

**GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER**

sacoord@mmrrc.org

800-910-2291 North America, +1-530-757-5710 International

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

- Open the original graphic in the program that created it
- Choose File, Save As
- Select No Compression in the save options.
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- Switch to Word, choose Insert, Picture, From File and choose the newly saved picture.

These instructions are very generic. The menu options for your graphics program may be different.

Donating Investigator/PI		
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tmglaser@ucdavis.edu or jbmiesfeld@ucdavis.edu		
Telephone	530-752-8520 (lab)	
530-752-9575 (office)		
Strain Name "Atoh7 KO mice (lacZ knock-in, C57BL/6J congenic)" more properly known as B6.SJL-Atoh7-tm1Gla1		MMRRC Stock Number not assigned yet

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NAME OF PCR: Atoh7-tm1Gla KO allele **MMRRC:** 0-CTR

Protocol: *(PCR protocol provided by Donating Investigator)*

Reagent/Constituent	Volume (μL)
Water	17.75 uL
10x Buffer (standard Roche Taq Pol buffer, includes MgCl ₂)	2.50 uL
MgCl ₂ (final concentration is 1.5 mM)	
Betaine (stock concentration is 5 M) <i>Optional</i> final 300 mM	1.50 uL
dNTPs (stock concentration is 10mM)	0.50 uL
Primer 1. (stock concentration is 20μM)	0.25 uL
Primer 2. (stock concentration is 20μM)	0.25 uL
Primer 3. (stock concentration is 20μM)	
Primer 4. (stock concentration is 20μM)	
Taq Polymerase 5Units/μL (standard Roche Taq Pol)	0.25
DNA (50-200ng/ μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	0.50
<i>The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume.</i>	
TOTAL VOLUME OF REACTION:	25.00 μL

Comments on protocol:

- We use 1X Masteramp (Epicentre) to obtain 300 mM betaine

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <i>always a good idea</i>	94	5 min	1
2. Denaturation	94	20 sec	
3. Annealing steps 2-3-4 cycle in sequence	56	30 sec	40
4. Elongation	70	60 sec	
5. Amplification	70	7 min	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Ag 1%	V:140
1. Neo 1	AGGATCTCCTGTCATCTCACCTTGCTCCT	Estimated	40 min.
2. Atoh7 REV	AATGGCCCCGAGGCTTAGCTG	Primer	Band
3.		1+2	986 bp
4.			bp
5.			bp
			Genotype
			WT

Comments:

- Atoh7 has one exon. The *lacZ* cassette was inserted in-frame into the *Ascl* site, within the coding region, such that an Atoh7-lacZ fusion protein is expressed, which has the N-terminus of Atoh7 (18 amino acids). See map.
- The PGK pro-neo-bGH pA cassette was inserted between *SmaI* restriction sites in the coding region, deleting 256 bp from the bHLH domain segment. See map.
- Generic Neo diagnostic PCRs can also be used for routine KO allele genotyping
- The Atoh7 (aka Math5) KO mice were described by Brown *et al.* 2001 *Development* 128:2497-2508.

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NAME OF PCR: Atoh7 WT allele **MMRRC:** 0-CTR

Protocol: *(PCR protocol provided by Donating Investigator)*

Reagent/Constituent	Volume (μL)
Water	19.25 uL
10x Buffer (standard Roche Taq Pol buffer, includes MgCl ₂)	2.50 uL
MgCl ₂ (final concentration is 1.5 mM)	
dNTPs (stock concentration is 10mM)	0.50 uL
Primer 1. (stock concentration is 20μM)	0.25 uL
Primer 2. (stock concentration is 20μM)	0.25 uL
Primer 3. (stock concentration is 20μM)	
Primer 4. (stock concentration is 20μM)	
Taq Polymerase 5Units/μL (standard Roche Taq Pol)	0.25
DNA (50-200ng/ μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	0.50
<i>The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume.</i>	
TOTAL VOLUME OF REACTION:	25.00 μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <i>always a good idea</i>	94	5 min	1
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4. Elongation	70	60 sec	
5. Amplification	70	7 min	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agaros 1%	V:140
1. Math5 FOR	CGCCGCATGCAGGGGCTGAACACG	Estimated	40 min.
2. anti-LP10	CATAATACAACCTTCGCCCAATAGGG	Primer	Band
3.		1+2	773 bp
4.			bp
5.			bp
			Genotype
			WT

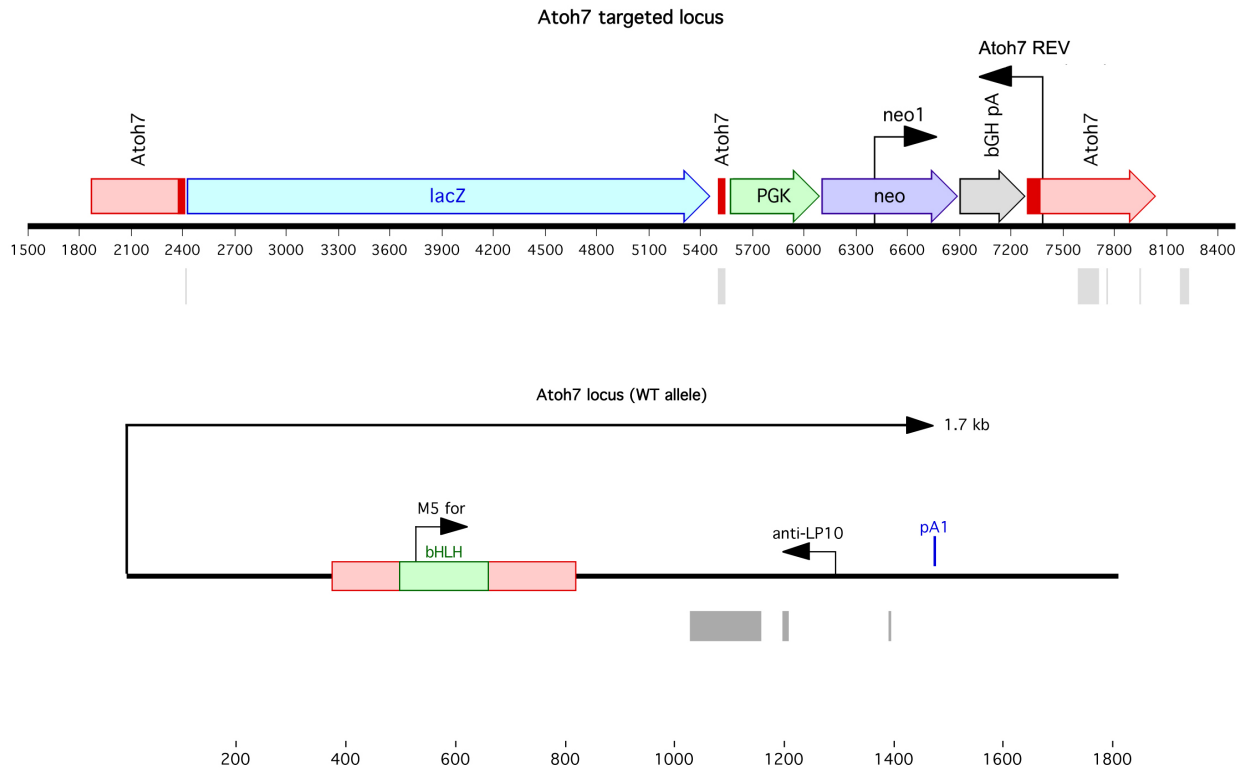
Comments:

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Locus map:



Gel picture:

