# Differential Regulation of Gene Isoforms During Myogenic Differentiation Using Long-Read RNA-Seq

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## Abstract

Alternative splicing (AS) has an important role in post-transcriptional regulation during myogenesis. In recent literature, genome-wide analysis have elucidated the scope of AS in biological transitions such as cellular differentiation. However, the extent of AS in cellular differentiation of myoblasts is still unknown. To assess the impact of AS in myogenesis, long-read RNA-sequencing was used to profile differential regulation of gene isoforms and expression in C2C12 cells for 72 hours. Analysis of the data identified genes and novel isoforms that were upregulated or downregulated during differentiation. These findings show that within cellular differentiation, AS has a major role in myogenesis.

## Introduction

C2C12 is a mouse muscle cell line used to model myogenesis in vitro. All C2C12 myoblasts undergo myogenesis. Myogenesis is the process of skeletal muscle development identified as the period following precursor cell proliferation where precursor myoblasts differentiate into myotubes during embryonic development[1]. During myogenesis, single-nucleated myoblasts align then fuse together to form multinucleated myotubes[2].

Alternative splicing (AS) is characterized by the process where certain exons in are included or excluded in precursor messenger ribonucleic acid (pre-mRNA) transcripts, resulting in unique protein isoforms[3]. Although they originate from the same gene, protein isoforms can have distinct functions. They are critical to encoding protein with varying activity and sequence[4].

In this work, we investigated the mechanisms involved in differentiation of myoblasts to myotubes over 72 h *in vitro*. We used long-read PacBio complementary DNA (cDNA) of the C2C12 line to precisely identify and compare the expression of genes and the creation of isoforms resulting from AS.

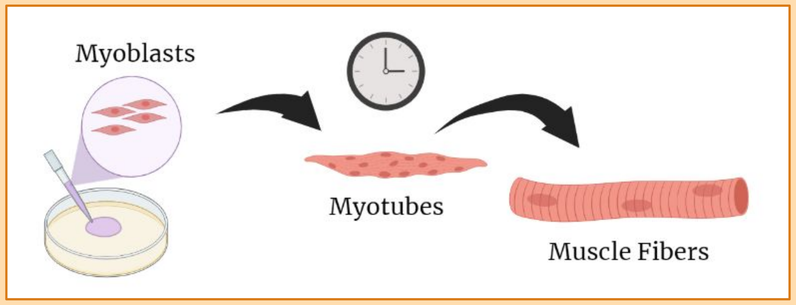



Figure 1: Diagram portraying the stages of myogenesis starting from myoblasts in the C2C12 line

## Methods

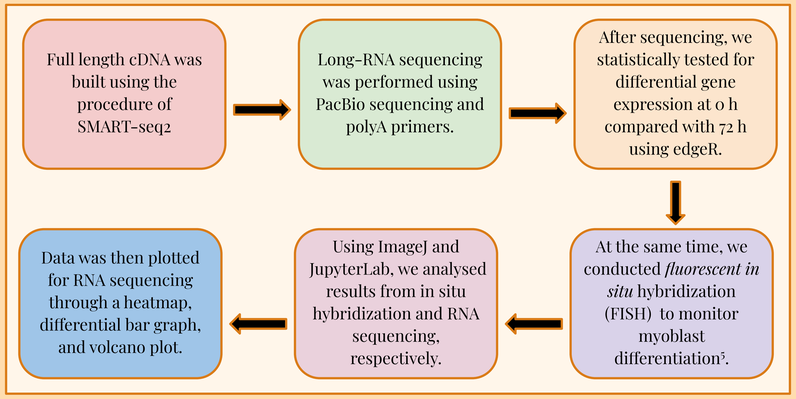



Figure 2: Flow chart showing the methods used during the process of RNA sequencing and RNA Scope

## Results

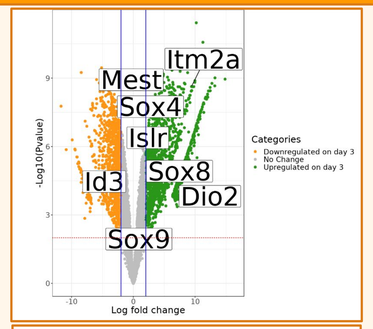



Figure 3: Volcano plot depicting the expression of Mest, Sox4, Sox8, Islr, Id3 and Itm2a on Day 3, upregulated being on the right and downregulated on the left.

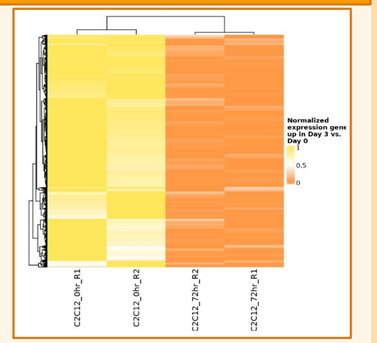



Figure 4: Heat map showing the genes in muscle cells and their expression over 3 days

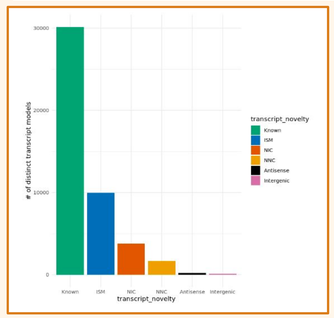



Figure 6: Bar graph displaying how many of the isoforms found in PacBio data were Known, ISM, NIC, NNC, Antisense, and Intergenic.

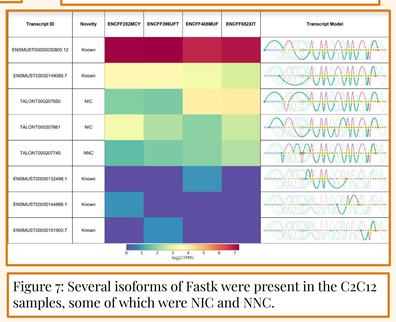



Figure 7: Several isoforms of Fastk were present in the C2C12 samples, some of which were NIC and NNC.

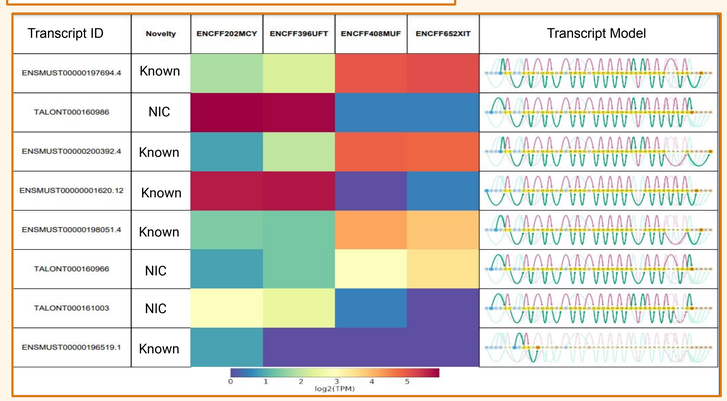



Figure 8: Swan Plot depicting isoforms of Fxr1, a RNA binding protein important to muscle development with multiple isoforms found in the PacBio samples.

## GO Terms Upregulated in PacBio Samples

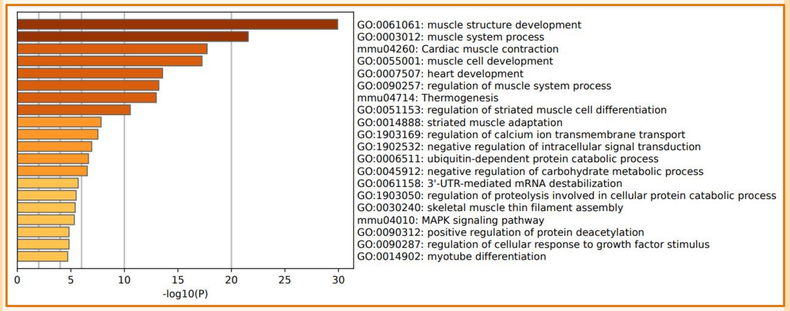
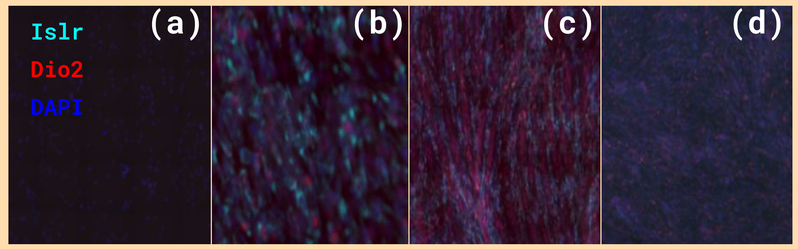



Figure 5: Gene Ontology Analysis; demonstrating a large expression of genes involved in muscle development and function.

## Fluorescent *In Situ* Hybridization




0 hours | 24 hours | 48 hours | 72 hours

Figure 9: Fluorescent *In Situ* Hybridization of Myoblast Differentiation. **(a)** 0 h of myoblast differentiation. DAPI nuclei are shown, but few genes are expressed. **(b)** 24 h of myoblast differentiation. Cells are homogenous, and Dio2 and Islr genes are expressed. Cell-fusion has not occurred. **(c)** 48 h of myoblast differentiation. Cell-fusion is occurring and long Myotubes are forming. Dio2 and Islr genes are upregulated. **(d)** 72 h of myoblast differentiation. Dio2 and Islr are highly expressed.

## Conclusion

Mest, Itm2a, Sox8, Islr, Sox4, and Dio2 are upregulated in differentiated myotubes as opposed to Id3 which is downregulated. Various Fxr1 isoforms are differentially expressed between the myoblasts and myotubes. By contrast, expression levels of Fastk isoforms are maintained throughout myogenesis. FISH later confirmed the upregulation of Islr and Dio2 mRNA transcripts during myogenesis in the differentiated C2C12 cells.

## Future Directions

- Repeat our experiment using Nanopore sequencing and compare those results to PacBio sequencing data
- Investigate which RNA splicing factors are contributing to the change in expression of the genes we analyzed
- Investigate additional myogenic regulatory genes such as Myog, Mymk
- Repeat our experiment using other cell lineages such as chondrocytes

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## References

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