Each innovation should list multiple references to papers to which you wish to draw a contrast. E.g., in Innovation 1, list all MPSA papers that use short read readout.

The titles of the innovations are too vague/general. You want the titles to be very specific. My suggestions are below.

Innovation

Our work will enable broader use of MPSAs to interrogate the mechanisms of splicing by extending the technique to work in the context of complex isoform distributions and correlated splicing outcomes. It will further enable wider use by providing end to end analysis and modeling software for MPSA data. This work is driven by innovations that will change the practices of the MPSA field and the broader splicing community.

An MPSA based on long-read sequencing

Innovation 1: ~~Using long read sequencing as a readout~~. The adoption of long read technologies in RNA sequencing is revolutionizing the study of alternative splicing.[26,36] By converting our sequencing approach from short reads to long reads, we open up our assay to a much broader array of use-cases and address common causes of error in MPSA data. **Aim 1** will develop protocols for LR-MPSA assays and **aim 2** will develop analysis software for this application.

An MPSA that quantifies diverse isoforms at nucleotide resolution.

Innovation 2: ~~Unbiased collection of spliced isoforms~~. Our work will prioritize maximizing our ability to collect and identify all spliced isoforms of a test transcript in an unbiased manner. This will reduce the noise in our measurements by cutting down on misassignment of isoforms. It will also open us to finer grained mechanistic interrogation of the system by allowing us to examine which variants cause shifts in the levels of each of the produced isoforms. **Aim 1** will develop protocols for unbiased sequencing of isoforms and **aim 2** will develop analysis software for detecting and quantifying isoforms by variant.

An MPSA for studying complex minigenes

too vague. You specifically mean introns.

Innovation 3: ~~A focus on maintaining regulatory context~~. Our work will focus on ensuring that the local intronic context for a splicing decision is maintained. This context can be essential for regulation of splicing patterns including the maintenance of mutually exclusivity between exons.[17,18,20–22] Further, it is common for variants to induce activation of cryptic intronic splice sites or to cause intron retention.[5,9,17,18] Our focus on maintaining local context will allow us to capture these effects. We will demonstrate this new focus in **aim 3.**

Fold into 1 innovation

Innovation 4: Broadening application to multi exon systems. MPSAs have largely focused on the spicing of a single intron or exon.[1–15] However, recent research has shown that mutually exclusive exon splicing and other forms of correlation across splicing decisions in a transcript is far more common that was previously understood.[23–26] Our work will allow us to apply MPSA techniques to studying these new phenomena. We will demonstrate this new capacity in **aim 3.**

Innovation 5: Using simulated data to vet analysis. The use of simulated data to vet data analysis pipelines is well established practice in other areas including variant calling and template assembly.[37–43] However, this approach has not been applied to MPSA data analysis pipelines. By testing our end-to-end analysis software on simulated reads in **aim 2**, we will be able to assess its accuracy at every step of the pipeline and its robustness across a wide variety of conditions.

Fold into 1 innovation

Robust software for analyzing MPSA data (say both PCR-based & nucleotide-resolution)

Innovation 6: ~~Packaging analysis software~~. Despite the common use of MPSAs to measure variant effects on splicing,[1–15] There is no MPSA analysis and modeling software. We will meet this need in **aim 2** by producing end to end MPSA analysis software and integrating with modeling software like MAVE-NN.