

Sequencing

Note: Double the minimum volume of sample for sequencing. Typically we make 20uL of diluted DNA and 4uL of plasmids in a microcentrifuge tube, doubling the specified amount by ACGT.

1. Label microcentrifuge tubes in pairs for forward and backwards sequencing primers for plasmids with >700bp
2. Dilute concentrated plasmid DNA for sequencing in a microcentrifuge tube (20ng/uL)
 - a. (use $C1 \times V1 = C2 \times V2$)
 - i. $C1$ = Current plasmid DNA concentration (ng/uL)
 - ii. $C2$ = Final plasmid DNA concentration (ng/uL)
 - iii. $V2$ = Diluted DNA volume (uL)
 - iv. $V1$ = Undiluted DNA volume (uL)
 - v. $V_{water} = \text{Final Volume (uL)} - V1$

Calculate V_{water} and $V1$. Then add water (V_{water}) to Undiluted DNA ($V1$) to dilute the DNA for LCO specified sequencing concentration. Total volume in the microcentrifuge tube should be 20uL.

3. Add resuspended primers to microcentrifuge tubes with diluted DNA.
 - a. Use IDT-DNA calculator to calculate if the initial concentration of the sequencing primers are not 100uM.
 - b. To dilute to 10pmol/uL from 100uM, add 0.4uL of sequencing primer and 3.6uL mQ water.
 - c. (Note: Doubled volume. Total volume in the microcentrifuge tube should be 24uL.)