Investigation of *RMRP* Expression in Response to Testosterone with respect to Familial Partial Lipodystrophy 2

Stephen Power

BSc Biomedical Science

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University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

The Academy of Clinical Science and Laboratory Medicine

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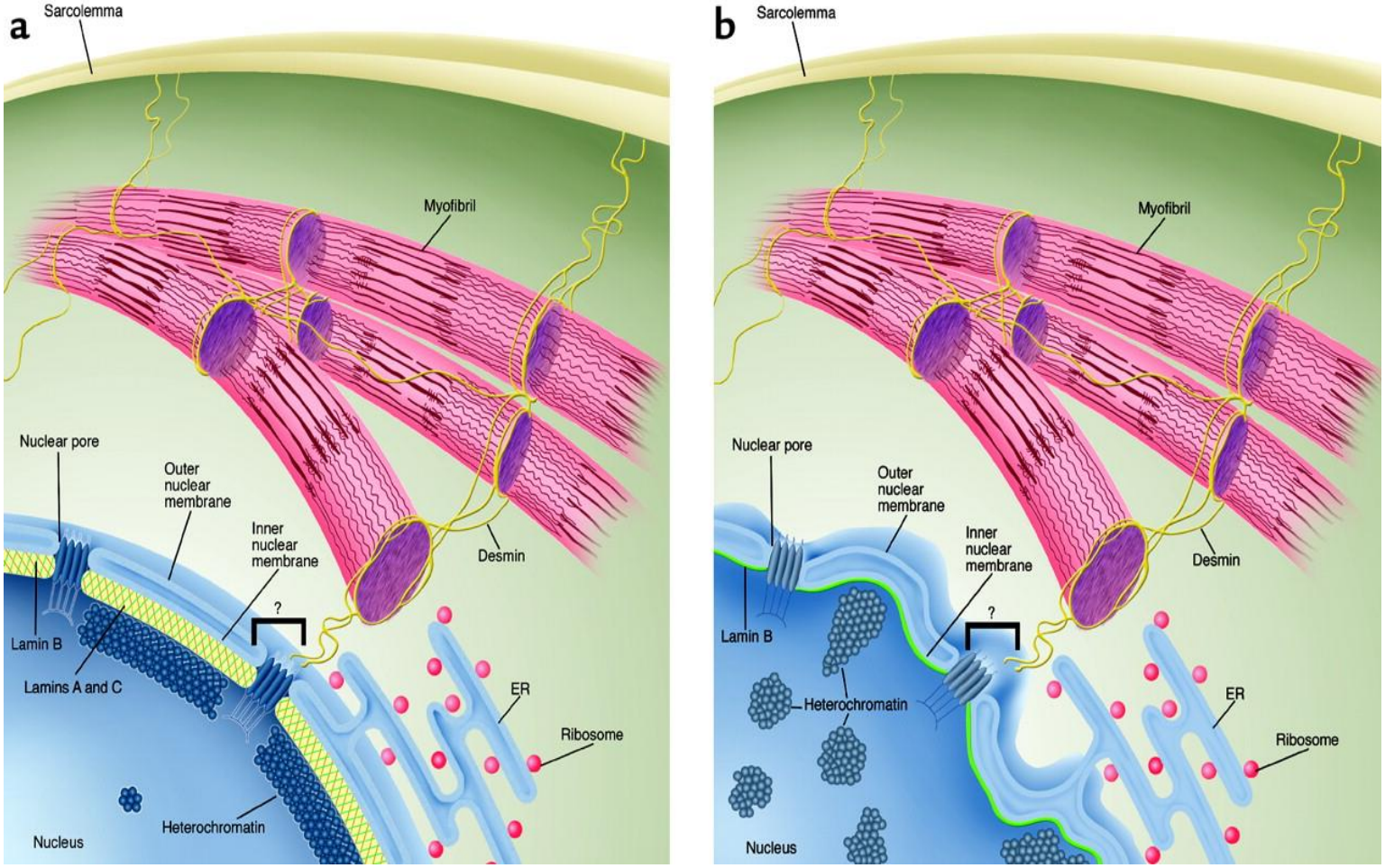
Familial Partial Lipodystrophy II (FPLD2)

- Autosomal dominant inheritance
- Progressive loss of subcutaneous adipose tissue
- Manifests at the onset of puberty
- Reduced adiponectin and increased TNF-ά levels
- Complications include diabetes, hypertriglyceridaemia, hepatic steatosis and premature atherosclerosis with an increased risk of coronary heart disease

- *LMNA* mutation at position 482, most commonly R482Q

Familial Partial Lipodystrophy II (FPLD2)



Worman, H. J. et al. J. Clin. Invest. 2004;113:349-351

LMNA

- The gene *LMNA* codes the production of a-type lamins, namely lamin A and lamin C

- Line the inner nuclear membrane

- Functions include
  - DNA replication
  - Chromatin organisation
  - Anchorage of nuclear membranes

- *LMNA* mutation causes FPLD2

RNA-Sequencing Analysis of 3T3-L1 cells overexpressing mutant *LMNA*

- Activity of approximately 250 genes affected in 3T3-L1 cells overexpressing mutant *LMNA*

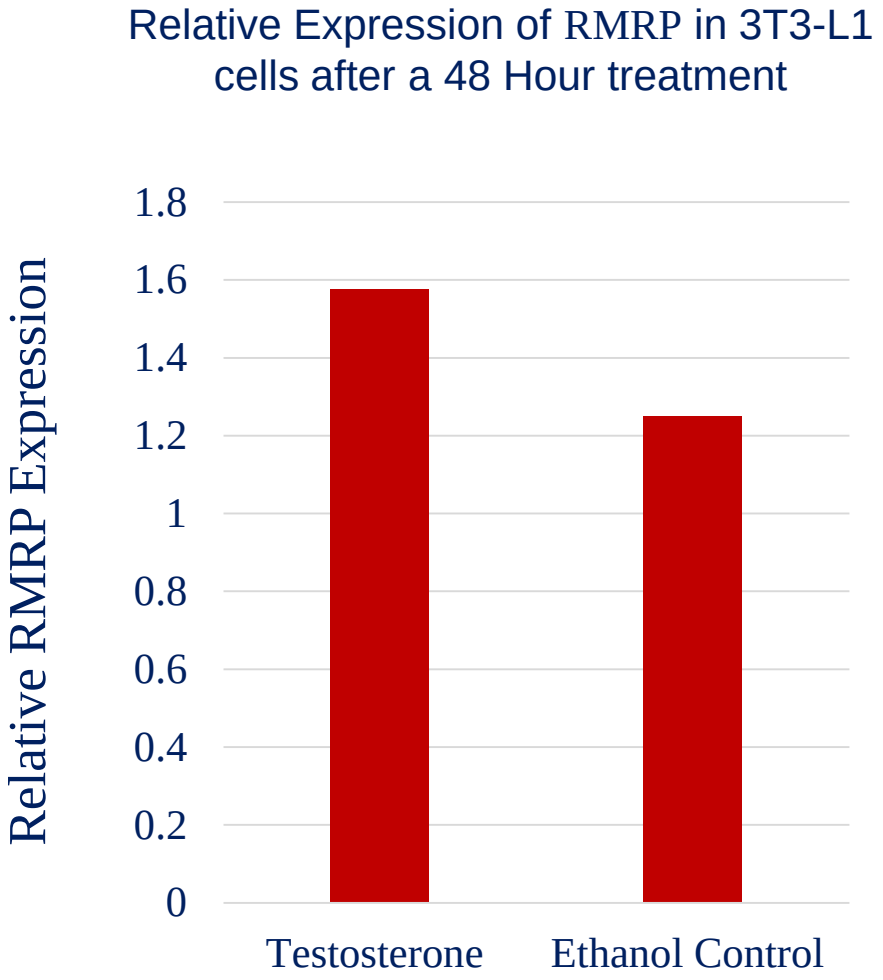
Gene	Wild Type	Wild Type Treated with Testosterone
RMRP	16.7	<0.1

RNase Mitochondrial RNA Processing Gene

- *RMRP* codes for the RNA component of the RNase mitochondrial RNA processing complex
- 267bp product
- Transcribed by RNA polymerase III
- Reservoir for silencing long non-coding RNAs
- Cartilage hair hypoplasia

Analysis of RMRP Transcript Levels in Response to Testosterone

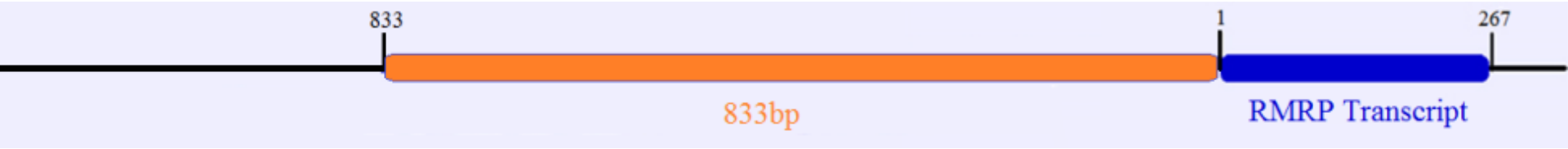
- 3T3-L1 cells were treated with 100nm testosterone in 100% ethanol (vehicle) or ethanol
- RNA extracted using phenol-chloroform method (Chomczynski and Sacchi, 1987).
- Converted to cDNA
- Analysed using quantitative real-time PCR
- Contrasts with existing RNA-Sequencing data



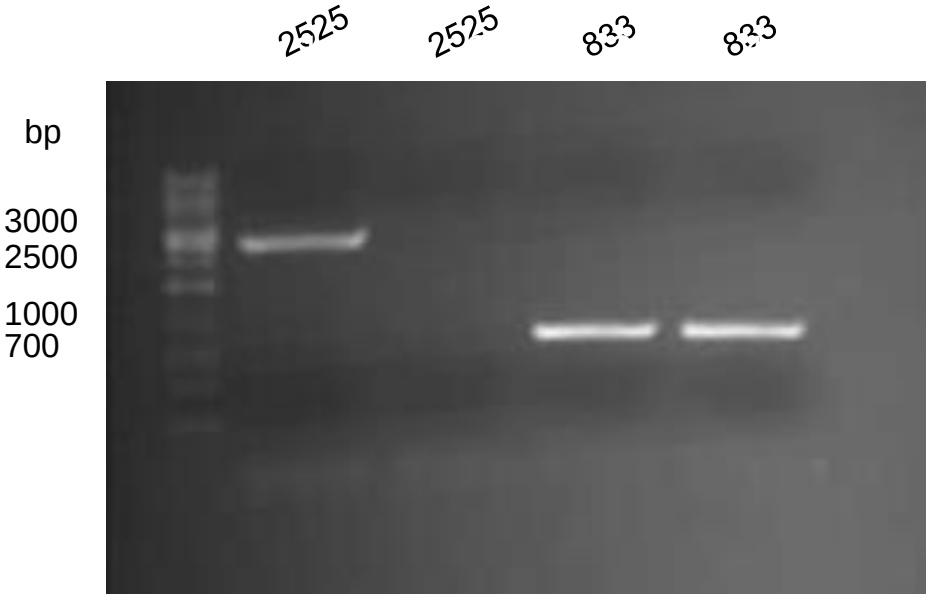
RMRP Promoter Analysis

- Designed forward and reverse primers to amplify 2525bp and 833bp regions of the *RMRP* promoter
- Successfully amplified and cloned the promoter segments into the pGLuc-basic luciferase reporter vector using Gibson Assembly Cloning
- Screened for the presence of the correct PCR product by restriction digest
- Sequenced the *RMRP* 833bp promoter segment

RMRP 833bp Promoter Segment



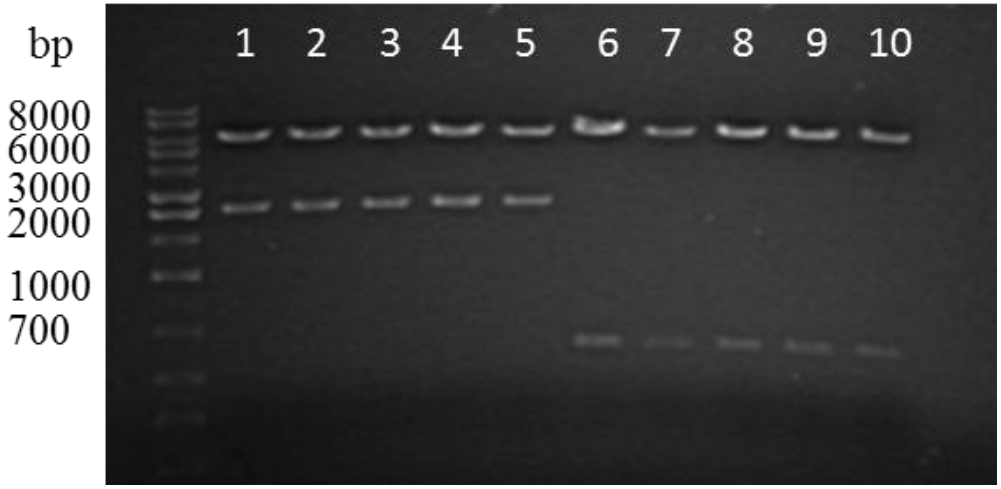
PCR of the RMRP Promoter Region



Amplified PCR products were inserted into pGLuc-basic luciferase reporter vectors using Gibson Assembly Cloning

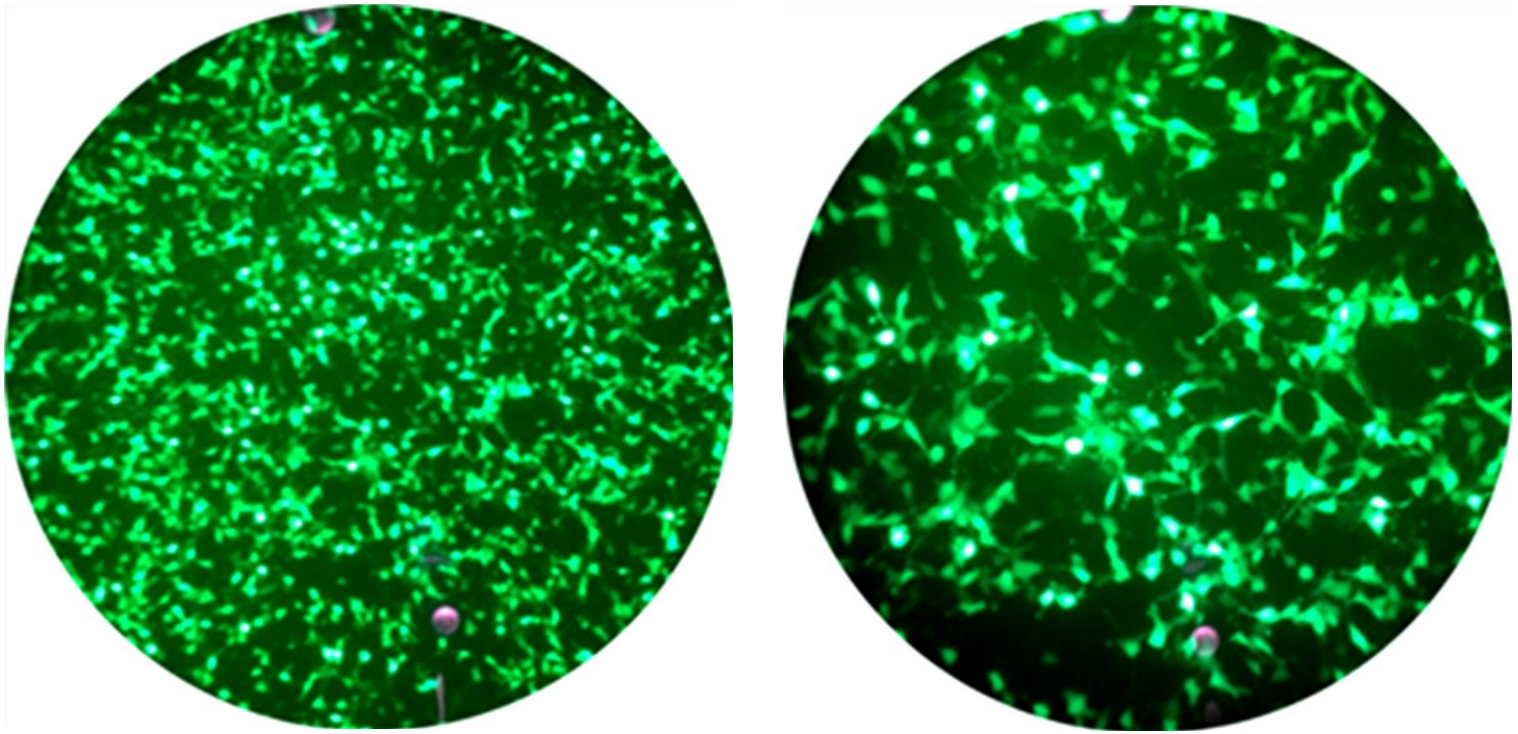
DH5α *E. coli* cells were transfected and selected for by plating on ampicillin agar

Restriction Digest of pGLuc-Basic



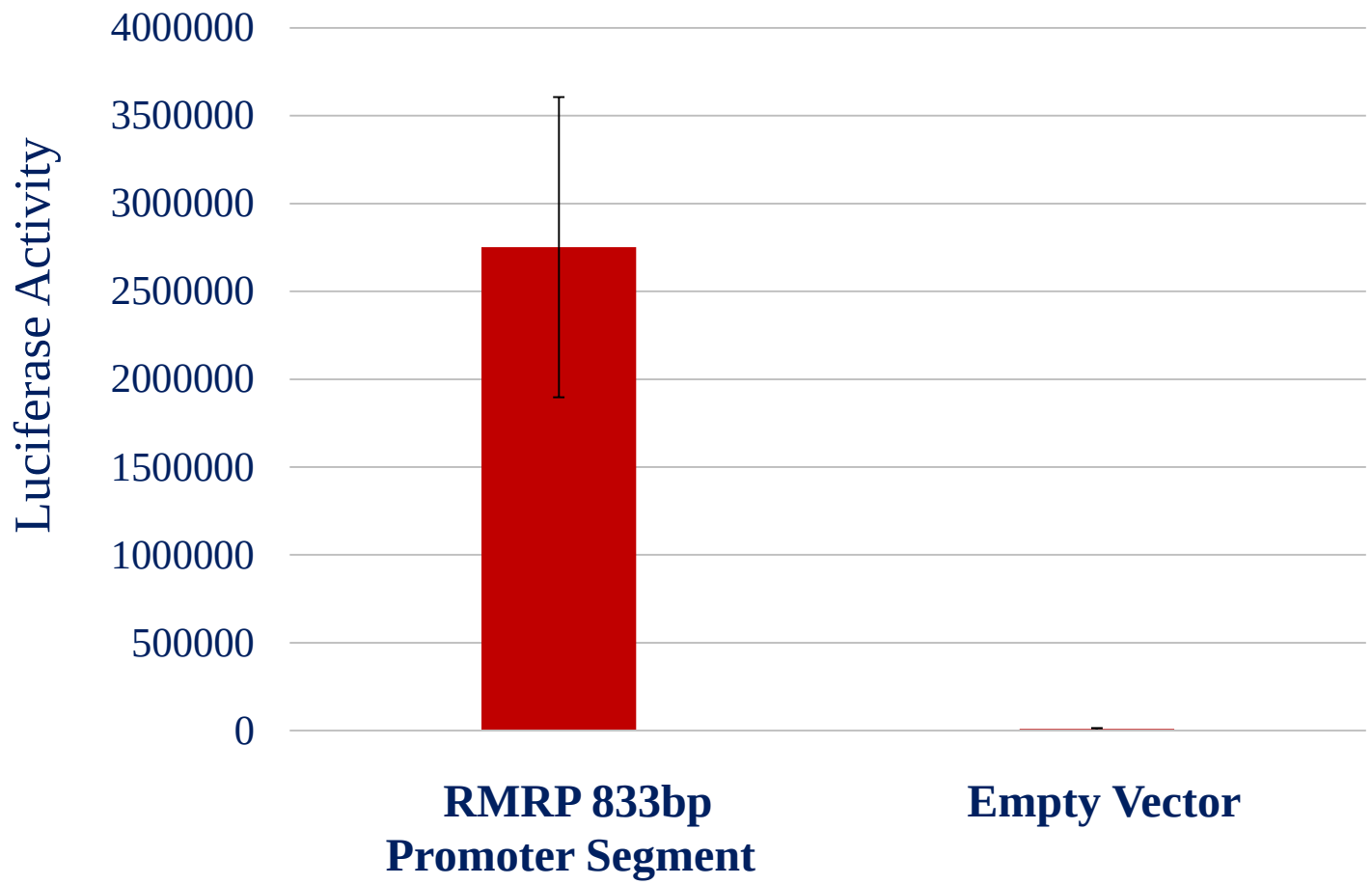
Minipreps of plasmids isolated from transformed bacteria were digested and analysed by running on electrophoretic gel

3T3-NIH Cell Transfection Efficiency

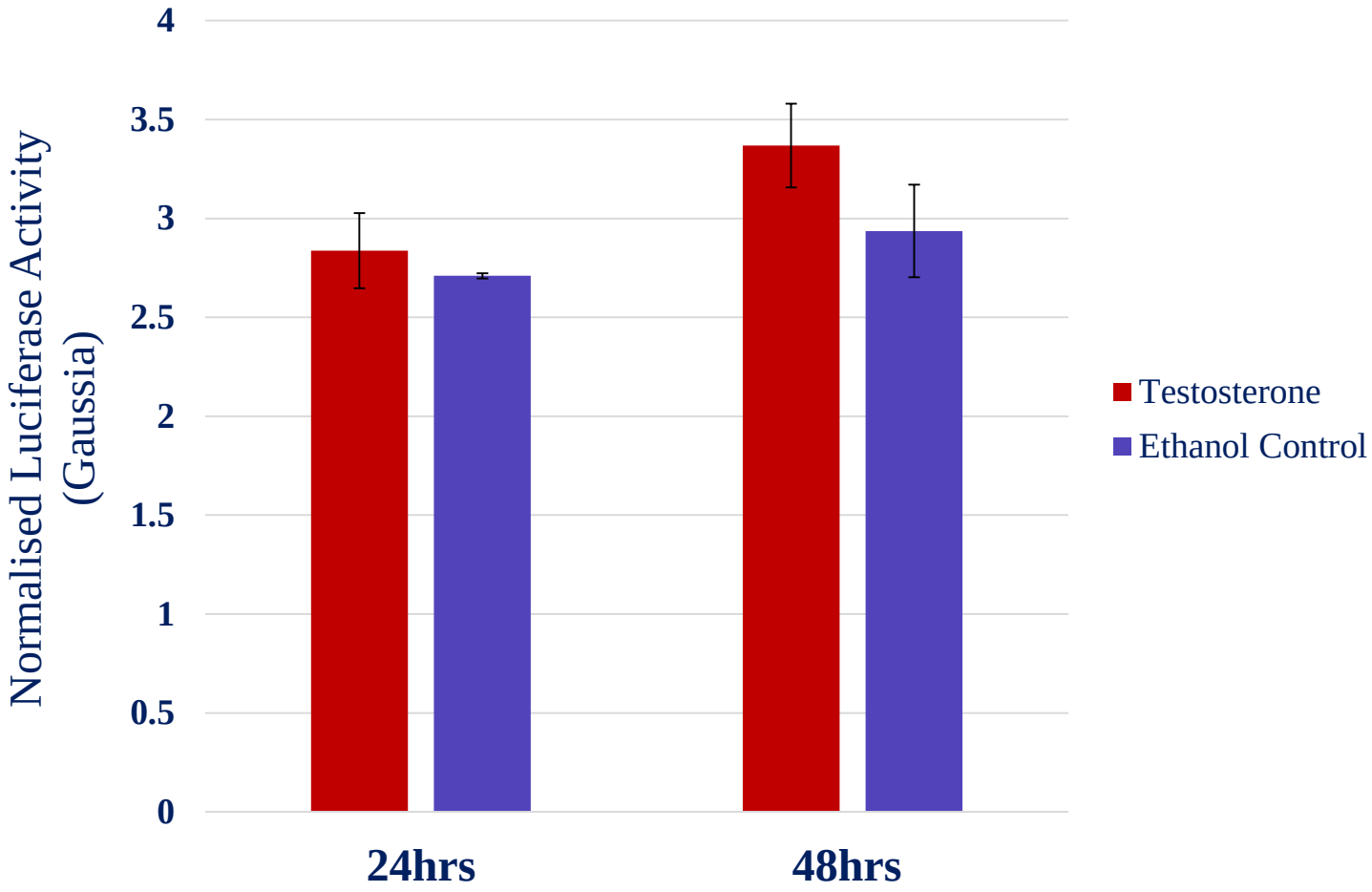


- To assess the efficiency of the TurboFect transfection reagent, green fluorescent protein (GFP) was transfected into 3T3-NIH cells

RMRP 833bp Promoter Segment Activity in 3T3-NIH Cells

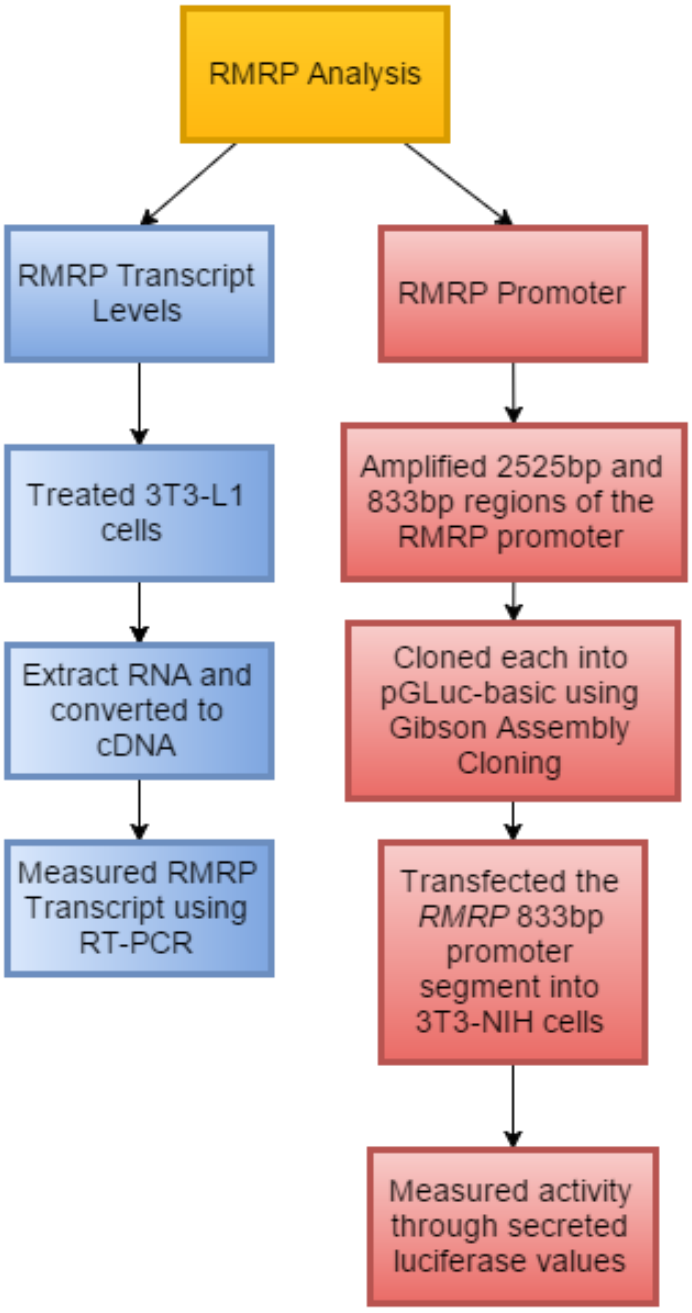


Luciferase assay findings demonstrating activity of the RMRP 833bp Promoter segment



Recap

- FPLD2
- *LMNA*
- Previous RNA-Seq analysis
- *RMRP*



Future Directions

- Repeat experiments for the purposes of statistical analysis
- 3T3-L1 cells
- Varying concentrations of testosterone over adipocyte differentiation
- Experimentally define the *RMRP* promoter

Thanks for Listening!

I would like to thank Noreen Casey and Jenny Duane for their expert technical assistance.

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