



STRAIN NAME :

CD1d1 KO

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Clinisciences)
- NcoI restriction enzyme 10u/μl (NEB)
- Primers 30μM (Eurofins)
- Mineral Oil (Fisher Scientific)
- DNA (extracted with Kapa Mouse Genotyping kit)

PCR REACTION MIX :

MIX	Quantity / well
Water	7,60 μl
2X Kapa Buffer	10,00 μl
CD1d1F	0,20 μl
CD1d1R	0,20 μl
DNA	2,00 μl
Final Quantity	20,00 μl

PCR PROGRAM:

3 min	95°C	
15 sec	94°C	
15 sec	60 °C	35 cycles
30 sec	72°C	
5 min	72°C	

MIGRATION : Migration on 1.5% agarose gel.

PRIMERS SEQUENCES

- CD1d1F                    5' CCCACTACTGCACAGAGCTT 3'
- CD1d1R                   5' GTCCTCCGGGTTTTGCTGG 3'

EXPECTED AMPLIFICATIONS : all alleles amplify at 261 bp

ENZYMATIC RESTRICTION REACTION MIX :

MIX	Quantity / well
Water	7,00 μl
10X Buffer r3.1	2,00 μl
NcoI	1,00 μl
PCR	10,00 μl
Final Quantity	20,00 μl

RESTRICTION PROGRAM: at least 3 hours at 37°C

EXPECTED FRAGMENTS : WT : 129 bp + 132 pb    CD1d1 mut : 261 bp (no cut)

*Perfection date : 16/05/2024*

*Update :*