

STRAIN NAME : **Dcx flox**

REAGENTS AND SOLUTIONS:

- Kapa Mouse Genotyping kit (Clinisciences)
- BamH1 restriction enzyme 10u/μl (NEB)
- Primers 30μM (Eurogentec)
- Mineral Oil (Fisher Scientific)
- DNA (extracted with Kapa Mouse Genotyping kit)

PCR REACTION MIX :

MIX	Quantity / well
Water	7,40 μl
2X Kapa Buffer	10,00 μl
DCX flox1	0,30 μl
DCX flox2	0,30 μl
DNA	2,00 μl
Final Quantity	20,00 μl

PCR PROGRAM:

3 min	95°C	
15 sec	94°C	
15 sec	55 °C	35 cycles
30 sec	72°C	
5 min	72°C	

MIGRATION : Migration on 1.5% agarose gel.

PRIMERS SEQUENCES

- DCX flox1 5' GGCTGAGCCTAACTTGCTTCCA 3'
- DCX flox2 5' CCAGTTTCTGTGATGCTCACTA 3'

EXPECTED AMPLIFICATIONS : all alleles amplify at 1000 bp

ENZYMATIC RESTRICTION REACTION MIX :

MIX	Quantity / well
Water	7,50 μl
10X Buffer 4	2,00 μl
BamH1	0,50 μl
PCR	10,00 μl
Final Quantity	20,00 μl

RESTRICTION PROGRAM: at least 3 hours at 37°C

EXPECTED FRAGMENTS : WT allele 1000 bp Mutant allele : 684 + 266 bp

Be careful : mutation is X-linked. Males are hemizygous.

Perfection date : 29/03/2012

Update :