

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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STRAIN NAME: B6;129S5-Stard3n^{tm1Lex}/Mmucd **MMRRC:** 032605-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	10.775
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)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Primer 3. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non-specific amplifications.

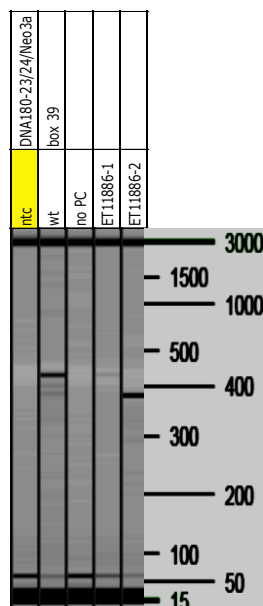
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA180-23	CTCATGGCCAGGATTGAGTC	Estimated Running Time: 90 min.		
2. DNA180-24	CAAGCCTGTGCACGATGACC	Primer Combination	Band (bp)	Genotype
3. Neo3a	GCAGCGCATCGCCTTCTATC	1 and 2	452	transgenic
		2 and 3	395	transgenic





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ855	Date of Submission:	1-19-05
Lexicon Contract Name:	DNA180A	Mutation Type:	X Standard Knock out
LexVision Name:	MEM485N1		☐ Conditional
Reference accessions:	NM_024270	Is this gene X-linked?	No

Required Materials: ☐ pKOS clone DNA(s) __pKOS71_____
☐ Target Vector DNA __KOS71TV_____
☐ Targeted ES Cell DNA __2A1_____
☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	19+20	15+16
Restriction Enzyme for Genomic Digest:	XbaI	EcoRV
Predicted Wild-type Band (kb):	8.7 kb	14.3 kb
Predicted Mutant Band (kb):	7.5 kb	12.5 kb
Probe Size:	615 bp	205 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:	DNA180-23	5' Primer Name:	Neo3A
3' Primer Name:	DNA180-24	3' Primer Name:	DNA180-24
Predicted Wild-type Band (bp):	452 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	395 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**

DNA180-19 5' – GGTGAGTGTTCCACGGCTTG
DNA180-20 5' – TAGCATGGTTGGCGTTCATT
DNA180-15 5' – TCCCTGTGCTGTTGCTCTGTG
DNA180-16 5' – CTTCTGCCTCTTGAGGTAACAC

PCR Genotyping

DNA180-23 5' – CTCATGGCCAGGATTGAGTC
DNA180-24 5' – CAAGCCTGTGCACGATGACC
Neo3A 5' – GCAGCGCATCGCCTTCTATC

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Email: kholt@lexgen.com

Genomic Sequence Deleted:

CTCCAGGATGAACCATCTTCCAGAACACATGGAGAACACTCTGACGGGGAGCCAGAGCTCACACGCTTCTCTGCGAGACATCCATTCCATCAACCCCTGCGCAGCTCATGGCCAGGATTGAGTCCTATGAAGGAAGGGAAAAGAAAGGCATTTCTGATGTCAGGAGGACTTTTTGTCTCTTTGTCACCTTTGACCTCTTATTTGTAACGTTACTGTGGATAATAGAGTTAAATGTAA GTTGGTTTTGCTCATGACTTGCTAAAACTGTTGAAATTTTTCTCATTATTTTGACTTATTTAGTGAATATCATCTCTTCAAATGATAA

Genomic Locus: *(The deleted sequence represents nt7597-7922-in the sequence below. KOS71 used to generate the TV represents nt 3684-13749 in the sequence below.)*

[illegible]

[illegible]

[illegible]

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