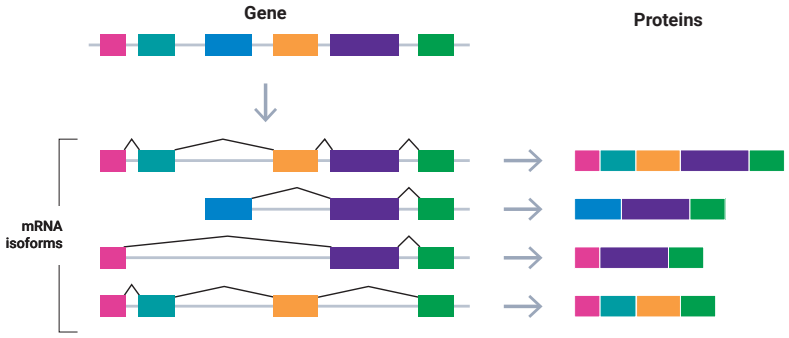
UNCOVER CANCER-SPECIFIC RNA ISOFORMS USING LONG-READ SEQUENCING

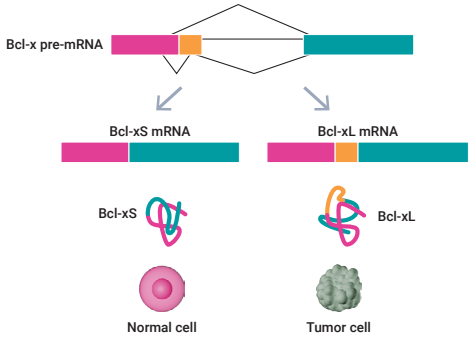
Novel biomarker and drug target discovery with the Iso-Seq® method

Alternative splicing affects protein function in cancer

Alternative splicing (AS) results in the generation of multiple RNA isoforms from a single gene, greatly increasing both transcriptomic and proteomic diversity. AS controls which introns are removed from pre-mRNAs and which exons are combined to form the final messenger RNA (mRNA).[1]

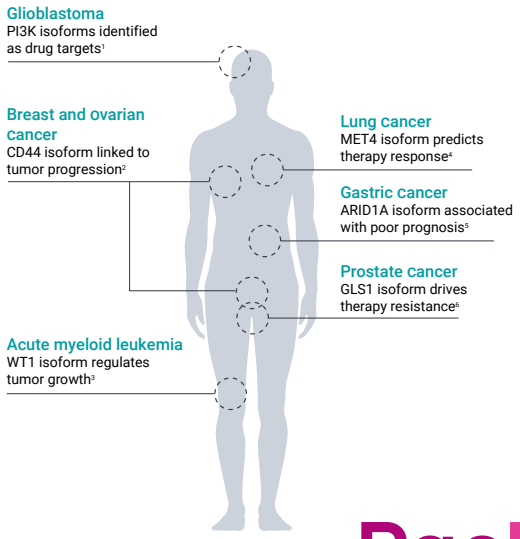


Cancers display widespread RNA dysregulation, including alternative splicing of transcripts. These aberrant transcripts are often translated into cancer-specific proteins and have been shown to affect cancer initiation, progression, metastasis, and drug resistance.[2] For example, the bcl-x gene expresses two isoforms: long and short. The short isoform is expressed in normal tissues and regulates apoptosis. The long form is over-expressed in several cancers and blocks apoptosis, promotes cancer progression, and has been shown to cause resistance to chemotherapy.



Much of the cancer isoform repertoire remains unexplored

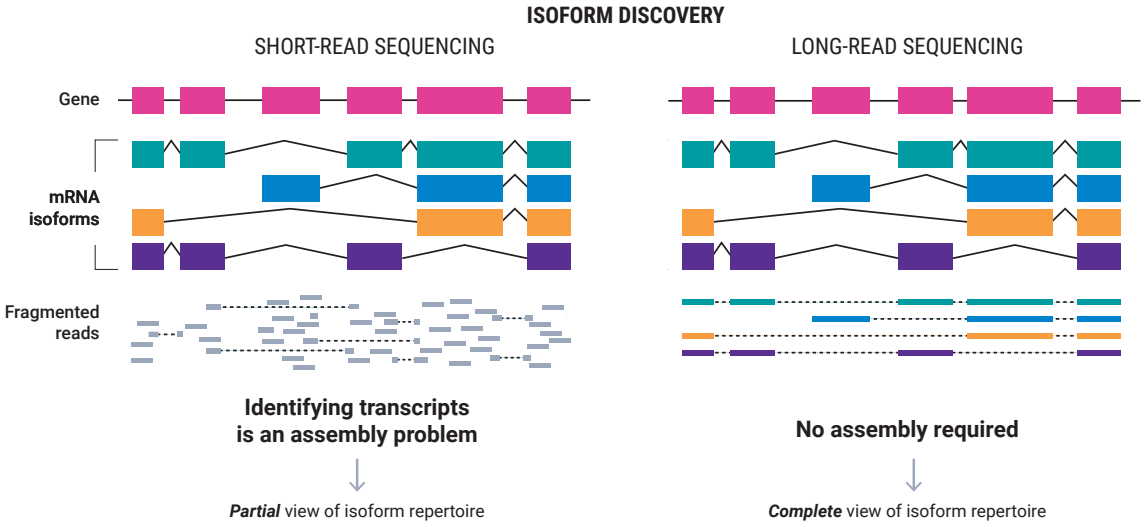
While it has been recently reported that cancer-specific isoforms can be used as novel prognostic biomarkers and serve as an untapped source of drug targets for immuno-oncology, the vast majority of cancer-specific isoforms remain unknown.[3,4,5] With at least 95% of genes undergoing alternative splicing and producing multiple isoforms, much remains to be discovered.[1]



PacBio

Long-read sequencing provides significantly more robust isoform discovery power

Current short-read sequencing approaches rely on the alignment of sequenced fragments to a reference genome and are only able to reconstruct an estimated 20–40% of the transcriptome.[6,7] A recent study showed that a short-read approach captured only a fraction (~41%) of truly novel isoforms identified by PacBio's long-read approach in cancer samples.[1] These intrinsic limitations in the discovery of novel isoforms likely underestimate the impact of isoforms in cancer and result in a partial view of the true biology of cancer.[8]



The Iso-Seq method utilizes long-read transcript sequencing to reliably capture full-length transcript isoforms without the need for computational assembly. PacBio's long-read RNA sequencing is also significantly less error-prone than other long-read sequencing technologies, offering more robust and accurate isoform discovery power.[9,10] Future research will continue to explore the complex and mostly unexplored landscape of isoforms across cancer types.

The Iso-Seq method offers an end-to-end approach for isoform discovery

Library prep	SMRT® sequencing	Data analysis
SMRTbell® express kit	Sequel® IIe system	SMRT® Link

For a detailed protocol about RNA sequencing best practices visit **pacb.com/RNAseq-bestpractices**

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KEY REFERENCES

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