

**FAX TRANSMITTAL FORM**

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To: MMRRC-ICC

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Genotyping protocol for MMRRC #10317 (dysferlin null mice)

## Dysferlin Genotyping (AT658)

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**Dysferlin Genotyping (AT658)**

Process tails using Campbell lab protocol, making sure to wash samples in 70% ethanol. Be sure to resuspend pellets in 500 uL TE (as opposed to 50 uL).

This reaction is very sensitive, therefore only 1 uL of DNA should be used. Additional DNA will cause the reaction to fail.

## KO Primers:

*KO allele size is 3 Kb*

PCRscrF1      database ID 2290      FORWARD: GCCAGACAAGCAAGGTTAGTGTGG  
 PCRscrR1      database ID 2291      REVERSE: GCTGACTCTAGAGCTTGCGGAACC

## WT Primers:

*WT allele size is 3 Kb*

5armF2          database ID 2867      FORWARD: GACTTCTGTTCATAGAGACCCGAG  
 GTR              database ID 2581      REVERSE: GCGGGCTCTCAGGCACAGTATCTGC

**PCR Reaction**

*NOTE: This genotyping requires use of the TaKaRa PCR kit.  
 WT and KO allele is about the same size. Therefore, rxns must be separated.*

dH <sub>2</sub> O	14 uL
Buffer	2.5 uL
MgCl <sub>2</sub>	2.5 uL
DNTPs	3 uL
Forward primer	1 uL
Reverse primer	1 uL
Tak Enzyme	0.25 uL
DNA	1 uL

Total Volume      25 uL

**PCR Thermocycler Program**

1=94 °C      1 minute  
 2=98 °C      30 seconds  
 3=68 °C      6 minutes  
 4=GOTO Step 2 (30 cycles)  
 5=72 °C      10 minutes  
 6=HOLD 15 °C