GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: B6N.129S5-Jamltm1Lex/Mmucd **MMRRC:** 032143-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
	TOTAL VOLUME
	15

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.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1x**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**30X**
7. Elongation		72	0:40	

Primers:

Name	Nucleotide Sequence (5' - 3')
1. UNQ722-12	CAGAATCTGGCTCACATTGG
2. IRESGTExt	GCTAGACTAGTCTAGCTAGAGCGG
3. UNQ722-3	ATACCCGTTGGTTTGTGAGATA
4. UNQ722-4	TGAGAACAGAGTTCCAGGCAAG

Electrophoresis Protocol:

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

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

Primer Combination	**Band** (bp)	Genotype
1 & 2	184	mutant
3 & 4	328	wildtype

Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ722	**Date of Submission:**	4/28/05
Lexicon Contract Name:	DNA377	**Mutation Type:**	X Standard Knock out
LexVision Name:	MEM766N1		☐ Conditional
Reference accessions:	NM_001005421.2	**Is this gene X-linked?**	no

Required Materials:
X pKOS clone DNA(s) __pKOS-56_____
X Target Vector DNA __pKOS-56TV_____
X Targeted ES Cell DNA __1E7_____
X Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' Internal	**3' External**
Name of Probe:	**23/24**	**29/30**
Restriction Enzyme for Genomic Digest:	**Bgl II**	**Acc65I**
Predicted Wild-type Band (kb):	**5.3**	**9.1**
Predicted Mutant Band (kb):	**9.6**	**13.4**
Probe Size (bp):	**352**	**222**

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:	3	5' Primer Name:	12
3' Primer Name:	4	3' Primer Name:	IRES-GT-Ext
Predicted Wild-type Band (bp):	328	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	184

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

23	5' – AGGAAATGGCCAGTGCTAGATG
24	5' – GTAGAACTAAGAATTGCGCTGT
29	5' – CCCAAGTCTCGATGTGTA
30	5' – CAGGCAAAGCTTAGACA

PCR Genotyping

3	5' – ATACCCGTTGGTTTGTGAGATA
4	5' – TGAGAACAGAGTTCCAGGCAAG
12	5' – CAGAATCTGGCTCACATTGG
IRESGTExt	5' – GCTAGACTAGTCTAGCTAGAGCGG

Genomic Sequence Deleted:

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Genomic Locus: (*The deleted sequence represents nt 6665-11185 in the sequence below. KOS56 used to generate the TV represents nt 5164-16450 in the sequence below.)*

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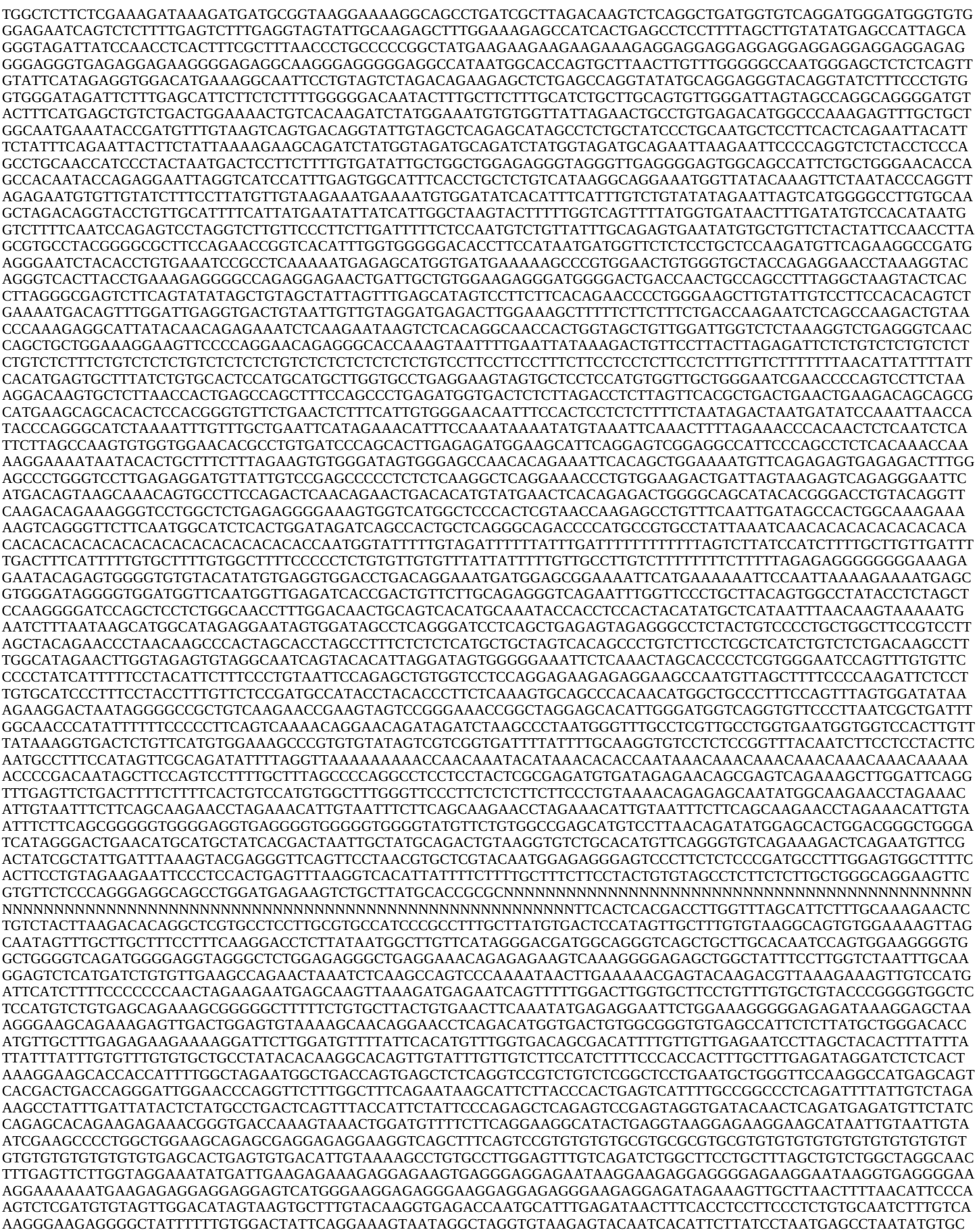

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Selection Cassette:

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Targeted Locus:

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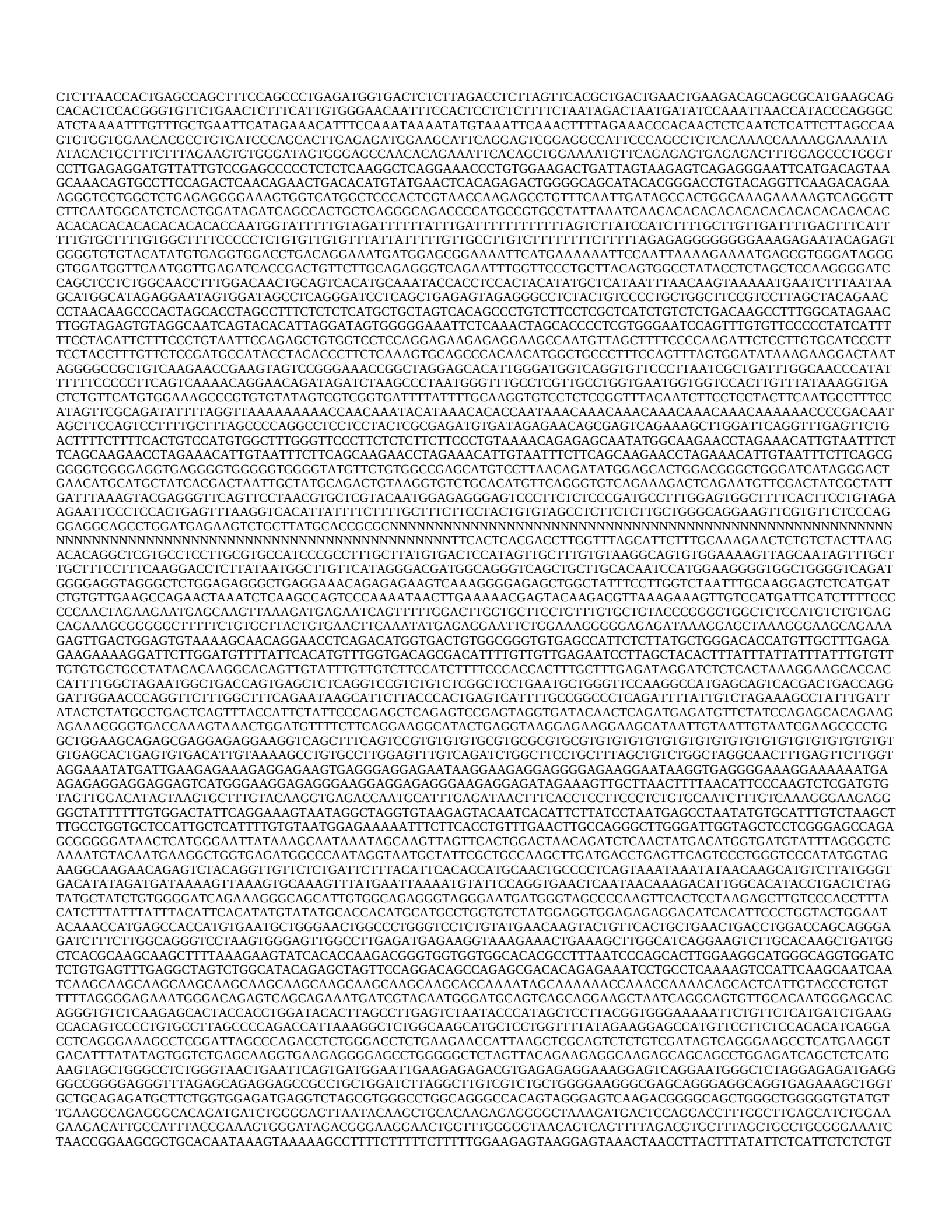

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