

Genotyping protocol for interleukin 1 receptor, type II gene (Il1r2^{tm1c(EUCOMM)Wtsi/Ph})

Protocol update: 18.3.2022

Type of PCR	Primer name	Sequence
Tm1c PCR	Il1r2_241249_F	5'-ACCTCTGGCCTTTTCTCAGTTC-3'
Tm1c PCR	Il1r2_241249_R	5'-AGAGTGCGGCTTACCTCCTG-3'

Master mix for tm1c PCR

reagent	volume
Dream Taq buffer 10x	2.0 µl
dNTPs (10mM)	0.5 µl
Primer forward (10µM)	0.5 µl
Primer reverse (10µM)	0.5 µl
Dream Taq polymerase, Fermentas	0.15 µl
DNA *	0.7 µl
Tk H ₂ O	15.65 µl
Total volume	20.0 µl

*DNA was isolated using DirectPCR Lysis PCR reagent (Mouse Tail), VIAGEN Biotech, cat.no. 102-T

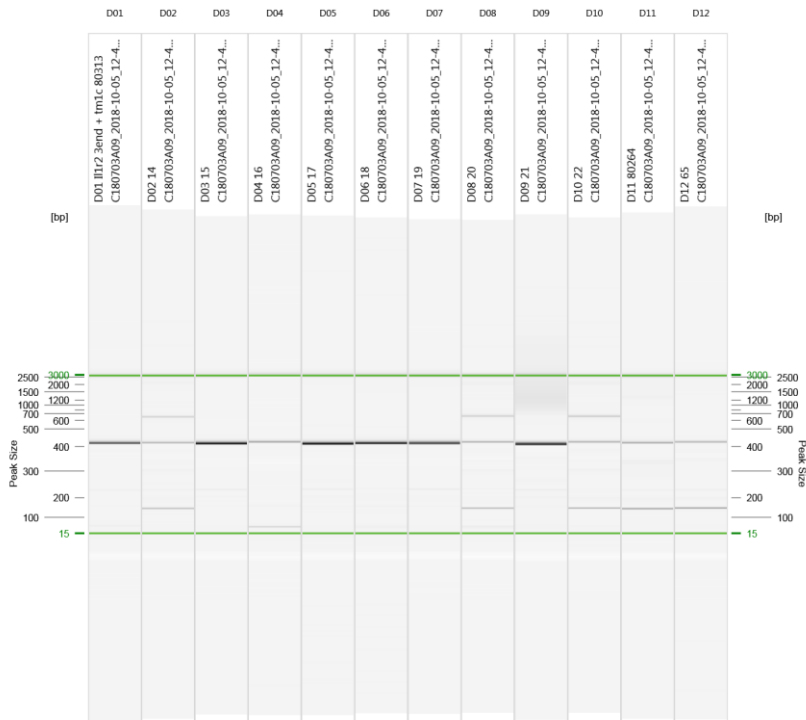
Tm1c PCR (36 cycles):

Denaturation	95°C	5 min
Denaturation	95C	30 sec
Annealing	55°C	40 sec
Elongation	72°C	40 sec
Final Elongation	72°C	5 min
End	12°C	forever

Gel electrophoresis	
Tm1c PCR	2% LD agarose, 90V, 45min

Type of PCR		Product size
Tm1c PCR	Wild-type allele	416 bp
Tm1c PCR	Tm1c allele	613 bp

Example picture tm1c-PCR



Chemicals:

- Taq Polymerase (5U/μl) – THERMO Scientific
- DNTPs (10mM) - Sigma Aldrich
- Primers - Sigma Aldrich
- SacI (20,000U/ml) - BioLabs