Customer Collaboration - PacBio Compatible Eden Protocols for SARS-CoV-2 Sequencing

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For sequencing on PacBio® Systems, the 2.5 kb Eden protocol for SARS-CoV-2 amplification can be followed exactly up to and including Part 4, Step 18. After Qubit™ fluorometer quantification in Step 18, the amplicons should be taken into PacBio library preparation and sequencing, using one of the two approaches described below.

- For initial tests (or low sample numbers), we recommend pooling the 14 amplicons from each sample to make one library per sample, following our Procedure & Checklist – Preparing SMRTbell Libraries using PacBio Barcoded Overhang Adapters for Multiplexing Amplicons. As noted in our Protocol & Checklist, multiple barcoded libraries can be pooled per SMRT® Cell run. This approach does not require any modifications to the existing protocol.

- For larger sample numbers after a successful pilot, we recommend using our 2-step PCR approach using barcoded universal primers, as delineated in our Procedure & Checklist – Preparing SMRTbell Libraries using PacBio Barcoded M13 Primers for Multiplex SMRT Sequencing for multiplexing up to 1,024 samples. Modified primers for using this approach can be found in this spreadsheet.

Please note that the 2.5 kb Eden protocol was created by the scientific community and has not been tested at PacBio. As such, we make no claims as to its reproducibility. Also be aware that the full protocol was not originally designed for PacBio sequencing, however, the 2.5 kb amplicons look good on the gel and should work well with full-length PacBio HiFi sequencing.

As a final note, in general, longer amplicons may be more difficult to obtain from samples with lower viral load or extensive viral RNA degradation. This protocol uses a very high number of PCR cycles (40), which is likely required to amplify low abundance *intact* virus. However, this strategy may not succeed in all cases. We understand from discussions with the community that RNA degradation due to sampling and storage conditions can be significant and may adversely affect long-insert PCR efficacies.

If you have questions about the protocols, please reach out to support@pacb.com and we will be happy to assist you.