PacBio DNA sequencing

The PacBio sequencer is a sequencer of the third generation. It sequences DNA as is, i.e. the cleaner and more intact the DNA is that you provide, the higher the data output and the read length. See quality recommendations here .

Short summary of quality parameters for best possible outcome:

Nanodrop 260/280	1.8 – 2.0
Nanodrop 260/230	> 2.0
Qubit concentration	> 150 ng/µl; Nanodrop should not be less than 50 % or more than 150 % of the Qubit concentration
Size on Gel (low voltage, low Agarose)/Tapestation	> 40 kb (DNA remains to a significant proportion in the gel pocket)

Neither PacBio nor us can give guarantees on sequencing output/SMRTCell. In perfect sequencing runs, one SMRTCell can give > 20 Gb of data, however, calculations should be made with around 12 Gb/SMRTCell IF all quality criteria are met.

To be considered during/before DNA extraction:

To extract the best possible DNA, the tissue/culture used for extraction optimally is fresh and processed immediately after sampling. If that is not possible, it should be flash-frozen immediately (thawed only once) or stored in RNA/DNA later.

RNA must be completely digested and removed.

Sequencing of Fungi (and some plants and protists) can be extremely difficult as they sometimes decorate their DNA with massive amounts of sugar-residues. If possible, try to have them on sugar-free medium for a while before they are extracted to reduce that problem.

DNA extracted from soil, sediment or feces are difficult as well, as the contamination level often is very high, even after clean-up.

There are special kits designed for extraction of DNA with contaminants or for high molecular weight DNA (e.g. Macherey Nagel (“Food” or “High Molecular Weight” for different organisms) or Qiagen (MagAttract, PowerSoil)).

Do not vortex extracted DNA to resuspend or mix.

Do not pipet up and down. DNA should be mixed by flicking the tube and pipetted slowly, if possible use wide-orifice tips.

Extracted DNA should not be frozen and thawed, therefore store it for < 2 weeks at 4 °C.

Sending HMW DNA to us in solution with mail is not optimal. Freeze the DNA once, send it on dry ice and we will thaw it once. In case you want to make extra-sure not to degrade your DNA or you are shipping over large distances and therefore shipment may take a long time: please contact us (ccga@ikmb.uni-kiel.de) to discuss the best options.