

## PCR FOR Tln1 wt

### PCR REACTION:

10X PCR buffer	2 $\lambda$
MgCL <sub>2</sub> 25 mM	2 $\lambda$
dNTP'S 2.5 mM	2 $\lambda$
Primer TLN1WTF	5 pmol per reaction (1 $\lambda$ )
Primer TLN1WTR	5 pmol per reaction (1 $\lambda$ )
Taq	0.4 $\lambda$
DNA	1 $\lambda$
H <sub>2</sub> O	Up to volume 20 $\lambda$ (10.6 $\lambda$ )

**Primer TLN1WTF:** 5'-CCTGTGGAAGGCTGATGGTT-3'

**Primer TLN1WTR:** 5'-GCAATGCCCCCAACCATTTT-3'

### PCR CONDITIONS:

1. 94<sup>0</sup>C for 5 min.
2. 94<sup>0</sup>C for 45 sec.
3. 60<sup>0</sup>C for 30 sec.
4. 72<sup>0</sup>C for 45 sec.
5. Steps 2-4 for 35 more cycles.
6. 72<sup>0</sup>C for 5 min.
7. 4<sup>0</sup>C for infinite

PCR product: wt: 222 bp

## PCR REACTION *Tln1 mut*

### PCR REACTION:

10X PCR buffer	2 $\lambda$
MgCL <sub>2</sub> 25 mM	2 $\lambda$
dNTP'S 2.5 mM	2 $\lambda$
Primer TLN1mutF	5 pmol per reaction (1 $\lambda$ )
Primer TLN1mutR	5 pmol per reaction (1 $\lambda$ )
Taq	0.4 $\lambda$
DNA	1 $\lambda$
H <sub>2</sub> O	Up to volume 20 $\lambda$ (10.6 $\lambda$ )

**Primer TLN1mutF:** 5'-CGCTCTGAAGTAGGGACAGC-3'

**Primer TLN1mutR:** 5'-CATAGGTAGCTGGGTAGGGGA-3'

### PCR CONDITIONS:

1. 94<sup>0</sup>C for 5 min.
2. 94<sup>0</sup>C for 45 sec.
3. 60<sup>0</sup>C for 30 sec.
4. 72<sup>0</sup>C for 45 sec.
5. Steps 2-4 for 35 more cycles.
6. 72<sup>0</sup>C for 5 min.
7. 4<sup>0</sup>C for infinite

PCR product: wt: 1671 bp  
mutant: 637 bp