

PCR FOR EHF

PCR REACTION:

10X PCR buffer	2 ul
MgCl ₂ 25 mM	Depends on Taq polymerase (usually 2 ul)
dNTP'S 2,5 mM	2 ul
Primer EHF-5	5 pmol per reaction (1 ul from 5pmole/ul stock)
Primer EHF-3	5 pmol per reaction (1 ul from 5pmole/ul stock)
Primer EHF-LAR-3	10 pmol per reaction (2 ul from 5pmole/ul stock)
DNA	1 ul
Taq	Variable (usually 0.3ul)
dd.H ₂ O	Up to volume (20λ) 8.7 ul

Primer EHF-5 → 5'-ACCAGGTGTGGGAATGGCTGCAGC-3'

Primer EHF-3 → 5'-CTCCTGAGAAGGAGCCCTACCTGC-3'

Primer EHF-LAR-3 → 5'-CAACGGGTTCTTCTGTTAGTCC-3'

PCR CONDITIONS:

1. 94°C for 5 min.
2. 94°C for 45 sec.
3. 60°C for 30 sec.
4. 72°C for 45 sec.
5. Steps 2-4 for 35 more cycles.
6. 72°C for 5 min.
7. 4°C for infinite.
8. End

PCR PRODUCT:

Ehf-5'arm	24	ACCAGGTGTGGGAATGGCTGCAGC	243 bp with wt DNA
Ehf-3 'arm	24	CTCCTGAGAAGGAGCCCTACCTGC	393 bp with mutant DNA
LAR3 primer	22	CAACGGGTTCTTCTGTTAGTCC	-150 bp difference

