

**GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**

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530-754-MMRRC

NAME OF PCR: B6;129S5-Cndp1^{tm1Lex}/Mmucd MMRRC # 032215-UCD

Protocol: Neo Tcrd Duplex used for Lexicon/Genentech gene trap lines.

Reagent/ Constituent	Volume (μL)
Water	10.275
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM) Neo TD F	0.5
Primer 2. (stock concentration is 20μM) Neo TD R	0.5
Primer 3. (stock concentration is 20μM) Tcrd F	0.5
Primer 4. (stock concentration is 20μM) Tcrd R	0.5
Taq Polymerase 5Units/μL	0.2
DNA extracted with <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input type="checkbox"/> Other:	1.0
TOTAL VOLUME OF REACTION:	25.000μL

Comments on protocol:

- This protocol only indicates the presence or absence of internal Neo vector; it does not distinguish heterozygous vs. knockout, nor is it specific to any single construct. TCRD is an internal control to verify DNA is present.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non specific amplifications.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	3:00	1
2. Denaturation	94	0:20	} 12x
3. Annealing } steps 2-3-4 will cycle in sequence	64	0:30	
4. Elongation	72	0:35	
5. Denaturation	94	0:20	
6. Annealing } steps 5-6-7 will cycle in sequence	58	0:30	} 25x
7. Elongation	72	0:35	
8. Amplification	72	2:00	
9. Finish	10	∞	n/a

Primers:

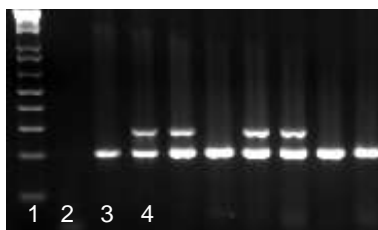
Name	Nucleotide Sequence (5' - 3')
1: Neo TD F	CTTGGGTGGAGAGGCTATTC
2: Neo TD R	AGGTGAGATGACAGGAGATC
3: Tcrd F	CAAATGTTGCTTGTCTGGTG
4: Tcrd R	GTCAGTCGAGTGCACAGTTT

Electrophoresis Protocol:

Agarose: 2% mV: 80

Estimated Running Time (min): 90

Expected Bands	Genotype
200 bp	WT
200 bp / 280 bp	Neo positive



Lanes:
1. 1Kb+ ladder
2. H₂O
3. Wild-type +/+
4. Neo +

Lexicon Gene Trap Protocol attached.



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ1915	Date of Submission:	7/28/04
Lexicon Contract Name:	DNA020	Mutation Type:	<input checked="" type="checkbox"/> Standard Knock out
LexVision Name:	PRT333N1		<input type="checkbox"/> Conditional
Reference accessions:	AK085308.1	Is this gene X-linked?	NO

Required Materials: X pKOS clone DNA(s) _KOS-93
 X Target Vector DNA _PRT333-93TV.1
 X Targeted ES Cell DNA _2B9
 X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' External	3' External
Name of Probe:	DNA020-13/14	DNA020-6/4
Restriction Enzyme for Genomic Digest:	KpnI	HindIII
Predicted Wild-type Band (kb):	16.6 kb	9.2 kb
Predicted Mutant Band (kb):	10.5 kb	7.2 kb
Probe Size:	543 bp	441 bp

PCR Strategies:*For standard knockouts, give wildtype and mutant-specific strategies**For conditionals, give 5' loxP and cre-excision strategies*

Wild type-specific (absent in targeted allele)		Mutation-specific product (absent in wt)	
5' Primer Name:	DNA020-17	5' Primer Name:	NEO3a
3' Primer Name:	DNA020-18	3' Primer Name:	DNA020-18
Predicted Wild-type Band (bp):	283 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	373 bp

5' loxP strategy		Distinguish Cre-excised and wt	
5' Primer Name:		5' Primer Name:	
3' Primer Name:		3' Primer Name:	
Predicted Wild-type Band (bp):		Predicted Wild-type Band (bp):	
Predicted mutant band (bp)		Predicted mutant band (bp)	

Primer sequences:**Southern probes**

DNA020-4 5' – GAATGTGGAAGATAGCAGTCC
DNA020-6 5' – GGCCTAGGACTCCACCC
DNA020-13 5' – CCTACAGCTTCTCCATGAC
DNA020-14 5' – CCTACGGAGACCACAGAAC

PCR Genotyping

DNA020-17 5' – CGTCTAGTCCCTACCATGTCTC
DNA020-18 5' – CCGCACCTCCAAATGGCTAGT
NEO3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

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Genomic Locus: (the deleted sequence represents nt5471-8288-in the sequence below)

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Selection Cassette:

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Targeted Locus:

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