

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

Protocol Name: B6;129S5-Angptl7tm1Lex/Mmucd MMRRC: 032149-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
<b>TOTAL VOLUME</b>	
15	

**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.

**Strategy:**

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	5:00	<b>1x</b>
2. Denaturation	94	0:15	
3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>	65 (↓1°C/cycle)	0:30	<b>10x</b>
4. Elongation	72	0:40	
5. Denaturation	94	0:15	
6. Annealing <span style="float: right;">steps 5-6-7 cycle in sequence</span>	55	0:30	<b>30X</b>
7. Elongation	72	0:40	

**Primers:**

**Electrophoresis Protocol:**

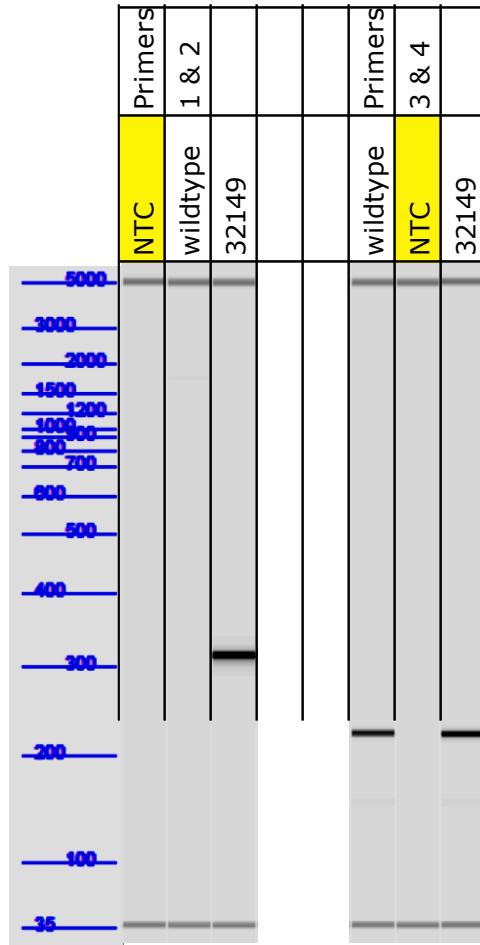
Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5%	V: 90		
1. DNA065-24	ACAATATTTAAGATGCTGCC	Estimated Running: Time: <b>90</b> min.			
2. GT-IRES	CCCTAGGAATGCTCGTCAAGA	Primer Combination	Band (bp)	Genotype	
3. DNA065-22	GAGGTACAGCAGCAACAATA	1 & 2	296	mutant	
4. DNA065-23	TGTTCCCTTAGAGTCT	3 & 4	207	wildtype	

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## Lexicon Genetics Incorporated – Genentech Project Materials

<b>Genentech ID:</b>	UNQ313	<b>Date of Submission:</b>	1/15/04
<b>Lexicon Contract Name:</b>	DNA065	<b>Mutation Type:</b>	<input checked="" type="checkbox"/> Standard Knock out
<b>LexVision Name:</b>	SEC429N1		<input type="checkbox"/> Conditional
<b>Reference accessions:</b>	ENSMUST00000030840, BC023373	<b>Is this gene X-linked?</b>	No

**Required Materials:**

- pKOS clone DNA(s) \_\_KOS91\_\_\_\_\_
- Target Vector DNA \_\_KOS91-FTV(Puro)\_\_\_\_\_
- Targeted ES Cell DNA \_\_1A1\_\_\_\_\_
- Genomic Map

**Southern Blot Analysis:**  
*External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	<b>19 + 20</b>	<b>15 + 16</b>
Restriction Enzyme for Genomic Digest:	<b>EcoRI</b>	<b>ScaI</b>
Predicted Wild-type Band (kb):	<b>9.4</b>	<b>12.1</b>
Predicted Mutant Band (kb):	<b>7.4</b>	<b>27.1</b>
Probe Size:	<b>252 bp</b>	<b>244 bp</b>

**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:	DNA065-22	5' Primer Name:	DNA065-24
3' Primer Name:	DNA065-23	3' Primer Name:	GT-IRES
Predicted Wild-type Band (bp):	208	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	296

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**

DNA065-19	5' – TAAATGGTGGGTGTGCTAGG
DNA065-20	5' – TCACACAGTCTCAAGTGCAC
DNA065-15	5' – TGTAAGTGTCCCATAGCGC
DNA065-16	5' – TCTCCATGGAGATCCTAACG

**PCR Genotyping**

DNA065-22	5' – GAGGTACAGCAGCAACAATA
DNA065-23	5' – TGTTCCCCTTAGAGTCT
DNA065-24	5' – ACAATATTTAAGATGCTGCC
GT-IRES	5' – CCCTAGGAATGCTCGTCAAGA

**Genomic Sequence Deleted:**

GTTCCATTTCAGAACTCTAGCTTTAAGAAAAGGCTAAGCAAGCACACAGAGGAAGGAGATCACGGGGAAGGAAGAAAACAGTCCAGTGTGGGTCAGAG  
AAAAAAGCTTCTACTCTCTCCAGGGACAGACTCTAAGGGGAACAGGCTGCACACCATGCTGAGGGAGACCTGGCTATGTTTATCCTTGTAGCCCTTT  
GTCAGCCACCCAGTGTGGCTGCAGAAAGCTCATAAAACGCAAGACACAGCTCAAAGCAGCCGGCTGTGTGAGGAGATGAGGGAGCTCAAAGCCCAGG  
TGGCCAACTCAGCAGTCTGCTGGGTGAGCTGAGCAGGAAGCAGGAGAGCGACTGGGTCAGTGTGGTTCATGCAGGTGATGGAGCTGGAGAGCAGCAG  
CAAGCACATGGAGTCTCGCTCAGCACTGCCGAGAGCAAGTACTCTGAGATGAACACCAGATTGACATCATGCAGCTGCAGGCTGCGCAGACCCTCA  
CGCAGACCTCGGCAGGTAAGTGCTGCCTCCACTCTCACCATGCGCTGCTCAAGGTCTCTGGCCCCAGCTCTGCTTCTGG

**Genomic Locus: (*the deleted sequence represents nt 10008 – 10579 in the sequence below. KOS91 used to generate the TV  
represents nt 5402 – 15939 in the sequence below.*)**

TCTGATGGACTAGAGAAGTTAACATCTGTGCATGTAATAATAGAATGTCTCATGTTTACGAAGCACAGTTGTAACAATCCTCCAGTCAATTCCTTGGC  
TTCACATAGCTGAGAAAAATCAGGAAAGAAAGGTTAGAATTAGTTCTCTCTATTATAATGTGGATGCCAAGTCAAAGAAAGGCCAAAAGCCCTAAACTC  
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GCAAGGCACTTGTGCCAAGCTGACACTGTAGCTTGACCCCCAGAACCCATGGTATCAGGAAAAACGGACTCTTGGAAAAACACATCAGCAGCAG  
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CCGCTGAGCAATGGAAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGGCTCCTTCGCTTTCTGGGCTCAGAGGGCTGGGAAGGGGTGGGTG  
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**Targeted Locus:**

TCTGATGGACTAGAGAAGTAAACATCTGTGCATGTAATAATAGAATGTCTCATGTTTACGGAAGCACAGTTGTAACAATCCTTCCAGTCAATTCCTTTGGC  
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CTAGCAAAGTTCTAAGAGCTAGATATTCAAAGTGTGGTCC