Thank you for considering our core facility for your IsoSeq project needs. We'd be glad to support your RNASeq (IsoSeq) experiments on the PacBio. A typical project pipeline and cost go as follows:

1. You submit either total RNA or full-length cDNA from your tissue of interest (please see attached sample requirements details). Since you presumably want full-length transcripts, RNA must be of high quality (RIN>8). It also MUST be free of any impurities that may inhibit downstream enzymatic reactions. We prefer to have at least 2 microgram of RNA as measured by fluorescence (NOT nanodrop). However, if you don't have this amount, please discuss alternatives with us before submitting samples. If you prefer to do the full-length reactions and optimization steps, you will need to submit 6-10 micrograms of cDNA. PacBio recommends the Clontech SMARTer PCR cDNA Synthesis Kit to generate full-length transcripts.
2. Once we get your sample, we do our independent QC/validation. If the sample passes all requirements by our own evaluation, we will construct the IsoSeq libraries. IsoSeq library construction for sequencing on the SEQUEL involves fractionation of cDNA into two different MW fractions (0.5-2.5 Kb and >2.5 Kb). Typically, an aliquote of the unfractionated cDNA is mixed equimolarly with the >2.5 kb fraction for library construction and sequencing. You can also request cDNA sequencing of the unfractionated/without re-pooling material if you are not too concerned about increased representation of the long transcripts in your sample.
3. The IsoSeq library is carefully quantified and prepared for SMRT sequencing on the PacBio SEQUEL. Typically, one SMRT cell per sample is a good starting point. A SEQUEL run generates 400k-600k reads of ~25-35 kbp average polymerase read length.
4. Greater sequencing depth may be desired, in which case an IsoSeq library can be loaded across as many as necessary SMRT cells. As of November, 2018, ICBR is using v3 sequencing chemistry in conjunction with SMRT Link 6.0 software. Conversely, cDNA samples can be barcoded and sequenced in multiplex.
5. Once sequencing runs are completed we provide all sequencing run files (BAM, fastq, and XML). If you also request (separately) data analysis services from the ICBR-bioinformatics group, the data will be directly available to them, and they would contact you to discuss analysis and results with you.

Please notice that the fee for IsoSeq library construction and sequencing services do NOT include data processing/analysis.

If desired, for data analysis support, please contact Brad Barbazuk (Barbazuk,William Bradley bbarbazuk@ufl.edu)

To begin, here is a blog talking about the tutorial on Iso-seq:

http://blog.pacificbiosciences.com/2015/05/tutorial-on-iso-seq-method-applications.html

Review:
http://www.sciencedirect.com/science/article/pii/S0959437X14001075/pdfft?md5=01fcc55d6d9ef6fe8dce80e5b446d0fc&pid=1-s2.0-S0959437X14001075-main.pdf

Poster:
https://s3.amazonaws.com/files.pacb.com/pdf/Single+Molecule+Real+Time+Sequencing+of+Full+length+cDNA+Transcripts+Uncovers+Novel+Alternatively+Spliced+Isoforms.pdf

Some of the recent publications:

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0117050

http://www.ncbi.nlm.nih.gov/pubmed/25912611

http://www.pnas.org/content/111/27/9869.abstract

http://www.cell.com/neuron/abstract/S0896-6273%2814%2900799-5