

## PCR analysis for LPAR1 mouse:

### Primers

Primer 309 F: 5' GCCAATCCAGCCAAGAAGTC 3' (Tm:53,8°C)

Primer 310 R1: 5' GGTATTCTTAATTCTAGAGGATCAGC 3' (Tm:50,7°C)

Primer 311 R2: 5' TATAGGAGTCTTGTGTTGCCTGTCC 3' (Tm:55,5°C)

### PCR mix (per reaction)

H<sub>2</sub>O: 10.4 µl

10X PCR BUFFER: 2 µl

MgCl<sub>2</sub> 25 mM: 1.2 µl

dNTPs 2.5 mM: 1 µl

Primer 309 (5 pmole/λ): 1 µl

Primer 310 (5 pmole/λ): 1 µl

Primer 311 (5 pmole/λ): 2 µl

Taq: 0.4 µl

Dna template 1 µl (80ng)

Final vol. = 20 µl

### "LPAR1"-program:

1. 5 min, 95°C

2. 30 sec, 95°C

3. 30 sec, 56°C

4. 2 min, 72°C

Steps 2-4 for 30 cycles.

5. 5 min 72 °C

6. 1 min, 16 °C

end store at 4°C

- Wild type will give you 348 bp band (309/310 primers), homozygous 227 bp band (309/311 primers) and heterozygous mutants will give both bands.