice and C57BL/6N mice

4 Reference

1. Suemizu H, Yagihashi C, Mizushima T, et al. Establishing EGFP Congenic Mice in a NOD/Shi-scid*IL2Rg*^{null} (NOG) Genetic Background Using a Marker-Assisted Selection Protocol (MASP)[J]. *Experimental Animals*, 2008, 57(5): 471-477.
2. Gurumurthy C B, Joshi P S, Kurz S G, et al. Validation of Simple Sequence Length Polymorphism Regions of Commonly Used Mouse Strains for Marker Assisted Speed Congenics Screening[J]. *Comparative and Functional Genomics*, 2015: 735845-735845.

5 Appendix1: Protocol for genomic DNA extraction from mouse's or rat's tails

5.1 Digestion buffer

Table 5: Concentration of digestion buffer

Reagent name	Concentration of stock solution
Tris-HCl (pH8.0)	1 M (10×)
EDTA-2Na (pH8.0)	0.5 M (100×)
NaCl	3 M (15×)
SDS	10% (50×)
Proteinase K	10 mg/mL (100×)

5.2 Digestion buffer preparation (for a 10-mL reaction)

Table 6: Preparation system of digestion buffer

Stock solution	Volume
Tris-HCl (pH8.0)	1 mL
EDTA -2Na (pH8.0)	100 μL
NaCl	667 μL
SDS	200 μL
Proteinase K	100 μL
Distilled water	7933 μL

5.3 Procedure

5.3.1 Cut 0.5 to 1 cm of tails from 2- to 3-week-old mouse or rat. Place the tissue into 1.5 mL microcentrifuge tubes on ice.

5.3.2 If the samples will not be used immediately, they can be stored at -20℃.

5.3.3 Add 500 μL of digestion buffer containing 5 μL of 10mg/ml Proteinase K solution to each tube.

5.3.4 Incubate the samples overnight at 55℃ in hybridization oven, with inverting samples to mix.

5.3.5 Remove the tubes from hybridization oven. Leave at room temperature for 10-15 minutes and then invert the tubes by hand to mix.

5.3.6 Centrifuge the tubes at 13000 rpm for 15 minutes at room temperature.

5.3.7 Remove 400 μL of the supernatant into a fresh microcentrifuge tube.

5.3.8 Add an equal volume of isopropanol to each tube and invert the tube until a stringy precipitate forms.

5.3.9 Centrifuge the tubes at 12000 rpm for 10 minutes and then discard the supernatant.

5.3.10 Rinse the DNA pellet with 700 μL of 75% ice cold ethanol and invert gently by hand.

5.3.11 Centrifuge the tubes at 12000 rpm for 5 minutes and then remove the supernatant with pipette thoroughly.

5.3.12 Air-dry the pellet for 3-5 minutes in super clean bench.

5.3.13 Resuspend the DNA pellet in 100 μL of distilled water. Incubate the tubes at 55℃ for 2 hours.

5.3.14 Measure the DNA concentration. Use 100 ng to 200 ng of DNA for PCR.

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