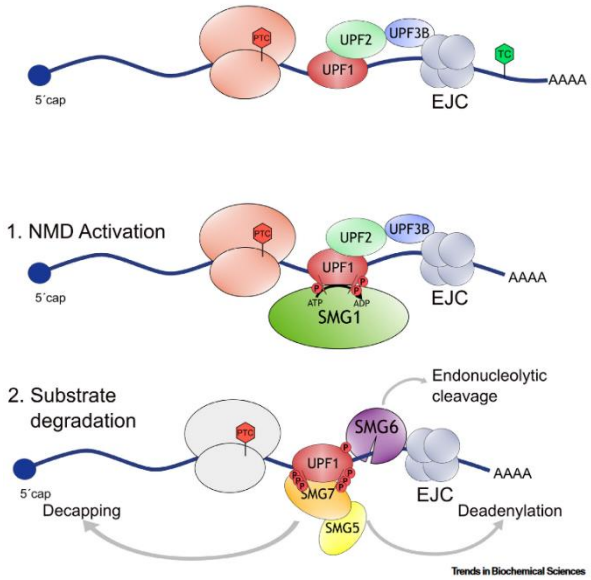
Insights into Nonsense-mediated mRNA decay by direct RNA sequencing

Project suggestions for Michael Cibien from Evan Karousis based on RNA sequencing data generated in October 2019.

Introduction

Nanopore direct RNA sequencing was applied to study the effects of NMD factors knockdown. We performed RNAi-mediated knockdowns of the two NMD factors SMG6/SMG7. These proteins are effector proteins which are active at the latest step of the pathway and lead to the degradation of mRNAs with premature termination codons or normal mRNAs with exons in the 3´UTR. You can find more info on NMD here.



We want to assess whether direct RNA sequencing provides enough reads to compare the expression levels and the features of mRNA isoforms that are sensitive to NMD versus the ones that are not affected by NMD.

So in Knockdown mouse we expect to find the NMD sensitive RNA not affected

Here are some analyses you can perform:

Quality Control (QC):

1. Check the quality of your sequencing data using tools like NanoPlot or FastQC.
2. Assess the read length distribution, sequencing error rates, and the number of reads.

Read Mapping:

1. Align your reads to a reference genome using tools designed for long reads, such as Minimap2 or GMAP. You can use the "NMD transcriptome that was generated in a previous work from the lab. (Karousis, 2022)
2. Evaluate mapping statistics to understand the proportion of reads that are successfully aligned to the genome.

Differential Gene Expression Analysis:

1. Compare the gene expression profiles between your knockdown samples and controls.
2. Use tools like DESeq2 or edgeR (though they are more commonly used for short-read data, they can be adapted for long-read analysis).

RNA Modification Analysis (would be completely new):

1. Nanopore sequencing can detect direct RNA modifications. Explore tools like Tombo or EpiNano to analyze potential RNA modifications.
2. As a control, assess if modifications are detected in well-characterized modified transcripts.

Alternative Splicing Analysis (to compare with previous approaches - Karousis, 2022):

1. Investigate changes in alternative splicing events due to the knockdown of NMD factors.
2. Tools like SplAdder or SUPPA2 can be useful for this purpose.

Nonsense-Mediated Decay Target Analysis (to compare with previous approaches - Karousis, 2022):

1. Identify potential NMD targets by looking for changes in transcript levels of genes with premature stop codons.
2. Use previous scripts or available bioinformatics tools to detect these features.

Transcript Isoform Analysis:

1. Use the data to identify and quantify different transcript isoforms.
2. Tools like FLAIR or StringTie can be helpful for isoform reconstruction and quantification.

Visualization:

1. Use genome browsers like IGV or UCSC Genome Browser to visualize your sequencing reads in the context of the genome. Some examples of NMD-sensitive isoforms: BAG1, TRA2B genes. More in the previous paper.
2. Visualize differential expression or alternative splicing results using tools like ggplot2 in R.

Functional Enrichment Analysis:

1. For genes or isoforms that show significant changes, perform functional enrichment analysis.
2. Use tools like DAVID, GSEA, or Enrichr to understand the biological significance of your findings.