

## Xrcc1 Knockout Mouse Genotyping

**PCR REACTION 1:** Detects wild type, knockout, and heterozygotes. However, detecting heterozygotes can be difficult since the wild type product, being much smaller, predominates over the knockout product and sometimes the knockout band is very faint. Please note that heterozygotes show the wild type band, the knockout band, and an intermediately sized band that is a heteroduplex of the two amplicons.

### PCR reaction mix:

water	37.2 $\mu$ l
10X buffer	5.0 $\mu$ l
50 mM MgSO <sub>4</sub>	3.0 $\mu$ l
10 mM dNTP	1.0 $\mu$ l
Primer TAR-C (20 $\mu$ M)	1.0 $\mu$ l
Primer TAR-D (20 $\mu$ M)	1.0 $\mu$ l
Platinum Taq (Invitrogen)	<u>0.3 <math>\mu</math>l</u>
TOTAL	48.5 $\mu$ l
Mouse DNA sample	1.5 $\mu$ l

### PCR cycle conditions:

<u>cycle</u>	<u>temperature</u>	<u>time</u>
1	94	1 min
2	94	45 sec
3	60	45 sec
4	68	2 min
5	repeat cycles 2-4 for a total of 30 cycles	
6	68	5 min
7	4	FOREVER

### PCR product sizes:

<u>allele</u>	<u>size</u>
wild type	441 nt
knockout	1597 nt

### PCR primers:

<u>Primer</u>	<u>Sequence</u>
TAR-C	5'-TGTCCTTCCATAGCCTCTACGAGTG
TAR-D	5'-ATTAGGTTGGTGCCCCATCGAG

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**PCR REACTION 2:** Detects the presence of a targeted allele. This reaction works very well, but it is a yes-no answer that either gives a PCR product or not and therefore has the potential to give false negatives if the PCR reaction failed due to technical error.

**PCR reaction mix:**

water	37.2	μl
10X buffer	5.0	μl
50 mM MgSO <sub>4</sub>	3.0	μl
10 mM dNTP	1.0	μl
Primer NEO-F01 (20 μM)	1.0	μl
Primer TAR-D (20 μM)	1.0	μl
Platinum Taq (Invitrogen)	<u>0.3</u>	<u>μl</u>
TOTAL	48.5	μl
Mouse DNA sample	1.5	μl

**PCR cycle conditions:**

<u>cycle</u>	<u>temperature</u>	<u>time</u>
1	94	1 min
2	94	30 sec
3	60	30 sec
4	68	45 sec
5	repeat cycles 2-4 for a total of 30 cycles	
6	68	3 min
7	4	FOREVER

**PCR product sizes:**

<u>allele</u>	<u>size</u>
wild type	no product
knockout	450 nt

**PCR primers:**

<u>Primer</u>	<u>Sequence</u>
NEO-F01	5'-GCTTGCCGAATATCATGGTG
TAR-D	5'-ATTAGGTTGGTGCCCCATCGAG