

Brca1^F



Liu X et al., PNAS 2007

Brca1 PCR:

FLOX

Primer 1: Brca1-int4-fwd: 5'- TAT CAC CAC TGA ATC TCT ACC G -3'
 Primer 2: Brca1-int4-rev: 5'- GAC CTC AAA CTC TGA GAT CCA C -3'
 Detection of 5' loxP site (located in intron 4): primers: P1 + P2

Wildtype product: 390 nt.
 Brca1F - 5' loxP product: 545 nt.

DEL

Primer 1: Brca1-int4-fwd: 5'- TAT CAC CAC TGA ATC TCT ACC G -3'
 Primer 4: Brca1-int13-rev: 5'- TCC ATA GCA TCT CCT TCT AAA C -3'
 Detection of deletion of exons 5-13: primers: P1 + P4

Wildtype product: none
 Brca1Del - ex. 5-13 product: 594 nt.

PCR reaction:

- DNA	1 μ l
- PCR buffer 10x	2 μ l
- dNTPs 2mM	2 μ l
- MgCl ₂ 50 mM	1 μ l
- P1 10 uM	1 μ l
- P2 or P4 10 uM	1 μ l
- Taq	0.2 μ l
- H ₂ O	11.8 μ l

PCR program:

1.	94°C	05:00
2.	94 °C	00:30
3.	58 °C	00:30
4.	72 °C	00:50
5.	goto 2	29x
6.	72 °C	10:00
7.	12 °C	forever