**Table 4.** Troubleshooting.

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| --- | --- | --- | --- |
| Step | Problem | Possible reason | Solution |
| 1 | Insufficient RNA recovery | Insufficient total RNA was used as a starting input | Use more total RNA as a starting point.  |
| 2 | Insufficient poly(A) RNA recovery | Insufficient total RNA was used a starting input | Note that only 1-3% of the total RNA is polydenylated. Use more total RNA (up to 1 mg) as a starting point |
| 4 | Low-molecular-weight DNA observed on the gel  | Degradation of RNA | Store your RNA at -80°C and make sure your working space is free of RNase contamination  |
| 8 | Short transcripts have are not being sequenced | Using size selection might affect the recovery of small molecules. | Be sure to specify the desired size selection before constructing a PacBio library  |
| 9 | The average size of the library is greater than 1 kb | The input DNA was not quantified correctly.  | The Nextera XT sample preparation kit has been optimized for 1 ng of double-stranded DNA as an input. Using more than 1 ng results in under-tagmentation of the sample and thus in larger DNA fragment sizes being observed on BioAnalyzer traces. |