SeqaulPrep PCR cleanup and normalization

Chang-Yu Chang

2021-02-11

Aim

Clean up and normalized PCR product.

Materials

- Duplicated PCR plates with PCR product.
- PCR plate (PCR_mix, unbound_DNA, chean_PCR) 3
- SequalPrep plate 1
- SequalPrep binding buffer 2 mL
- SequalPrep wash buffer 5 mL
- SequalPrep elution buffer 2 mL
- 20uL tip box 4
- 200uL tip box 2

Procedures

☐ Thaw the 2 PCR plates and spin down.
☐ Label 3 PCR plates like **XX PCR_mix**, **XX unbound_DNA**, and **XX chean_PCR**.
☐ Mix 20μL of each replicate PCR into each well of a skirted PCR plate PCR_mix (40 uL in total).
☐ Add 20μL SequalPrep Binding buffer to each well of SequalPrep plate.
☐ Add 20μL mixed PCR product into each well of SequalPrep plate.
☐ Cover with PCR lid, vortex 30 sec, and centrifuge briefly.
☐ Incubate for 1 hour at room temperature. Note: extra incubation time will not improve yield but will not decrease it either. Overnight incubation is fine if necessary.
☐ Transfer unbound DNA to fresh skirted PCR plate unbound_DNA. Be careful not to touch the sides of the wells. Discard or foil and save at -20C for up to 30 days and reuse for further SequalPrep.
☐ Add 50 uL SequalPrep Wash buffer to the SequalPrep plate containing bound DNA and pipette up and down twice to mix. Vortex.
☐ Remove buffer from wells by pipetting. Tap plate on paper towel gently to remove remaining buffer. Spin down the plate.
☐ To elute, add 20μL SequalPrep Elution buffer to each well.
☐ Cover with lid, vortex 30 sec, and centrifuge briefly.
☐ Incubate at room temperature for 5 minutes.
☐ Elute DNA from each well into a new skirted PCR plate clean_PCR and store at -20C. The final product is 20μL 1.25 ng/uL DNA in the PCR plate clean_normalized_PCR.