



Gene Editing Rat Resource Center

Genotyping Your Model



Date:	04/06/2020		
Mutant Strain:	LE-FMR1 ^{em2Mcwi}	Mutant Strain RGD ID:	11553873
Gene Targeted:	FMR1	RGD Gene ID:	2623
Parental Strain:	Long Evans	RGD Parental Strain ID:	2308852
Mutation Description:	Net 2bp insertion in exon 8		
Deletion:	TCTAGCTATTGGTA ^c TCATGGTGCTAATAT (deletion in red)		
Insertion	TCTAGCTATTGGTA ^{gca} TCATGGTGCTAAT (insertion in red)		
Mutation Region:	(RGSC 6.0/rn6): chrX:154,703,634-154,703,635		
Target Site:	GGTCTAGCTATTGGTACTCA		

Genotyping Primers:	
Forward:	GTTTATTTGCTTCTCTGAGGG
Reverse:	ACCTTTTAAATGGCATAGACCT
WT PCR Size:	413 bp
Mutant Size:	415 bp

Derivation Information:

This strain was produced by injecting a CRISPR targeting the sequence **GGTCTAGCTATTGGTACTCA** into Long Evans rat embryos. The resulting mutation is a net 2-bp frameshift insertion in exon 8. Founder animals were genotyped by the Cel-1 assay and confirmed by sanger sequencing. The founders were then backcrossed to the parental strain and subsequent litters were genotyped by fluorescent genotyping.

Suggested Genotyping:

Suggested genotyping is to Direct sequence PCR products or fluorescent capillary genotyping. Another option is to design mutant specific primers along with WT primers that overlap mutation.

Funding Source:	
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Additional Notes:

Questions? Send an email to: mcwcustomrats@mcw.edu

PCR

5.0 ul of template (approximately 200ng)

12.5 μ l of Accuprime Supermix II (Invitrogen, Carlsbad, CA)

1.0 μ l of primer set at 10 μ M

8.5 μ l of water

25 uL / rxn

PCR cycling parameters:

95°C- 5 min

35 cycles of:

95°C- 30 sec

60°C- 30 sec

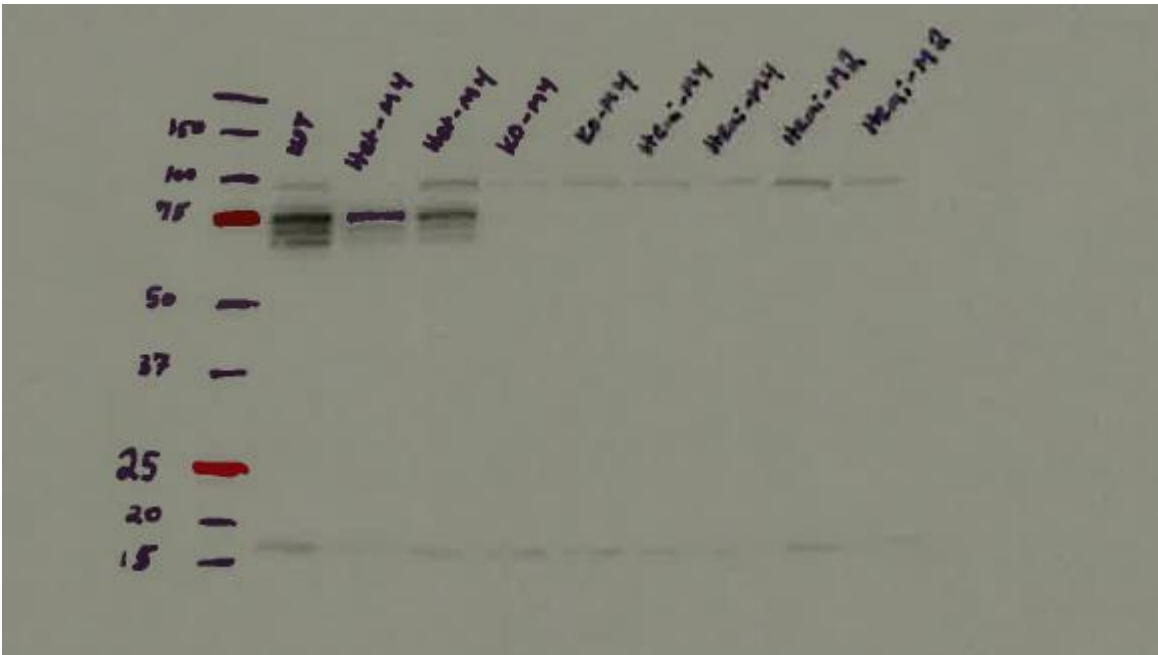
68°C- 45 sec

68°C- 5 min

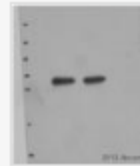
4°C- forever

Western Showing Depletion of FMR1 Protein (<http://www.abcam.com/fmrp-antibody-ab17722.html>)

25ug protein (whole Brain)



Application	Western blot
Loading amount	10 µg
Gel Running Conditions	Reduced Denaturing (10%)
Sample	Human Cell lysate - whole cell (megakaryocytes)
Specification	megakaryocytes
Blocking step	Milk as blocking agent for 2 hour(s) and 0 minute(s) · Concentration: 5% · Temperature: 21°C



Other product details

Incubation time	16 hour(s) and 0 minute(s) · Temperature: 4°C · Diluent: 5% milk
Dilution	1/2000

Secondary antibody

Name	Non-Abcam antibody was used: Goat Anti-Rabbit IgG (H + L)-HRP Conjugate Host species: Goat Clonality: Polyclonal Conjugation: Horse Radish Peroxidase
Dilution	1/3000

Detection

Detection method	ecl
Bands	Specific: 71 kDa
Exposure	5 minute(s) and 0 second(s)

Additional data

Additional Notes	In the image you can see the result with 10 and 30 ug of total proteins.
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