LRGC: NGS Customer DNA Dilution Protocol

Protocol: Perform serial dilutions on the DNA samples over the course of X days to obtain a final concentration of 20 ng/μL

1. Measure the stock concentration and quality of genomic DNA using a full-spectrum spectrophotometer (Ex: NanoDrop). Record original concentration, 260/280 and 260/230.
2. Based on the concentration measured, dilute the DNA to ~25 ng/μL using 10 mM Tris buffer pH 8.5 (Note: The concentration of the sample should be >20 ng/μL because of the lower sensitivity of the spectrophotometer).
3. Allow the sample to sit for 2-3 days at 4°C.
4. Measure DNA concentration with a fluorometer appropriate for the quantification of DNA (Ex: Qubit 2.0).
5. Based on the concentration measured, dilute the DNA to 20 ng/μL using 10 mM Tris buffer pH 8.5.
6. Allow the sample to sit overnight at 4°C.
7. Measure DNA concentration with the fluorometer to confirm desired concentration (~20 ng/μL) is obtained.
8. If necessary, repeat steps 5-7 until desired concentration (~20 ng/μL) is obtained.

Tips:

- This process takes time (~1 week).
- Recommended timeline:
  - Thursday: Steps 1, 2
  - Monday: Step 4, 5
  - Tuesday: Step 7, 8 (if applicable)
- Never go below the desired concentration.
- A 260/280 of ~1.9 is ideal.
  - 1.6 is too low to continue
  - 2.3 is too high to continue

Questions:

- What is too low 260/230 to continue with sample?
  - 260/230 isn't as critical
  - 260/280 should be around 1.9. 1.6 is too low to continue, 2.3 is too high
- What is the buffer?
  - 10 mM Tris buffer pH 8.5
- Is there too big of a jump?
  - Not strict – it's flexible, but getting closer makes following dilutions easier to be precise