GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

STRAIN NAME: **B6;129S5-*Slc25a25*^{tm1Lex}/Mmucd** **MMRRC: 032579-UCD**

Protocol:

Reagent/Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (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 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust

Strategy:

Steps		Temp (ºC)	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 (↓1ºC/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		15	∞	n/a

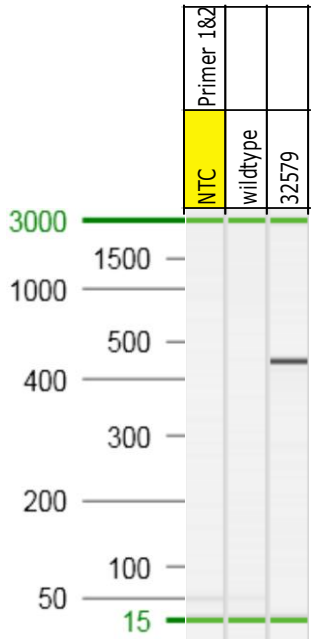
Primers:

Name	Nucleotide Sequence (5' - 3')
1. DNA311-19	GCTTCCAGTACAGGATGATC
2. Neo3a	GCAGCGCATCGCCTTCTATC
3. DNA311-1	CTTCTGCTAGTACTTGCTCC
4. DNA311-2	CCATGACAGTGACGTACAAC

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	Band (bp)	Genotype
1 & 2	466	Mutant
3 & 4	379	Wild-type



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Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ549	**Date of Submission:**	10/05/04
Lexicon Contract Name:	DNA311	**Mutation Type:**	X Standard Knock out
LexVision Name:	TRA347N1		☐ Conditional
Reference accessions:	NM_146118	**Is this gene X-linked?**	No

Required Materials:
- ✓ pKOS clone DNA(s) __pKOS38_____
- ✓ Target Vector DNA __pKOS38 FTV_____
- ✓ Targeted ES Cell DNA __1D8 _____
- ✓ Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' External	**3' External**
Name of Probe:	**13/14**	**17/18**
Restriction Enzyme for Genomic Digest:	**HindIII**	**EcoRI**
Predicted Wild-type Band (kb):	**6.5 Kb**	**12.1 Kb**
Predicted Mutant Band (kb):	**5.2 Kb**	**8.1 Kb**
Probe Size:	**395 bp**	**402 bp**

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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:	1	5' Primer Name:	Neo3a
3' Primer Name:	2	3' Primer Name:	19
Predicted Wild-type Band (bp):	379	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	466

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

DNA311-13	5' – CTCTGTGACATTCGACTTCG
DNA311-14	5' – CCAGGATTGTGCTCATTGTG
DNA311-17	5' – CATCGACCTAGCTGTCTATG
DNA311-18	5' – CTTGCTGAACTCCTGACTTG

PCR Genotyping

DNA311-1	5' – CTTCTGCTAGTACTTGCTCC
DNA311-2	5' –CCATGACAGTGACGTACAAC
DNA311-19	5' –GCTTCCAGTACAGGATGATC
Neo3a	5' – GCAGCGCATCGCCTTCTATC

Contact Information:
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Genomic Sequence Deleted:

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Genomic Locus: ***(the deleted sequence represents nt 10000 – 13337 in the sequence below)***

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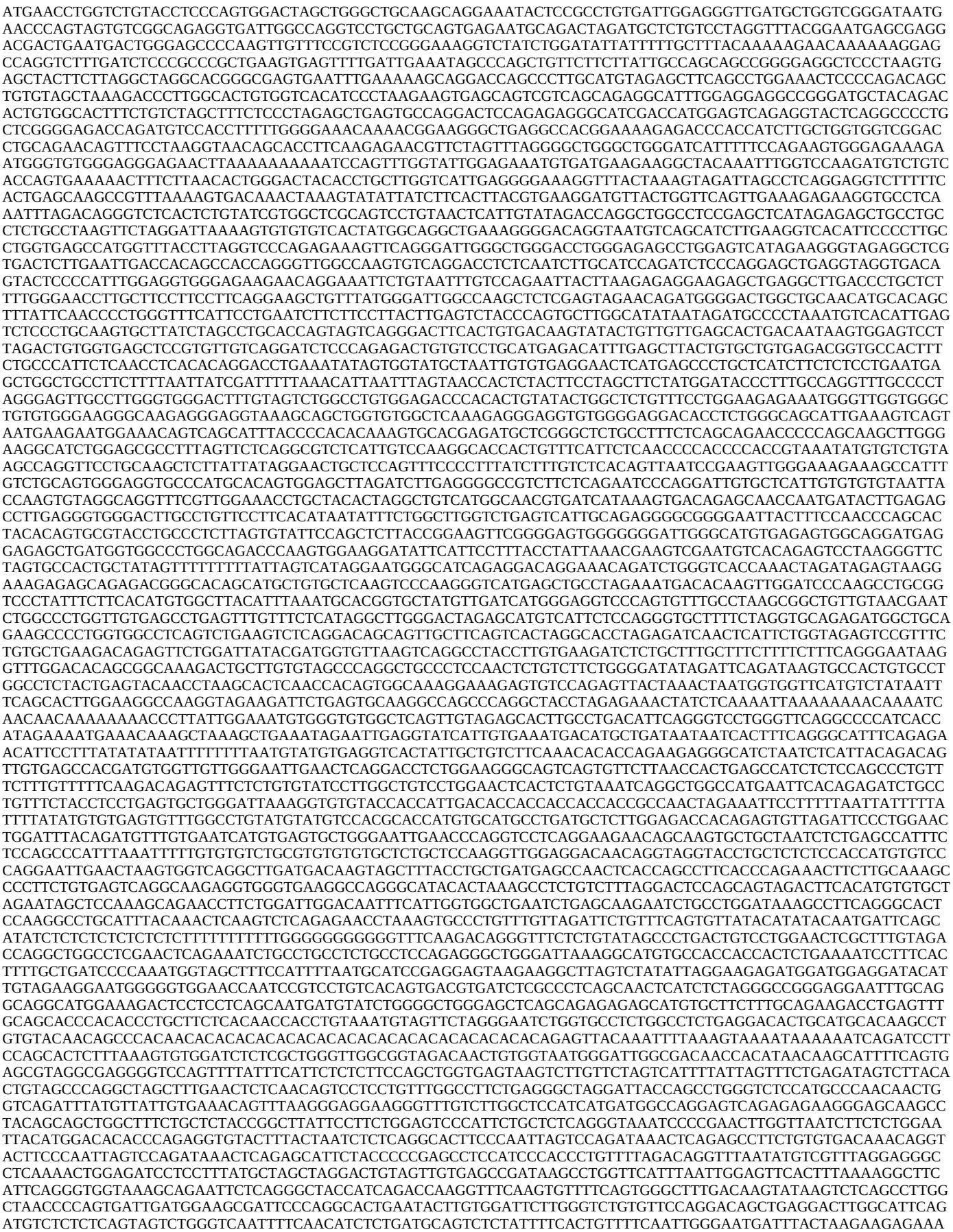

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Targeted Locus:

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