GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: Sanger MirKO ES Cell Line Mir130a - LRPCR **MMRRC #** 034430-UCD

Protocol: SequelPrep long PCR kit, Invitrogen A10498

Reagent/ Constituent	Volume (µL)
Sterile H20	13.24
10X Buffer (green tube)	2
Enhancer A (Red tube)	1
Enhancer B (Yellow tube)	1
DMSO (brown tube)	.2
gene specific primer (10uM)	0.5
universal primer (10uM)	0.5
Enzyme (black tube)	0.36
DNA extracted with ☐ NaOH ☒ Proteinase K ☒ Other: Qiagen DNEasy	1
TOTAL VOLUME OF REACTION:	**20µL**

Comments on protocol:

May decrease Elongation time for shorter PCR fragments per manufacturers suggestion i.e. 1 minute/kb of sequence.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	93	3:00	**1**
2. Denaturation	steps 2-3-4 will cycle in sequence	93	0:15	**8x**
3. Annealing		68 to 60 (↓1°C/cycle)	0:30	
4. Elongation		68	9:00	
5. Denaturation	steps 5-6-7 will cycle in sequence	93	0:15	**32x**
6. Annealing		60	0:30	
7. Elongation		68	9:00 (↑20sec/cycle	
8. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: 5' common rev	atagcatacattatacgaagttatcactgg
2: 5' gene-specific (LR1)	cacttcctaagtctctctttaacttcttct**
3: 3' common fwd	tctagaaagtataggaacttccatggtc
4: 3' gene-specific (LR4)	gtagcagtcagggaaatacagaacag

Electrophoresis Protocol:

% Agarose: 0.8 **V:** 90

Estimated Running Time (min): 90

Primer Combination	Band (kb)	Genotype
1 and 2 (5')	5413	Targeted
3 and 4 (3')	3087	Targeted

**Targeting confirmation at the 5' end has not been successful with these primers; primer re-design is in progress. Please contact mmrrc@ucdavis.edu for details.

Animal Genotyping - Designing Primers for Short Range PCR (SRPCR)

- Go to http://www.knockoutmouse.org/martsearch/search?query=mir+TV database and search the gene name i.e. Mir100
- In IKMC Targeted Projects—click on the "View Details"
- Under "# Targeted Non-Conditional Clones"—click on the "Genbank file" and copy into a sequence analysis software such as VectorNTI (invitrogen) or other analysis tool.
- Locate the vector specific fwd primer "tctagaaagtataggaacttccatggtc" and design a reverse compliment ~ 200-400 bases downstream (within homology arm). Name "3' gsp rev" and note expected amplicon size.
- Locate the "LR2" region on the sequence file and design a fwd primer ~ 100-200 bp upstream and name "5' gsp fwd". This will pair with the 3'gsp rev and give a reaction on the wildtype (WT) allele only with this protocol and always slightly larger by a few hundred bp compared to the KO reaction.
- To calculate the expected WT amplicon size: Blast this entire sequence against the mouse genome rather in NCBI or Ensembl and retrieve the contig sequence. Input the contig sequence into your DNA sequence analysis software and determine the exact amplicon size of the 3' gsp rev and 3' gsp fwd reaction.
- Contact Brandon Willis at bjwillis@ucdavis.edu at anytime for assistance.