

**Protocol for using quantitative PCR (Taqman 7700 QPCR Assay) to assess neo dosage in knockout mice**

Reaction conditions:

2 × Universal PCR Master Mix (ABI 4304437)	5 µl	1 ×
20 µM (oligonucleotide) oIMR1080	0.4 µl	0.8 µ M
20 µM (oligonucleotide) oIMR1081	0.4 µl	0.8 µ M
20 µM (oligonucleotide) oIMR1082	0.4 µl	0.8 µ M
20 µM (oligonucleotide) oIMR1083	0.4 µl	0.8 µ M
5 µM probe TMoIMR001	0.4 µl	0.2 µ M
5 µM probe TMoIMR002	0.4 µl	0.2 µ M
dH <sub>2</sub> O	1.6 µl	
DNA	1 µl	1 ng
 Total volume	 10 µl	

optical plate (ABIN801-0560) can be sealed with optical caps (ABIN801-0935) or with sealing tape (ABI4313663)

Cycling:

50°C	2 minutes
95°C	10 minutes
95°C	15 seconds*
60°C	1 minute*
	*40 cycles

Oligonucleotides and probes were designed by JAX Molecular Genotyping Laboratory.

Oligonucleotides synthesized by Invitrogen (Gibco/BRL).

oIMR1080 5 –Cgg CTg CAT ACg CTT gAT C–3 Neo primer

oIMR1081 5 –CgA CAA gAC Cgg CTT CCA T–3 Neo primer

oIMR1082 5 –TgC ATA gCg TAA TgT CCA TGT Tg–3 Ngf primer

oIMR1083 5 –TCT CCT TCT ggg ACA TTg CTA TC–3 Ngf primer

Taqman Probes synthesized by Applied Biosystems.

TMoIMR001 6FAM–CATCgCATCgAgCgAgCACgTACT–TAMRA neomycin

TMoIMR002 VIC–ACggTTCTgCCTgTACgCCgATCA–TAMRA Ngf

Procedure

Do each sample in triplicate

1. Set up reaction plate with reaction mixture from above and DNA (1ng/ul).
2. For easier data analysis and handling, set samples in adjacent wells on the long axis. Data output recognizes well A1 as sample 1, A2 as sample 2, ...well H12 as sample 96.

Sample Plate Sheet

	1	2	3	4	5	6	7	8	9	10	11	12	
<b>A</b>	H72			H73			H74			H75			<b>12</b>
<b>B</b>	H76			H77			H78			H79			<b>24</b>
<b>C</b>	H80			H81			H82			H83			<b>36</b>
<b>D</b>	H84			H85			H86			IM1			<b>48</b>
<b>E</b>	Im2			Im3			Im4			Im0			<b>60</b>
<b>F</b>	If1			If2			If3			If0			<b>72</b>
<b>G</b>	Cf0			amo			am1			ntc	ntc	ntc	<b>84</b>
<b>H</b>													<b>96</b>
	<b>8</b>	<b>16</b>	<b>24</b>	<b>32</b>	<b>40</b>	<b>48</b>	<b>56</b>	<b>64</b>	<b>72</b>	<b>80</b>	<b>88</b>	<b>96</b>	

Pss2 Taqman 7700 multiplexed reactions

3. Using Taqman 7700 Sequence Detector Software, assign sample type to individual wells in both VIC dye layer and FAM dye layer. Assign wells containing reaction mix but no DNA “No Template Controls.” Assign all other wells, including empty wells, “Unknown.” Be sure that the well assignment is identical for both dye layers.
4. Set thermocycler conditions (as noted above).
5. Begin run. When run is completed, save to appropriate file. DO NOT USE “SAVE AS”!! This is a software glitch with Sequence Detector V1.6.3 that will not save data.
6. Analyze data with Sequence Detector v1.6.3. Set the threshold at higher than 10 times the background standard deviation or clearly within the logarithmic phase of PCR amplification.
7. Export results.

8. You can set up a Microsoft Excel spreadsheet to calculate change in Ct values (Ct) and standard deviations.
  - a. Cut and paste Ct values into a new spreadsheet, with FAM (neo) Ct values in one column and VIC (ngf-normalizer) in another.
  - b. In a third column, calculate Delta Ct Values by subtracting the Normalizer Ct (ngf) from the Target Ct (neo).
  - c. Obtain the mean and standard deviation for each sample run in triplicate.

Example:

Sample	Ct(Neo)	Ct(Ngf)	Ct	Mean ± SD
H81	25.75	27.63	-1.88	
H81	25.61	27.6	-1.99	-1.89
H81	25.59	27.4	-1.81	±0.09
IF1	26.23	27.87	-1.64	
IF1	26.33	28.14	-1.81	-1.75
IF1	26.31	28.1	-1.79	±0.09
IF2	24.17	26.98	-2.81	
IF2	24.21	26.95	-2.74	-2.76
IF2	24.14	26.86	-2.72	±0.05
IF3	23.33	26.21	-2.88	
IF3	23.28	26.13	-2.85	-2.84
IF3	23.26	26.05	-2.79	±0.05
IF0	26.79	28.58	-1.79	
IF0	26.55	28.36	-1.81	-1.82
IF0	26.64	28.5	-1.86	±0.04

9. Plot the Ct values using a graphing program (e.g., Cricket Graph). Samples on the x-axis and the Ct values on the y-axis. Be sure to include standard deviations. Two distinct populations should be observed, with the difference between the two populations being approximately 1 Ct.

Example: Samples H81–H86, IF1, and IF0 are heterozygous.  
Samples IM0–IM4, IF2, and IF3 are homozygous.

