

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

STRAIN NAME: B6;129S5-Mmp27^{tm1Lex}/Mmucd **MMRRC:** 032471-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:

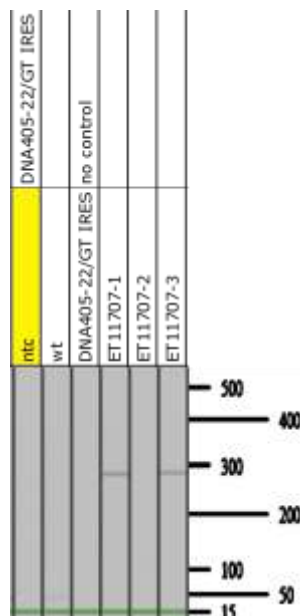
- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust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

Name	Nucleotide Sequence (5' - 3')	Electrophoresis Protocol:		
1. DNA405-22	TGATTACTTCATGCCACGTCTC	Argarose: 1.5% V: 90		
2. GT IRES	CCCTAGGAATGCTCGTCAAGA	Estimated Running Time: 90 min.		
3. DNA405-23	TAGCCAAATCACACCCCATAC	Primer Combination	Band (bp)	Genotype
		1 and 2	288	Mutant
		1 and 3	462	WT





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2503	Date of Submission:	4-3-06
Lexicon Contract Name:	DNA405	Mutation Type:	X Standard Knock out
LexVision Name:	SEC491N1		☐ Conditional
Reference accessions:	NM_001030289.1	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS40, pKOS67
X Target Vector DNA __pKOS40TVpuro_____
X Targeted ES Cell DNA __1B9_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	14+15	16+17
Restriction Enzyme for Genomic Digest:	AvrII	ScaI
Predicted Wild-type Band (kb):	9.7	7.7
Predicted Mutant Band (kb):	6.2	13.6
Probe Size:	202 bp	218 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:	DNA405-22	5' Primer Name:	DNA405-22
3' Primer Name:	DNA405-23	3' Primer Name:	GT-Ires
Predicted Wild-type Band (bp):	462	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	288

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**

DNA405-14 5' – CTGCCCTCATCCTAGATATG
 DNA405-15 5' – CCGAGAAAGACAAGGACGTCAC
 DNA405-16 5' – GTACTTAGGTAAGACCCTGATG
 DNA405-17 5' – GCTGGAGTGTCAAGGGAATAG

PCR Genotyping

DNA405-22 5' – TGATTACTTCATGCCACGTCTC
 DNA405-23 5' – TAGCCAAATCACACACCCATAC
 GT-Ires 5' – CCCTAGGAATGCTCGTCAAGA

Contact Information:

Laura L. Kirkpatrick, Ph.D.
 Senior Scientific Group Leader, Molecular Genetics
 Lexicon Genetics, Inc.
 8800 Technology Forest Place
 The Woodlands, TX 77381-1160
 Phone: 281-863-3016
 Email: laurak@lexgen.com

Additional Contact:

Kathleen Holt, Ph.D.
 Senior Director Molecular Genetics & Alliance Management
 Lexicon Genetics, Inc.
 8800 Technology Forest Place
 The Woodlands, TX 77381-1160
 Phone: 281-863-3013
 Email: kholt@lexgen.com

Genomic Sequence Deleted:

[illegible]

Genomic Locus: *(The deleted sequence represents nt2244-9171-in the sequence below. pKOS40 represents nt 1893-12871 in the sequence below.)*

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