

## Introduction

### Repeat Expansion Diseases

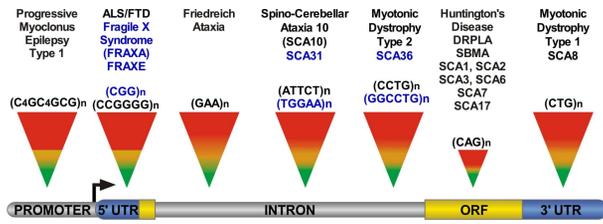


Figure 1: Repeat Expansion Diseases.

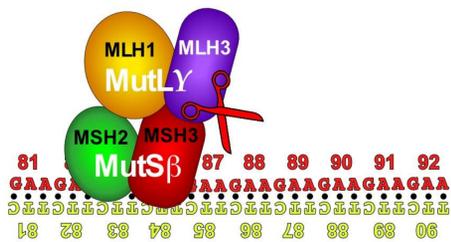
### MLH3, Exon 7, and Repeat Expansion

- Structures form during transcription that attract mismatch repair proteins that, in turn, attract MLH3.
- Isoform 1 of MLH3 cuts DNA in a way that leads to DNA expansion. An alternate isoform, MLH3 isoform 2, lacks exon 7 and the ability to cut DNA<sup>1</sup>.

**A** During transcription the strands of a DNA repeat are separated and the non-template strand may re-anneal out of register and form a loop



**B** If the loop is 3-12 bases MutSβ may bind and recruit MutLγ



**C** MutLγ cuts the DNA leading to incremental expansion



Figure 2: Model of MLH3 leading to repeat expansion.

## Isoform Analysis

### Alternative Way to Perform Isoform Analysis

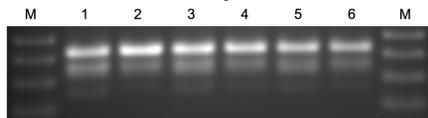


Figure 3: Endogenous MLH3 Isoforms in Human Cell lines. Reverse transcriptase PCR of human cell lines showing MLH3 isoforms. Cell lines analyzed include HEK293 (1), LAS CPC-01 (2), CN95 (3), SH-SY-5Y (4), BE<sub>2</sub>-M17 (5), and CoG-N-519 (6). Human MLH3 isoform 1 is shown at 434 bp and isoform 2 is shown at 362 bp. M is a 1 Kb Plus DNA Ladder showing 500 bp, 400 bp, 300 bp, and 200 bp bands.

## Preparation and Analysis

### cDNA Isolation

- Generated from RNA isolated from 5 human cell lines including embryonic kidney, prostate cancer, and neuroblastoma

### EPI2ME Labs: wf-transcriptomes

- A Docker-based Nextflow pipeline
- Mapped reads to the human genome using Minimap2
- Used Stringtie to differentiate isoform 1 and 2 via the supplied genome annotation

### ggSashimi

- Used Docker container version of ggSashimi<sup>2</sup>
- Generated Sashimi plots

### Percent Retained and Percent Spliced

- Calculated based on output of ggSashimi

## Sashimi Plots

### Sashimi plots show alternative splicing patterns

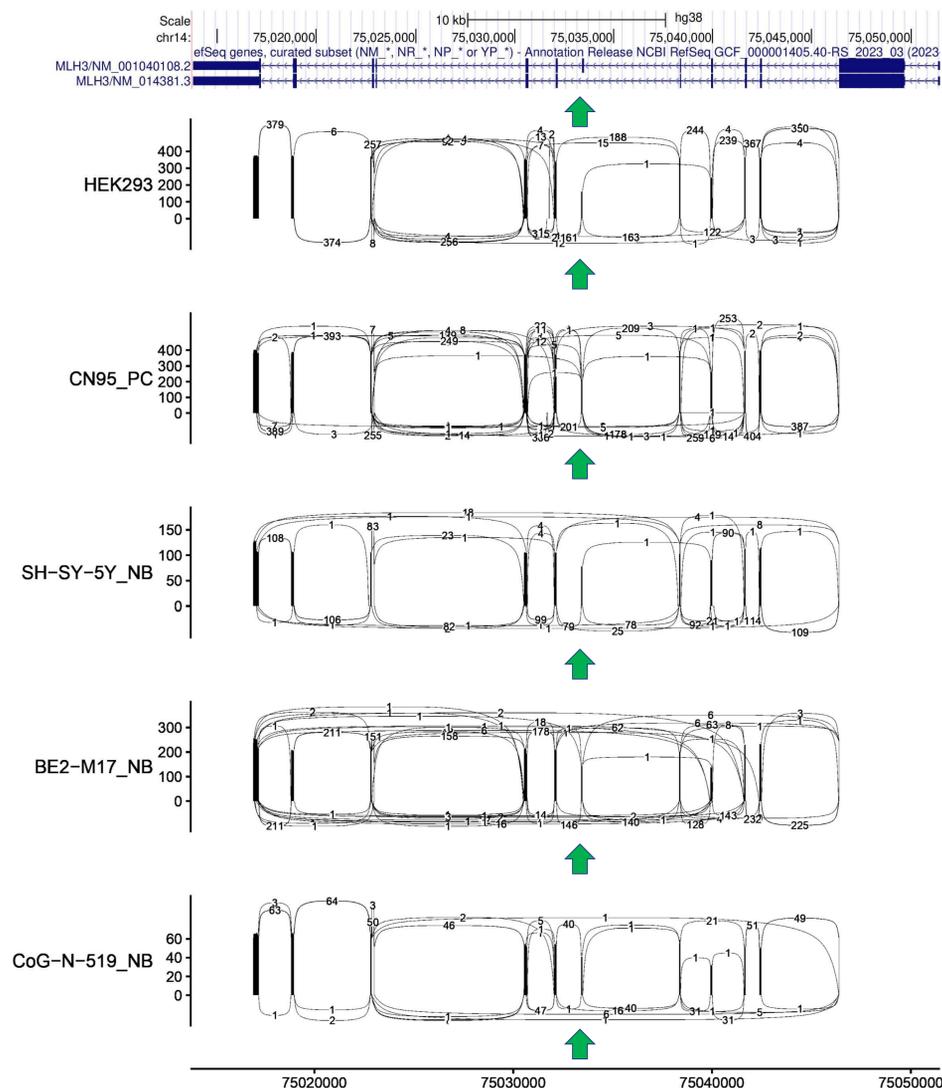


Figure 4: Sashimi plots depict MLH3 splicing on five human cell lines. Cell lines include human embryonic kidney (HEK293), prostate cancer (CN95), and neuroblastoma (SH-SY-5Y\_NB, BE2-M17\_NB, and CoG-N-519\_NB)

## Results

### Exon 7 Skipping in Human cells

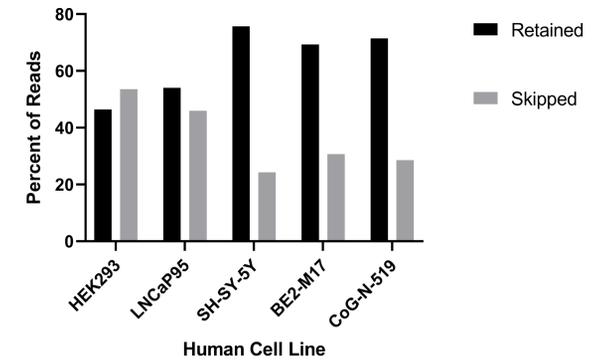


Figure 5: Exon 7 Skipping in Human Cells. Percent MLH3 transcripts retaining exon 7 and the percent skipping exon 7 were calculated for human embryonic kidney (HEK293), prostate cancer (CN95), and neuroblastoma (SH-SY-5Y\_NB, BE2-M17\_NB, and CoG-N-519\_NB).

- Raw data was extracted from Sashimi plots and analyzed to create this graph.

- The goal of future interventions is to increase the percent skipped.

## Conclusions

### The inclusion and exclusion of exon 7 in MLH3 can be quantified

- Sashimi plots provide a visual representation of the splicing that occurs to create various isoforms of MLH3.
- The expression of various MLH3 isoforms is quantified for several different cell lines and a baseline measure for the malign exon 7 has been established.

## Future Directions

### Longitudinal study of repeat expansion in respect to MLH3 isoforms is now possible

- Sashimi plots can be utilized as a visual representation of splicing in MLH3 expression.
- The raw data extracted from wf-transcriptomes and ggSashimi can be used to determine exon 7 skipping in human cells.
- This protocol can be used with other experiments to monitor their effects on the expression of MLH3 isoforms.
- Redirecting splicing to remove exon 7 slows the expansion of trinucleotide repeats and may delay the onset of symptoms<sup>1</sup>.

## Sources

- Halabi A, Fuselier KTB, Grabczyk E. GAA•TTC repeat expansion in human cells is mediated by mismatch repair complex MutLγ and depends upon the endonuclease domain in MLH3 isoform one. *Nucleic Acids Res.* 2018 May 4;46(8):4022-4032. doi: 10.1093/nar/gky143. PMID: 29529236; PMCID: PMC5934671.
- Garrido-Martin, D., Palumbo, E., Guigó, R., & Breschi, A. (2018). ggsashimi: Sashimi plot revised for browser-and annotation-independent splicing visualization. *PLoS computational biology*, 14(8), e1006360.