Whitman Lab

Lab Members/Woolet/16S-Cates/Kranz/Re-run Library Prep/SequalPlate

PDF Version generated by

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on

Dec 13, 2017 @10:34 AM CST

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SequalPlate

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Date: 08 Dec 2017

SequalPrep Normalization

Goal: To purify and normalize PCR 16S amplicons from Cates and Kranz projects, and CNL and WB2015 re-runs.

Materials:

SequalPrep Normalizaion Plate Kit

PCR reactions

Pipets

Pipet tips

Procedure:

Pool PCR amplicons

1. Binding Step
    a. Transfer 25ul PCR product to the **SequalPrep Normalization Plate** in the following lineup:

	1	2	3	4	5	6	7	8	9	10	11	12
A	5M-2	U3M-2	CNL_180	CNL_PyOM-2	SC1-1	SC5-1	56M	1M	Cates14	Cates27	Cates39	Cates48
B	5O-2	U3O-1	CNL_181	CNL_PyOM-3	SC1-2	SC5-2	52T	Cates1	Cates16	Cates28	Cates40	Cates49
C	8M-1	U5O-2	CNL_182	CNL_PyOM-4	SC2-1	SC6-1	52M	Cates4	Cates18	Cates32	Cates41	Cates50
D	7O-1	U7M-1	CNL_183	CNL_Bm-1	SC2-2	SC6-2	15T	Cates5	Cates19	Cates33	Cates42	Cates51
E	B6	CNL_MUC	CNL_184	CNL_Bm-2	SC3-1	B20	15M	Cates7	Cates20	Cates34	Cates43	Cates52
F	43O-1	CNL_089	CNL_185	CNL_Bm-3	SC3-2	SC4-2	4T	Cates8	Cates22	Cates35	Cates44	Cates53
G	39O-1	CNL_178	CNL_186	CNL_Bm-4	SC4-1	58M	4M	Cates10	Cates24	Cates36	Cates45	Cates54
H	50O-2	CNL_179	CNL_PyOM-1	CNL_Blank	58T	56T	1T	Cates12	Cates25	Cates37	Cates46	Cates55

    b.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cates56	Cates64										
B	Cates57											
C	Cates58											
D	Cates59											
E	Cates60											
F	Cates61											
G	Cates62											
H	Cates63											

    c. Add 25ul of **SequalPrep Normalization Binding Buffer**
    d. Mix completely by pipetting up and down
    e. Incubate the plate for 1 hour at room temperature- mixing not necessary
2. Washing Step
    a. Aspirate liquid from the wells- be sure to not scrape the well sides
    b. Add 50 uL of **SequalPrep Normalization Wash Buffer** to the wells
    c. Mix by pipetting up and down twice to remove contaminants
    d. Completely aspirate buffer from wells and discard- tap the plate on a paper towel to remove excess wash buffer
3. Elution Step
    a. Add 20 uL **SequalPrep Normalization Elution Buffer** to each well of the plate
    b. Put foil seal on each plate and mix by vortexing. Use the plate spinner to ensure all the liquid is at the bottom of each well
    c. Incubate at room temperature for 5 minutes
    d. Transfer and pool purified DNA into a 5ml centrifuge tube
4. Aliquot the pooled and purified DNA into 4 centrifuge tubes. Poke 2 holes in each centrifuge tube cap. Freeze the samples prior to concentrating.
5. Concentrate the normalized DNA with the Pederson SpeedVac for ~2.5hours (starting volume ~2100ul; ending volume ~800ul)