Quantifying Concentration of Target Enrichment Panels

BACKGROUND AND QUANTIFICATION PRINCIPLE

Optical density (OD) is a measure of the light-absorbing properties of a chemical compound in solution. A spectrophotometer measuring absorbance can be used to estimate the concentration of that solution using the Beer-Lambert law. This formula relates the concentration of a compound to the absorbance of light at a specific wavelength given it is passed through a solution at a fixed distance. In simple terms related to target enrichment panels, the absorbance in OD units can calculate the nanogram mass of oligos when paired with the correct coefficients for the oligo compound.

Typically, obtaining an accurate value for a specific oligo sequence would require a correction value called the extinction coefficient (EC_{260}) which can be determined based on the specific composition of the nucleic acid sequence and any added modifications. However, given that Twist Bioscience© typically provides target enrichment probes as a complex mixture of many different sequences paired with a terminal biotin modification, we can assume a random composition of sequences of a specific length of bases and still arrive at a relatively accurate determination of the probe sequences in solution.

Many laboratories utilize NanoDrop® spectrophotometer instruments, which alleviates the need for using cuvettes and can directly measure the absorbance from only 1–2 µl of sample. While very convenient, these instruments have some caveats that should be noted. From our empirical testing, the accuracy of the measurements can be variable at the upper and lower bounds of the detection range. Twist Bioscience uses a coefficient of “40” in the NanoDrop software for our probes in solution. It is also advised that the total probe concentration be greater than 15 ng/µl to ensure an accurate reading. Below this limit, it is possible that the OD would vary from expected despite the correct molar concentration of oligonucleotides in solution. Similarly, we advise that the probe concentration not exceed 10,000 ng/µl for accurate quantification of Twist Bioscience’s probes in solution. Additionally, measuring concentration using fluorescence-based methods is not recommended as the secondary structure of the probes will change the signal significantly.

CALCULATION OF THE EXPECTED DNA MASS IN SOLUTION

The expected concentration of probes in solution can be determined by taking the total mass per probe ordered (typically 0.2 fmol/probe/hyb) multiplied by the total number of unique probes for uniform capture panels or the number of probes requested for uniform probe panels.

Expected nanograms per hybridization = [Probe concentration in fmol/probe/hyb x Probe Count x 1e-6 unit conversion] x [330 mass coefficient x (probe length in bases + 40 for oligo modification)] x (1 hybridization reaction per 4 µl)

For example, a probe panel consisting of uniform probe concentration at 0.2 fmol per probe per hybridization with 15,000 probes which are 120 bases in length would be calculated as follows: Expected nanograms per hybridization = [0.2 fmol/probe/hyb x 15,000 probes x 1e-6 unit conversion] x [330 mass coefficient x (120 bases per probe + 40 for oligo modification)] x (1 hybridization reaction per 4 µl) = 158.4 ng of probe per hybridization (4 µl) or 39.6 ng per microliter.

INSTRUCTIONS FOR QUANTIFYING CONCENTRATION OF TARGET ENRICHMENT PANELS

Twist Bioscience develops various target enrichment panels to optimize next-generation sequencing workflows. In order to ensure the reliability of sequencing results when using target enrichment panels, quantifying the concentration can be performed at intake of the panel. This guideline outlines the materials required and the steps involved in quantifying target enrichment panels.

MATERIALS AND EQUIPMENT REQUIRED

EQUIPMENT
NanoDrop Spectrophotometer
Pipet-Lite™ LTS Pipette L-2XLS+ or equivalent

CONSUMABLES
P20 pipette filtered tips
TechniCloth® Non-woven Wipers or similar lab wipes
HyClone™ HyPure water or similar molecular biology grade water
TE Buffer (1x) pH 8.0 low EDTA

STEP 1: SETUP

1.1 In the NanoDrop software, select the Nucleic Acid application. Select the 'Custom Factor' setting.

1.2 Apply the following settings:

- Custom Factor: 40.00
- Baseline correction: 340 nm checked

1.3 Raise the arm of the NanoDrop.

1.4 Clean the NanoDrop pedestals with HyClone HyPure water applied to a TechniCloth dry wipe by wiping the pedestal and where the arm makes contact with the pedestal.

CAUTION: Do not use ethanol, isopropanol, or detergent to clean the pedestals.

1.5 Pipette 2 µl of molecular biology grade water (blanking agent) directly onto the measurement pedestal.

1.6 Lower the sampling arm and initiate a "blanking" of the instrument. Do not proceed until the instrument prompts you to load your sample.

1.7 Using a TechniCloth dry wipe, wipe the molecular biology grade water from both the pedestal and the arm before proceeding.

STEP 2: QUANTIFYING SAMPLES

2.1 Pipette 2 µl of the sample onto the bottom pedestal.

2.2 Lower the sampling arm and initiate a spectral measurement of the sample. After a moment, data results will populate the screen within the software. Record the results in ng/µl.

2.3 Raise the sampling arm.

2.4 Wipe the surface of the upper and lower pedestals with a dry, lint-free laboratory wipe.

2.5 Pipette 2 µl of molecular biology grade water directly onto the measurement pedestal and lower the sampling arm. Raise the arm and wipe with a dry, lint-free laboratory wipe. Then lower the pedestal and the sampling arm.

2.6 Repeat steps 2.1 to 2.5 for the remaining samples, recording the results each time. Twist Bioscience recommends collecting 3 or more data points for each oligo solution and averaging the results to ensure an accurate measurement.

HAVE FURTHER QUESTIONS?

For additional support please contact Twist Bioscience's support team at **customersupport@twistbioscience.com.**