Contaminants

Chemical components used in DNA extraction methods can be carried over from the extraction procedure and contaminate the extracted DNA sample. These contaminants can have a significant effect on downstream library preparation efficiency, and therefore sequencing throughput.

Below, we present data on the effects of several common contaminants on: Nanodrop spectra, Nanodrop A260/280 and A260/230 ratios, and library prep efficiency with the Ligation Sequencing Kit and the Rapid Sequencing Kit. We recommend customers are mindful of such contaminants and ensure that the extracted DNA is as pure as possible.

Ethanol effects:

The presence of residual ethanol in extracted DNA can lead an overestimation of the concentration of DNA that is present in your sample. Also, it can lead to a reduction in the measured A260/280 and A260/230 ratios. We found that the presence of some ethanol in the input sample can be tolerated by both the Ligation Sequencing Kit and the Rapid Sequencing Kit, before performance might start to be adversely affected: the Ligation Sequencing Kit can tolerate up to 20% ethanol contamination and the Rapid Sequencing Kit can tolerate up to ~7.5% ethanol contamination.

Isopropanol effects:

The presence of residual isopropanol in extracted DNA can lead an overestimation of the concentration of DNA that is present in your sample. Also, it can lead to a reduction in the measured A260/280 and A260/230 ratios. We found that the presence of up to ~7.5% isopropanol in the input sample can be tolerated by the Rapid Sequencing Kit, before performance might start to be adversely effected, but the presence of any isopropanol might adversely affect the performance of the Ligation Sequencing Kit.

EDTA effects:

The presence of EDTA in extracted DNA can lead large perturbations in the nanodrop spectrum and A260/280 and A260/230 ratios. It can also lead to an overestimation of the concentration of DNA that is present in your sample. We found that the presence of some EDTA in the input sample can be tolerated by both the Ligation Sequencing Kit and the Rapid Sequencing Kit, before performance might start to be significantly affected: the Ligation Sequencing Kit can tolerate up to 10 mM EDTA contamination and the Rapid Sequencing Kit can tolerate up to 5 mM EDTA contamination

NaCl effects:

The presence of NaCl in extracted DNA does not appear to perturbation nanodrop spectra or A260/280 and A260/230 ratios. We found that the presence of up to 100 mM NaCl in the input sample can be tolerated by both the Ligation Sequencing Kit and the Rapid Sequencing Kit, before performance might start to be significantly affected.

Guanidinium chloride effects:

The presence of guanidinium chloride in extracted DNA can significantly perturb nanodrop spectra, particularly with respect to the A260/230 ratio. We found that the presence of up to 100 mM guanidinium chloride in the input sample can be tolerated by both the Ligation Sequencing Kit and the Rapid Sequencing Kit, before performance might start to be significantly affected.

Guanidinium isothiocyanate effects:

The presence of guanidinium isothiocyanate in extracted DNA can significantly perturb nanodrop spectra, giving atypical A260/280 and A260/230 ratios, and leading to mis-quantification of the DNA concentration. We found that the presence of up to 50 mM guanidinium isothiocyanate in the input sample can be tolerated by both the Ligation Sequencing Kit and the Rapid Sequencing Kit, before performance might start to be significantly affected.

Phenol effects:

The presence of residual phenol in extracted DNA can significantly perturb nanodrop spectra, giving atypical A260/280 and A260/230 ratios, and lead to overestimation of the DNA concentration. We found that the presence of up to 1% phenol in the input sample can be tolerated by both the Ligation Sequencing Kit and the Rapid Sequencing Kit, before performance might start to be significantly affected.