PRIMER PREPARATION PROTOCOL

PREPARING PRIMER SOLUTION
- Step A. TAKING OD
- Step B. CALCULATING FINAL OD

Preparing Primer solution

STEP A: TAKING OD

1. Add 500µl H_2O to initial Primer pellet & Mix well.
2. Take 15µl to a new 1.5ml tube, add 1485µl H_2O bringing it to a 1:100 ratio. Mix well.
3. Check for OD using ssDNA program.

Prepare Computer:

- Turn Vision light ON
- Turn UV light ON
- Select Assay type:
  - ssDNA (for primers)
- Set Dilution Factor (100.00)

Prime machine:

- 'Flush' Trace Clear for ~10seconds (for cleaning)
- 'Flush' autoclaved H_20 for ~10 seconds (cleaning).
- 'Fill Blank' with H_20
- Click on the sample number to insert the Sample ID #
- 'Fill Read' with samples for absorption reading
- Repeat for all Samples
- Print readings to calculate the Final OD

STEP B: CALCULATING FINAL OD

4. Calculate: $M_1V_1 = M_2V_2$
   - M_1: Final OD desired = 2 OD/ml
   - V_1: UNKNOWN = Amount of H_20 add to make [primer] 2 OD.
   - M_2: Primer stock solution OD from part A
   - V_2: 500µl (Final volume of stock solution want to dilute)
5. After calculating volume V_1, subtract this amount from the total volume of 500ul to determine the amount of primer stock solution to add.

6. In a new ependorf tube, add the appropriate amount of primer stock solution and H_20 to bring volume to 500µl (OD of 2)
7. Vortex and spin samples briefly – store Primer solutions at -20° C.