

Gel purification of amplicons for PacBio sequencing

- Never use Ethidium bromide to stain gel fragments
- Do not use UV to visualize amplicons during cutting of desired fragments

UV will damage DNA and will make fragments non-sequenceable on the Pacbio. Even a few seconds of UV irradiation appears to render DNA non-sequenceable on the SEQUEL.

Our recommendations for handling samples for PacBio sequencing can be found in the MSword attachment. For gel-based size fractionation we have tested the SYBR safe method with blue light visualization (see <https://www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/nucleic-acid-gel-electrophoresis/dna-stains/sybr-safe.html>). It seems that RedSafe can also be used in this same manner (i.e., without UV).