

Genetic Background Detection Report

Report No. TR-MT-20240429-001

Genotyping Strain B-NDG mice

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Report Date 2024.04.29



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- <i>scid</i>	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 μ L)

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

Table 3: KOD-FX DNA Polymerase PCR Procedure

Step	Temp	Time	Cycles
1	94°C	2 min	1
2	98°C	10 sec	
3	62°C	30 sec	30
4	68°C	30 sec	
5	68°C	10 min	1
6	4°C	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 16.23bp) (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 Biocytogen, 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- <i>scid</i>	C57BL/6N	B-NDG/ NOD- <i>scid</i> ¹	B-NDG/ C57BL/6N ²
D1Mit303	34.8	114	114	124	0	10
D1Mit132	43.1	160	160	141	0	19
D1Mit150	81.08	121	121	132	0	11
D2Mit42	54.85	125	125	140	0	15
D2Mit311	83.1	115	115	127	0	12
D2Mit346	91.8	100	100	93	0	7
D3Mit189	43.89	156	156	132	0	24
D3Mit85	72.9	216	216	210	0	6
D3Mit89	86.1	211	211	217	0	6
D4Mit308	57.66	117	117	78	0	39
D4Mit203	63.26	111	111	105	0	6
D4Mit256	82.7	129	129	134	0	5
D5Mit146	1	124	124	120	0	4
D5Mit158	55.99	323	323	135	0	188
D5Mit161	65.34	156	156	118	0	38
D6Mit296	2.25	110	110	99	0	11
D6Mit100	41.03	95	95	80	0	15
D6Mit304	75	103	104	113	1	10
D7Mit267	11	180	180	194	0	14
D7Mit220	55.69	116	116	129	0	13
D7Mit189	72.4	116	116	132	0	16
D8Mit155	1	158	158	162	0	4
D8Mit80	43.06	120	120	105	0	15
D8Mit88	58	125	125	112	0	13
D9Mit83	6	128	128	132	0	4
D9Mit97	29	157	157	147	0	10
D9Mit52	72	171	171	169	0	2
D10Mit2	16	134	134	128	0	6
D10Mit230	45.28	138	138	111	0	27
D10Mit266	62	76	76	85	0	9
D11mit151	15.29	151	151	134	0	17
D11mit298	42.76	217	217	193	0	24
D11Mit48	77	125	125	130	0	5
D11mit303	82.9	104	104	106	0	2
D12Mit12	8.49	166	166	139	0	27
D12mit2	18.94	146	146	133	0	13
D12Mit133	56	95	94	113	1	18
D13Mit51	11.94	142	142	140	0	2
D13mit191	45.05	121	121	115	0	6
D13mit78	67.21	203	203	225	0	22

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D14mit126	11.94	128	128	134	0	6
D14Mit225	42.5	95	95	114	0	19
D14mit95	57.2	163	163	121	0	42
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	164	165	178	1	14
D16Mit140	40.3	157	157	140	0	17
D16Mit106	71.5	134	134	146	0	12
D17mit164	2.11	88	88	127	0	39
D17Mit68	23.55	168	168	130	0	38
D17Mit93	44.5	142	142	155	0	13
D18Mit12	17	132	132	119	0	13
D18Mit91	29	137	137	140	0	3
D18Mit187	47	109	109	112	0	3
D19mit45	16.14	135	135	139	0	4
D19mit1	50.32	143	143	122	0	21
DXMit55	1.4	129	129	135	0	6
DXMit48	25.51	98	98	105	0	7
DXMit179	53.17	114	114	123	0	9
average	42.96	139.55	139.57	134.12	0.03	16.23

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*/L2R g^{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°C.

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°C 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 μ L 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 μ L 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 μ L of distilled water. Incubate the tubes at 55°C for 2 hours.
- 5.3.14 Measure the DNA concentration. Use 100 ng to 200 ng of DNA for PCR.

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Data: Apr 29th, 2024

Data: Apr 29th, 2024

Data: Apr 26th, 2024

