Best Practices: Preparation of Amplicon DNA for PacBio® Sequencing

Amplicon DNA of high integrity and purity is necessary to obtain the best possible sequencing data on the PacBio® Sequel® platform. Please consider the following guidelines when preparing PCR products for submission to GENEWIZ.

- ❑ Optimize the PCR reaction.
  - Use high-quality DNA as a template.
  - Use a high-fidelity polymerase (Taq polymerase is not recommended).
  - Use fresh primers.
  - Minimize the time spent above 60°C.
  - Ensure extension times are sufficient for complete amplification of the desired locus.

- ❑ Avoid non-specific PCR products or multiple bands on a gel.
  - Any non-specific products in the sample will be sequenced.
  - Shorter products tend to sequence more efficiently and thus may reduce the number of on-target reads.
  - Amplicons sequenced on the same SMRT® cell should be ±10% in size.

- ❑ If size selection is required, we recommend using the BluePippin system.
  - GENEWIZ may perform this size selection for an additional fee.
  - Alternatively, use a gel and visualize with SYBR Safe dye and blue light.

- ❑ Purify the PCR products prior to sample submission.

- ❑ Do not expose the DNA to ethidium bromide or UV light.

- ❑ Avoid heating or over-drying the DNA.

- ❑ Submit amplicons in 10 mM Tris-HCl, pH 8.
  - We do not recommend eluting DNA in nuclease-free water or buffer containing >0.1 mM EDTA.

- ❑ Avoid freeze-thaw cycles, as this can cause shearing of the DNA.
  - DNA can be stored short-term at 4°C or aliquoted for long-term storage at -20°C.

- ❑ Ship samples to GENEWIZ overnight on blue or dry ice.

CONTACT GENEWIZ:
Ph.D. experts available to discuss your project:
+1 (877) 436-3949 ext. 1
ngs@genewiz.com
genewiz.com