

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

**NAME OF PCR:** B6;129S5-Igfbp2<sup>Gt(OST365171)Lex</sup>/Mmucd **MMRRC:** 011721-UCD

**Protocol:**

Reagent/Constituent	Volume (μL)
Water	10.275
10x Buffer	2.5
MgCl <sub>2</sub> (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
<b>TOTAL VOLUME OF REACTION:</b>	<b>25.000 μL</b>

**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

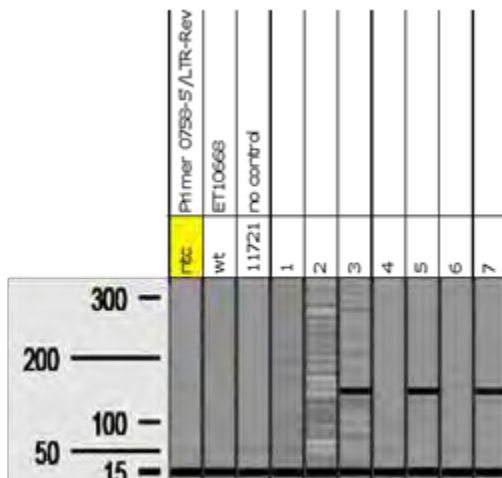
**Strategy:**

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>	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. 0758-5-prime	GGTTCCTCCTGGCTGGTGACTC	Estimated Running Time: 90 min.	
2. LTR-rev	ATAAACCTCTTGCAGTTGCATC	<b>Primer Combination</b>	<b>Band (bp)</b>
3. 0758-3-prime	GAGTCTCCCTGGATCTGATTAAGG	1 and 2	155
		1 and 3	109
			<b>Genotype</b>
			Mutant
			WT



## NIH-0758 Genotyping Strategies

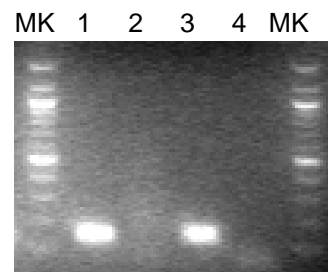
Reaction Components	Vol (ul)
5x Phusion buffer	8
25mM MgCl <sub>2</sub>	3.2
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
Phusion Enzyme	0.1
Water	20.7
Total mix volume	35
Tail lysate (1:20 dilution)	5
Total reaction volume	40

Step	Temp	Time	Note
1	96C	17"	
2	65C	15"	Decrease 1C/cycle
3	72C	15"	Go to 1, 6 cycles
4	96C	17"	
5	60C	15"	
6	72C	15"	Go to 4, 30 cycles

Primer Sequences (5' to 3')	
Mutant PCR: Primer 0758-5' and Primer LTR-rev, 155 bp	
Recommended Wt PCR: Primer 0758-5' and Primer 0758-3', 109 bp	
Primer 0758-5'	GGGTTCTCCTGGCTGGTGACTC
Primer LTR-rev	ATAAACCCCTCTTGCA GTTGCATC
Primer 0758-3'	GAGTCTCCCTGGATCTGATTAAGG

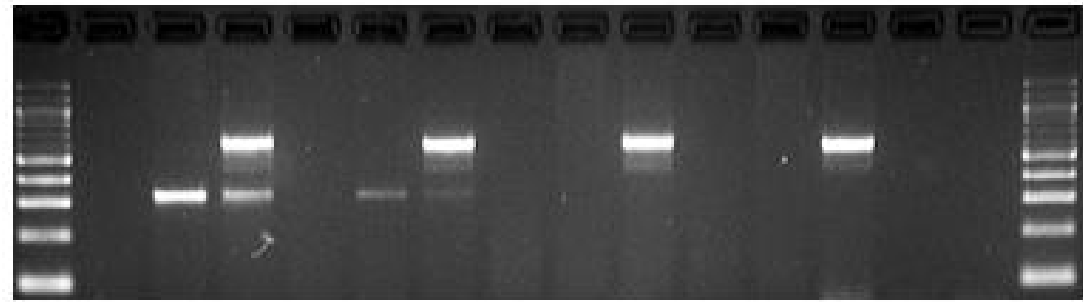
Well	Sample	Genotype
1	49	het
2	wt lysate	wt
3	es DNA	het
4	water	no amp

### Mutant PCR



# QC Expression

RT	-	+	+	-	+	+	-	+	+	-	+	+	-	-
Actin Primers	-	-	+	-	-	+	-	-	+	-	-	+	-	+



← Actin  
← Gene Specific

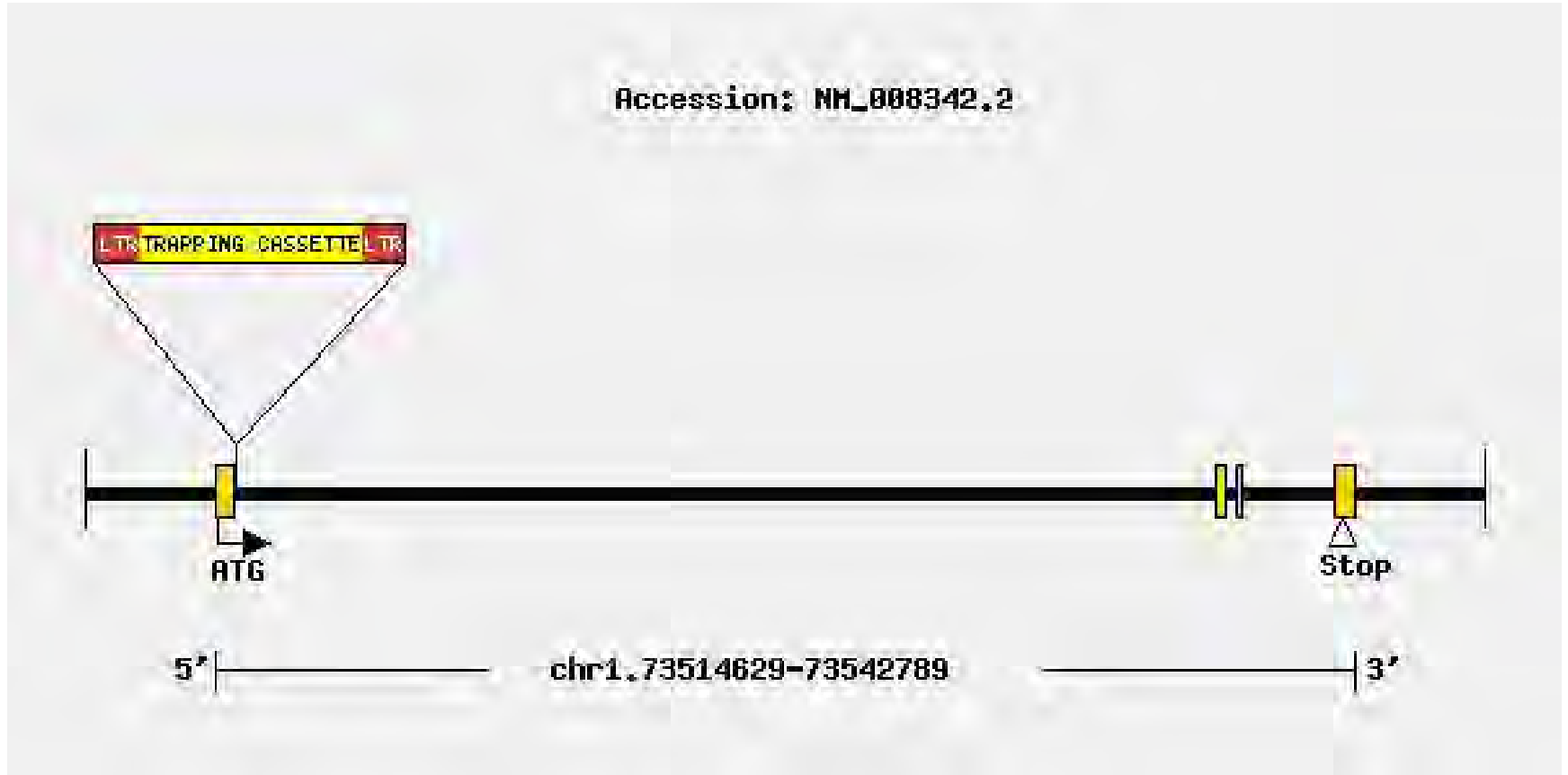
Genotype	+/+		-/-		No
Tissue	Kidney	Spleen	Kidney	Spleen	Template

PCR 35 cycles  
Primers: 1&2

Mouse ID 127

# QC Image

Accession: NM\_008342.2



# RT-PCR WT Expression

mouse random primed cDNA with Primers: 1,2



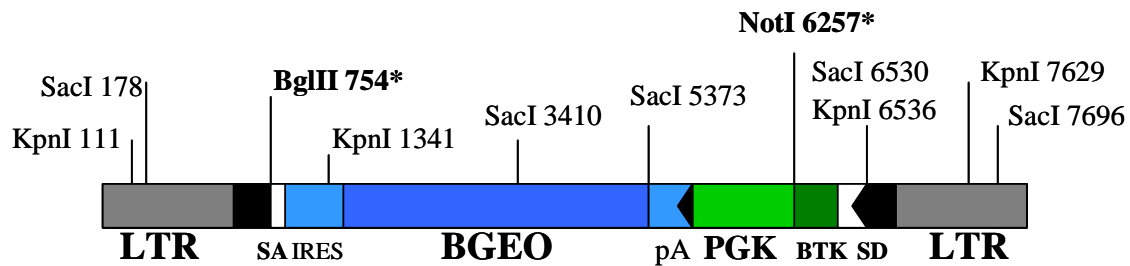
01/13/2004

**Note:** Expected band size denoted by arrow adjacent to 100bp ladder/marker.

## Mouse cDNA Tissues

- 1) Brain
- 2) Spinal Cord
- 3) Eye
- 4) Thymus
- 5) Spleen
- 6) Lung
- 7) Kidney
- 8) Liver
- 9) Skeletal Muscle
- 10) Bone
- 11) Stomach, Small Intestine & Colon
- 12) Heart
- 13) Adipose
- 14) (-) Control
- 15) (+) Control- ES cell cDNA
- 16) (+) Control- Genomic/Lex1 DNA
- 17) 100 bp ladder/marker

## VICTR 37 Omnibank Vector



**Total Size:** 8108 nucleotides

**Non-Cutters:** SacII, DraI, XhoI

\* Unique sites

### Location of components in VICTR 37:

LTR (viral long terminal repeat): 1-590, 7519-8108

SA (splice acceptor): 759-888

IRES: 885-1469

Bgeo: 1471-5365

pA: 5370-5655

frt sites: 5670-5717, 6546-6593

PGK promoter: 5738-6254

BTK exon: 6289-6514

>VICTR 37

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TGAAAGACCCCGCTGACGGGTAGTCAATCACTCAGAGGAGACCCTCCCAAG
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