Submission of externally prepared libraries for PacBio sequencing

The CGR cannot guarantee the performance of externally prepared libraries. Unfortunately, we have received externally prepared PacBio libraries in the past that look as we would expect during library QC but have performed poorly during sequencing. We will carry out projects exactly as described on the quote uploaded during project submission and will charge for any work undertaken, regardless of the quality of results.

Maximising sample quality

For best results in library preparation, it is essential that your input DNA samples:

- are double-stranded. Single-stranded DNA is not compatible with PacBio library preps.
- have been stored at 4°C (short term) or -20/-80°C (long term) and have not undergone multiple freeze-thaw cycles, which can affect DNA quality.
- have not been exposed to high temperatures or extremes of pH.
- have a 260:280 ratio of 1.8-2.0 and a 260:230 ratio of 2.0-2.2.
- do not contain insoluble material.
- are free from RNA contamination.
- have been eluted and stored in a neutral, buffered solution, preferably QIAGEN EB Buffer with no EDTA. Avoid storing samples in unbuffered solutions, RNase-free water or AE Buffer.
- have not been vortexed or shaken, as this can cause shearing of the DNA.
- have not been exposed to intercalating fluorescent dyes or ultraviolet radiation. SYBR dyes do not damage DNA, but we would strongly advise against using ethidium bromide.
- do not contain denaturants (such as guanidinium salts or phenol), divalent metal cations (such as Mg2+) or detergents (such as SDS or Triton-X100).
- do not contain contamination from the original organism/tissue (haeme, humic acid, polyphenols, etc.).

Maximising library quality

- Libraries should be free from contaminants. We recommend purifying libraries using AMPure PB beads.
- Library quantification should be performed using a dye-based method such as Qubit, rather than a spectrophotometric method such as NanoDrop.

Library submission requirements

Library loading requirements for PacBio platforms differ significantly depending on the quality of the library, so please contact us at CGR_Lab@liverpool.ac.uk for advice before shipping.

Please supply each library or pool of libraries in a tube labelled with the sample number and/or name exactly as given on the online order form. If more than one tube is provided, please label them in numerical order for ease of sample identification. Please underline any numbers that could be misread upside-down (e.g. 6/9, 16/91).