

Sample Preparation Guidelines for Nanopore Sequencing

- Please refer to published literature for plasmid extraction protocols. Submit the final purified plasmids in elution buffer (10 mM Tris, pH 8.5) or nuclease-free water; avoid buffers containing EDTA (e.g. TE or AE buffer) whenever possible.
- For PCR samples (size ≥ 0.5 kb) please also refer to published literature for PCR purification protocols.
- **Note:** *If the plasmid or PCR product is larger than 30 kb, please contact us at support@poochonscientific.com.*

Quality Requirements:

OD_{260/280} Ratio: ≥ 1.8 , ≤ 1.95

OD_{260/230} Ratio: ≥ 2.0 , ≤ 2.2

Plasmids – Minimum Volume and Concentration:

1 kb - 3 kb Plasmids: 10 μ l minimum volume; Concentration ≥ 10 ng/ μ l

3 kb - 15 kb Plasmids: 10 μ l minimum volume; Concentration ≥ 25 ng/ μ l

15 kb - 30 kb Plasmids: 10 μ l minimum volume; Concentration ≥ 50 ng/ μ l

PCR Products – Minimum Volume and Concentration:

0.5 kb - 3 kb Amplicon: 10 μ l minimum volume; Concentration ≥ 5 ng/ μ l

3 kb - 15 kb Plasmids: 10 μ l minimum volume; Concentration ≥ 25 ng/ μ l

15 kb - 30 kb Plasmids: 10 μ l minimum volume; Concentration ≥ 50 ng/ μ l

For quality results, samples should NOT contain any of the following:

- 1) RNA (RNase treatment is recommended during extraction)
- 2) Denaturants (guanidinium salts, phenol, etc.) or detergents (SDS, Triton-X100, etc.)
- 3) Residual contaminants from the organism (heme, humic acid, polyphenols, etc.)
- 4) Insoluble material, colors, or cloudiness
- 5) Samples should also be “pure” in the sense that they should only contain copies of a single clonal plasmid DNA molecule, or a single clonal PCR DNA molecule
- 6) Sending mixtures of molecular species cannot guarantee assembly of a consensus contig and is at your own risk!

Our low sequencing prices and fast turnaround times do not include DNA extraction or quality control (QC) services, so please verify with full QC that your samples meet the requirements prior to shipping. If the samples do not meet the requirements, high quality data cannot be guaranteed.