GDF5 genotyping

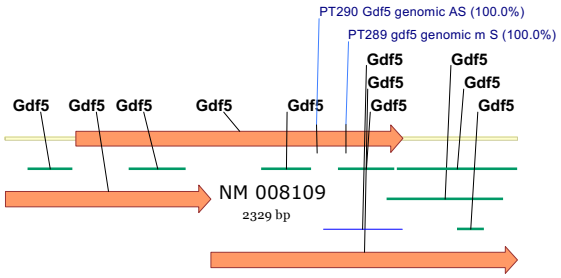
The GDF5 genotyping is done by 2 PCR's followed by sequencing.
The first PCR is done to amplify a fragment around the insertion mutation, the second PCR is the sequence PCR which amplifies a single strand from the fragment of the first PCR and after the second PCR this single strand is sequenced.

PRIMERS:
PT289_gdf5_genomic_m_S ctcaagaggtgcgatgatccagtc
PT290_Gdf5_genomic_AS atttgttcagccagcggcg

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_2O (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 60 °C - 10 sec 72 °C - 20 sec	}	35 cycles
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 1st PCR)	1

PCR program: Sequence PCR (pre-programmed).

95 °C - 4 minutes

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

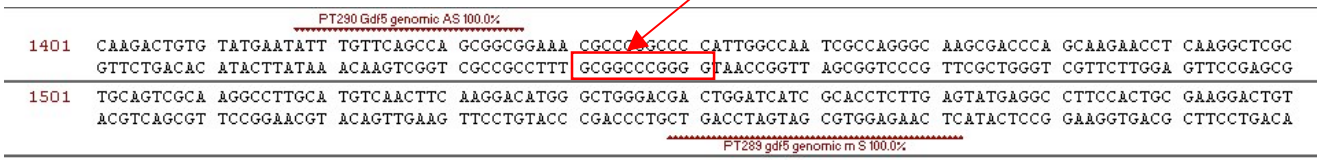
4 °C - ∞

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



Results: