**Significance**

1. Mechanisms of alternative splicing are important
   1. Alternative splicing is very prevalent (>90% of human genes)
   2. Targeting alternate splicing offers the potential for targeting diseases
      1. SMN2, Dystrophin etc
   3. Alternative splicing regulation is still poorly understood
2. Mutually exclusive splicing is important
   1. MXEs are important in many processes
   2. MXE mutations drive disease
   3. MXEs are enriched for disease causing SNPs
   4. Mutually exclusive splicing was once though uncommon
   5. Recent work has shown it is much more common than previously thought
   6. Mechanisms that are known for MXEs
   7. Most MXEs still have no known mechanism
3. PKM is particularly important
   1. Differentially regulated across cell types
   2. Can translocate to the nucleus and act as a regulator
   3. Driver of the Warburg effect
   4. Has a role in cardiovascular disease, Alzheimer’s, inflammation, and immune function
4. Current MPSAs have shown promise but are deeply flawed
   1. MPSAs allow rapid characterization of splicing mechanisms
   2. Dissection of MPSA techniques
      1. Known isoforms only
         1. Sort-seq
            1. Nonnative context
            2. Low isoform resolution
            3. Low PSI resolution
         2. PCR-seq
            1. Low isoform resolution
            2. PSI resolution limited by isoform collapse
         3. Gel cut seq
            1. Low isoform resolution
            2. Assumes RNA levels are even
      2. All isoforms
         1. Junction sequencing
            1. Can only differentiate isoforms below read length

Requires non native or small system

Cannot distinguish isoforms with differences beyond the read length

* + - * 1. PCR or RT may cause crossover noise
        2. Interior primer sites may miss isoforms that loose those sites
  1. These techniques are not good fits for large systems or for differentiating isoforms that maybe large
  2. Native context is important
  3. Analysis pipelines have not been made as publicly available software reducing acceptance and spread of the methods

1. How my study fits
   1. Long reads will allow junction sequencing over larger constructs
      1. Native contexts
      2. More complicated situations
         1. MXEs
         2. Cryptic sites
         3. Intron retention
      3. Reduced count noise
   2. Open-source software will democratize the method
      1. Allow robust verification of analysis pipelines on synthetic datasets
      2. Allow experimentalists to go directly from reads to processed data
   3. Applications of these methods to PKM will help understand it’s complicated regulation
      1. Interesting basic biology
      2. Disease relevance
      3. Form a good test ground for the techniques

**Approach**