



# Gene Editing Rat Resource Center

## Genotyping Your Model



Date:	04/06/2020		
Mutant Strain:	LE-FMR1 <sup>em4Mcowi</sup>	Mutant Strain RGD ID:	11553875
Gene Targeted:	FMR1	RGD Gene ID:	2623
Parental Strain:	Long Evans	RGD Parental Strain ID:	2308852
Mutation Description:	2bp del in exon 8		
Deletion:	TAGCTATTGGTA <sup>ct</sup> CATGGTGCTAATATTC (deletion in red)		
Insertion	NA		
Mutation Region:	(RGSC 6.0/rn6): chrX:154,703,634-154,703,635		
Target Site:	GGTCTAGCTATTGGTACTCA		

Genotyping Primers:	
Forward:	GTTTATTGCTTCTCTGAGGG
Reverse:	ACCTTTTAAATGGCATAGACCT
WT PCR Size:	413 bp
Mutant Size:	411 bp

### Derivation Information:

This strain was produced by injecting a CRISPR targeting the sequence **GGTCTAGCTATTGGTACTCA** into Long Evans rat embryos. The resulting mutation is 2-bp frameshift deletion 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.

Funding Source:	
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### Additional Notes:

Questions? Send an email to: [mcwcustomrats@mcw.edu](mailto:mcwcustomrats@mcw.edu)

## PCR

5.0  $\mu$ l of template (approximately 200ng)

12.5  $\mu$ l of Accuprime Supermix II (Invitrogen, Carlsbad, CA)

1.0  $\mu$ l of primer set at 10  $\mu$ M

8.5  $\mu$ l of water

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25  $\mu$ L / rxn

## PCR cycling parameters:

95°C- 5 min

35 cycles of:

95°C- 30 sec

60°C- 30 sec

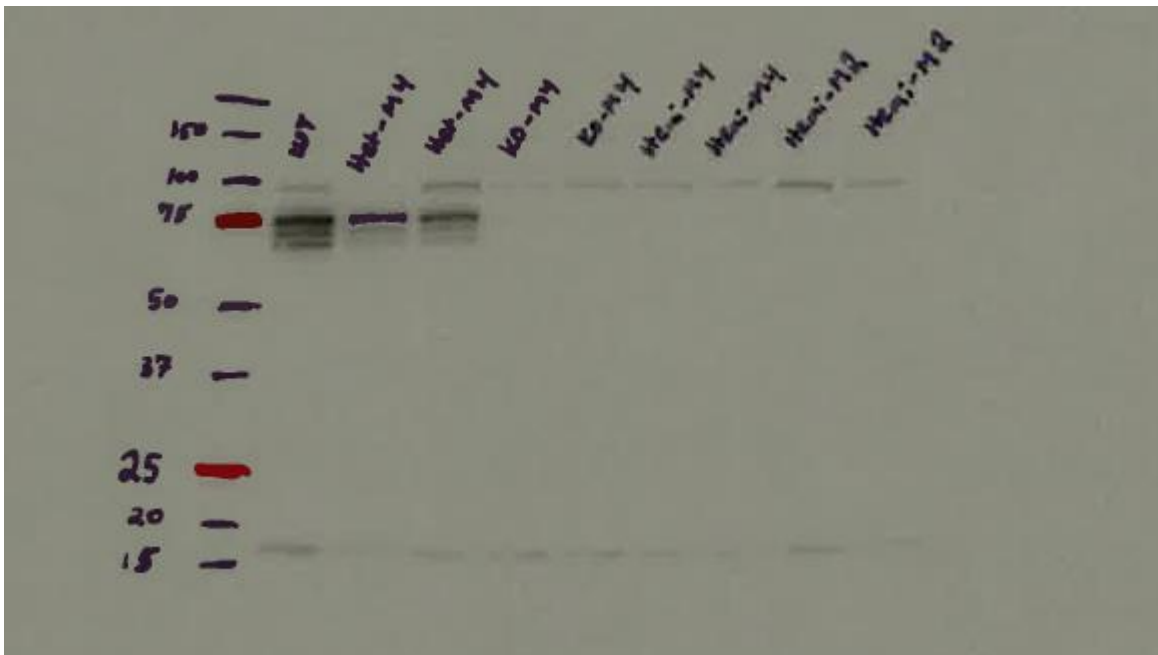
68°C- 45 sec

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)



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



### Other product details

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

### Secondary antibody

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

### Detection

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

### Additional data

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