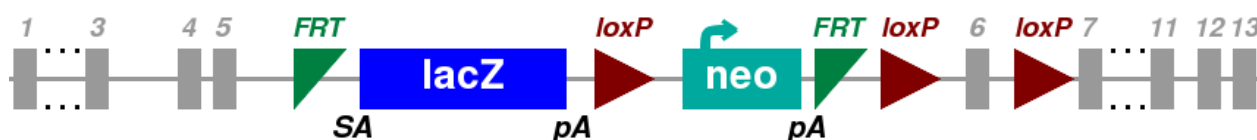


Genotyping protocol for transmembrane protein 237 (Tmem237^{tm1a(EUCOMM)Hmgu})

Protocol update: 22.8.2017



Type of PCR	Primer name	Sequence
3'end SR-PCR	3'Tmem237 fw	5'-ggggttgaaaagaatagaacactg-3'
3'end SR-PCR	3'Tmem237 rv	5'-gtgattggttattctttgtagtcatttttag-3'
5'end SR-PCR	5'Tmem237 fw	5'-gctctggtcctctgttttg-3'
5'end SR-PCR	5'Atf2-Rv	5'-ctacatagttggcagtgtttg-3'

Master mix for 3'end SR-PCR and 5'end SR-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 QuickExtract DNA Extraction solution from EPICENTRE, GeneTiCA, cat. number QE09050. 50µl of solution was added to 0,5cm long tail biopsy.

3'end SR-PCR (32 cycles):

Denaturation	95°C	5 min
Denaturation	95°C	30 sec
Annealing	60°C	40 sec
Elongation	72°C	1 min
Final Elongation	72°C	5 min
End	12°C	forever

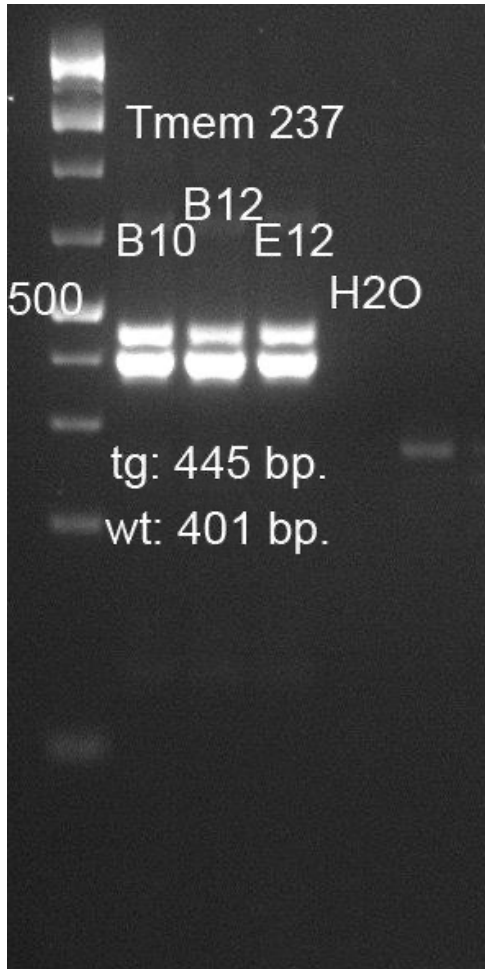
5'end SR- PCR (32 cycles):

Denaturation	95°C	5 min
Denaturation	95°C	30 sec
Annealing	57,5°C	40 sec
Elongation	72°C	1 min
Final Elongation	72°C	5 min
End	12°C	forever

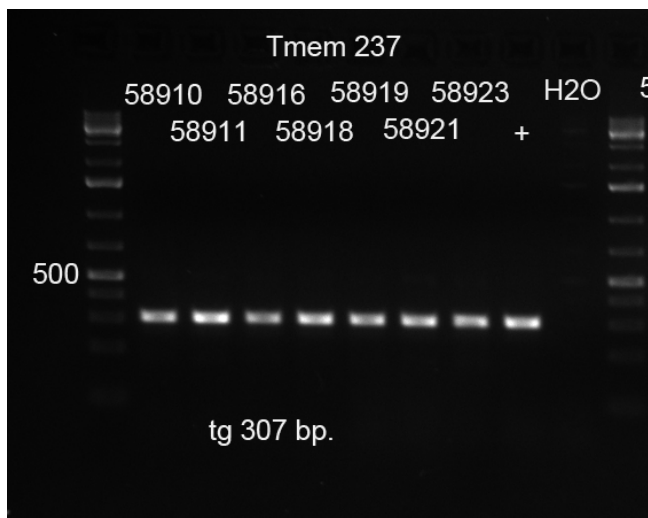
Gel electrophoresis	
3'end SR-PCR	3% agarose, 90V, 30min
5'end SR-PCR	2% LD agarose, 90V, 30min

Type of PCR		Product size
3'end SR-PCR	Wild-type allele	401bp
3'end SR-PCR	Tmem237 floxed allele	445bp
5'end SR-PCR		307bp

Example picture 3'end SR-PCR



Example picture 5'end SR-PCR



Chemicals:

Taq Polymerase (5U/ μ l) – THERMO Scientific

DNTPs (10mM) - Sigma Aldrich

Primers - Sigma Aldrich

Sacl (20,000U/ml) - BioLabs
