Genotyping of NDRG1 KO mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping NDRG1 KO mice. This method is a little bit modified from the original (see the next section).

1.1) Primer sequences:

- Primer1: NDRG5'
  - Sequence: 5'- CCGCCTCTGTCAAATTAGTAGCTG -3' (24-mer)
- Primer2: NDRG3'
  - Sequence: 5'- GGGAGAGCTGAAGGCTGTTCTAGG -3' (24-mer)
- Primer3: NULL5'
  - Sequence: 5'- AGCAGGCTCTTAAAGCGGCTCC -3' (22-mer)

1.2) Reaction mixture:

	For wild allele	For KO allele
	Tube 1 (µL)	Tube 2 (µL)
Water	8	8
Primer1 (NDRG5', 10 µM)	0.5	-
Primer2 (NDRG3', 10 µM)	0.5	0.5
Primer3 (NULL5', 10 µM)	-	0.5
Taq polymerase (U/µL)	10	10
DNA extracted from tail (purified)	1	1
total	20	20

Taq polymerase: HotStarTaq Master Mix Kit (Qiagen). The enzyme is a chemically modified Taq polymerase for hot start PCR and needs 15-min incubation at 95 °C for activation. Master Mix contains enzyme, dNTP, Mg, etc at 2 x concentration. Please see Qiagen's website for details (http://www1.qiagen.com/Products/Pcr/HotStarTaqSystem/HotStarTaqMasterMix.aspx).

1.3) Thermal cycles:

95 °C	15 min	Enzyme activation and first denature
94 °C	15 sec	35 cycles
60 °C	15 sec	
72 °C	20 sec	
72 °C	3 min	once
4 °C	∞	

Thermal cycler:Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: approx. 200 bp for wild-type alleles
Primers 2 and 3: approx. 270 bp for KO alleles

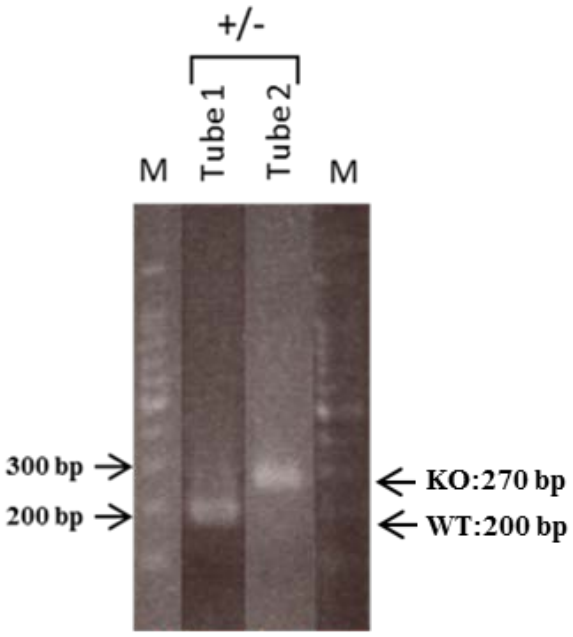

Fig.1. Electropherogram of PCR products from heterozygous mice.

1.5) Reference

Okuda T, Higashi Y, Kokame K, Tanaka C, Kondoh H, Miyata T. Ndrg1-deficient mice exhibit a progressive demyelinating disorder of peripheral nerves. Mol Cell Biol. 2004 May;24(9):3949-56. PMID: 15082788

Nbio_ID=062;05/30/2012