

Genotyping by copy number: setting up reactions for qPCR with Neo and TfrC assays manually in multiplex – 96 well plate

- The following protocol deals purely with the process of setting up reactions for copy number genotyping and qPCR. Please see other protocols for DNA extraction, setting up plate records and running the qPCR machine.
- In this case the endogenous control is the TfrC gene, and the target reporter is the Neo gene present in most gene trap constructs and EUComm/KOMP alleles. Details for an assay to LacZ are also given
- This method can also be used as a basis for 384well setup using automation

Reagents:

- DNA prepared using the ABI sample-to-SNP method (stored at -20°C)
- ABI GTXpress master mix (stored at 4°C)
- TfrC assay 20x conc (stored at -20°C)
- Neo assay 60x conc (aliquoted and stored at -20°C)

Protocol:

1. Fully thaw out assay aliquots and store on ice along with the master mix. DNA should be thawed out and left on the bench.
2. Centrifuge the DNA plate on the Eppendorf 5810R at 4000rpm for 1 min.
3. Reactions should be set up as a 10µl volume with the following reagents (one assay per well).
 - a. When removing the DNA, pipette up and down 2-3 times prior to aspiration. This ensures proper re-suspension of the DNA without disturbing the ear clip remains.
4. Once the plate is set up, seal it with the ABI optical clear film. Warning - the film is not very sticky and requires the use of one of the plastic plate spatulas to stick to the plate. Use the spatula to hold the plate seal on when removing the tabs at the end of the seal. Make sure not to touch the top of the seal more than is necessary.
5. Centrifuge the plate briefly to ensure any air bubbles are removed from the bottom of the well. There may be some left at the top which is okay.
6. Load the plate on to the qPCR machine and run the samples as described elsewhere on the $\Delta\Delta C_t$ (RQ) module.

	Cassette 60x / TfrC 20x
2x buffer	5
WT Probe	0.166
Water	3.334
TfrC probe	0.5
DNA	1

Part numbers:

• TaqMan® GTXpress™ Master Mix, 1-Pack (10 mL)	4401892	4000 reactions
• TaqMan® GTXpress™ Master Mix, 5-Pack (50 mL)	4401890	20000 reactions
• MicroAmp® Optical Adhesive Film	4311971	100 pack
• MicroAmp Fast Optical 96-Well Reaction Plate 0.1 ml	4346906	20 pack
• MicroAmp® Optical 384-Well Reaction Plate	4309849	50 pack
• TaqMan® Copy Number Reference Assay, Mouse, Tfrc,	4458367	3000 reactions

Sequences:

Forward Primer Name	Forward Primer Seq.
LacZ-RegF	GGAGTGCGATCTTCCTGAGG
NeoF	GGTGGAGAGGCTATTCGGC

Reverse Primer Name	Reverse Primer Seq.
LacZ-RegR	CGCATCGTAACCGTGCATC
NeoR	GAACACGGCGGCATCAG

Reporter 1 Name	Reporter 1 Dye	Reporter 1 Sequence
LacZ-RegM1	FAM	CGATACTGTCGTCGTCCCCTCAAACCTG
NeoM1	FAM	TGGGCACAACAGACAATCGGCTG

Date last reviewed:**Read by:**