

GDF5 genotyping

The GDF5 genotyping is done by 2 PCR's followed by sequencing.

The first PCR is done to amplify a fragment around the insertion mutation, the second PCR is the sequence PCR which amplifies a single strand from the fragment of the first PCR and after the second PCR this single strand is sequenced.

PRIMERS:

PT289_gdf5_genomic_m_S ctcaagaggtgcatgatccagtc

PT290_Gdf5_genomic_AS attgttcagccagcgcg

PRODUCTS:

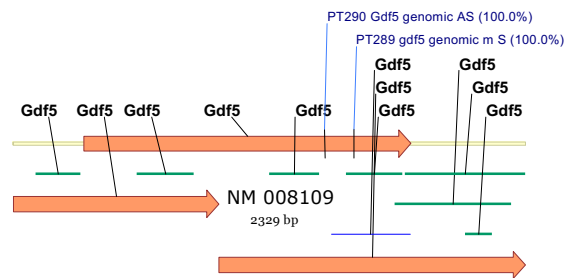
Buffer, MgCl₂, Taq: Bioline Biotaq polymerase 2500 units; cat nr BIO-21060

dNTP: Life technologies 10mM dntp mix; cat nr 18427-013

Prepare the mix on ice.

PCR mix per sample (µl)

H ₂ O (Fresh Milli-Q)	6.5
Buffer	1
MgCl ₂ 50mM	0.5
dNTP 1mM	1
primers 289 x 290	1 x 1
Enzyme (Taq-Pol.)	0.2
Template DNA	1



Mix by pipetting

PCR program: 3-step PCR.

95 °C - 3 minute	
98 °C - 20 seconds	} 35 cycles
60 °C - 10 sec	
72 °C - 20 sec	
72 °C - 3 min	
4 °C - ∞	

Expected size:

155 basepairs

Mix 2 µl of the PCR product with 1 µl Loading Buffer and run it on a gel to see if the PCR amplified a fragment in all samples.

Results:

All samples should have a fragment of 155 basepairs

Sequence PCR

Prepare the mix on ice

PCR mix per sample:

	μl
Terminator Cycle Sequencing Ready Reaction	1
primer 289 (= Anti-Sense primer)	2
water	1
DNA (from 1 st PCR)	1

PCR program: Sequence PCR (pre-programmed).

95 °C - 4 minutes	
95 °C - 10 seconds	} 35 cycles
55 °C - 10 sec	
60 °C - 4 minutes	
4 °C - ∞	

Precipitation + Sequencing

- Add 15 μl of 100% ethanol + 1.25 μl 125 mM EDTA
- Incubate for 15 minutes at RT
- Centrifugate for 30 minutes at 4000 RPM at 4 °C
- Take the caps of, cover with paper towel, turn the tubes upside-down and centrifugate for 3 minutes at 700 RPM
- Add 15 μl Formamide
- Denature in the PCR machine for 2 minutes at 95 °C
- Take the tubes out of the machine and put them directly on ice (be careful, the caps might explode)
- Put the samples in the sequencer

Remarks:

Use buffer, MgCl_2 and enzyme from the same kit (Lot#).

If purification by hand (without columns), see my notebook Page 20

Insertion of extra C, if working with PT289 reverse primer

PT290 Gdf5 genomic AS 100.0%	
1401	CAAGACTGTG TATGAATATT TGTTGAGCCA GCGGCGGAAA CGCCCGCC CATTGGCCAA TCGCCAGGGC AAGCGACCCA GCAAGAACCT CAAGGCTCGC
	GTTCTGACAC ATACTTATAA ACAAGTCGGT CGCCGCCTTT GCGGCCCGG GFAACCGTT AGCGGTCCCG TTCGCTGGGT CGTTCTTGA GTTCCGAGCG
1501	TGCAGTCGCA AGGCCTTGCA TGTCAACTTC AAGGACATGG GCTGGGACGA CTGGATCATC GCACCTCTTG AGTATGAGGC CTTCCACTGC GAAGGACTGT
	ACGTCAGCGT TCCGGAACGT ACAGTTGAAG TTCCTGTACC CGACCCTGCT GACCTAGTAG CGTGGAGAAC TCATACTCCG GAAGGTGACG CTTCTGACA
PT289 gdf5 genomic m S 100.0%	

Results:

