

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: Sanger MirKO ES Cell Line Mir325 - LRPCR MMRRC # 034471-UCD

Protocol: SequelPrep long PCR kit, Invitrogen A10498

Reagent/ Constituent	Volume (µL)
Sterile H2O	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 <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input checked="" type="checkbox"/> 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	HOT START? <input type="checkbox"/>	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting		93	3:00	1
2. Denaturation		93	0:15	
3. Annealing	steps 2-3-4 will cycle in sequence	68 to 60 (↓1°C/cycle)	0:30	8x
4. Elongation		68	9:00	
5. Denaturation		93	0:15	
6. Annealing	steps 5-6-7 will cycle in sequence	60	0:30	32x
7. Elongation		68	9:00 (↑20sec/cycle)	
8. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: 5' common rev	atagcatacattatacgaaggttatcactgg
2: 5' gene-specific (LR1)	tatattggatatttagtcccstatctgattc
3: 3' common fwd	tctagaaagtataggaacttccatggtc
4: 3' gene-specific (LR4)	caatattaaaaatgaaattgcaatgaac

Electrophoresis Protocol:

% Agarose: 0.8 V: 90

Estimated Running Time (min): 90

Primer Combination	Band (kb)	Genotype
1 and 2 (5')	5191	Targeted
3 and 4 (3')	2855	Targeted

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 “tctagaaaagtataggaacttccatggtc” 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.