

Identification of HuR -/- HuR +/- and HuR +/+ mice (After mating with a Cre mice)

Primers:

Sense: 5' – GTT CCA TGG CTC CCC ATA TC- 3'

Antisense: 5' - TGG CAC TCA CTG AAC TGG AA – 3'

PCR reaction

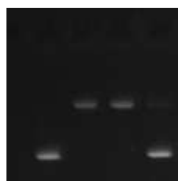
- ~300ngr DNA from the tissue of interest (where Cre is expressed)
- 2µl from 10x PCR buffer
- 1.5µl from 25mM MgCl₂
- 1µl from 2.5mM DNTPs
- 1µl from 5µM from each primer
- 0.3µl from 5units Taq polymerase
- H₂O

PCR program

1. 94° C 5min
2. 94° C 1min
3. 57° C 1min
4. 72° C 1min
5. Go to step 2, repeat 5x
6. 94° C 10sec
7. 55.5° C 50sec
8. 72° C 1:30min
9. Go to step 6, repeat 25x
10. 72° C 10min
11. 16° C 5min

Expected bands: HuR +: 1122bp, HuR - : 459bp

Example:



1st well: HuR -/-

2nd and 3rd well: HuR +/+

4th well: HuR +/-

Please be very careful with identification of HuR +/- mice since in the above PCR there is preference in amplification of the lower mutant band (as you can see in the example as well)