Whitman Lab

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

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Wizard SV Gel and PCR Clean-up System

Date: 11 Dec 2017

Goal: Use Wizard Gel cleanup to extract and purify Cates/JackPine/WB2015reruns/SaltPlains products that were normalized using SequalPrep

Materials:

Wizard SV Gel and PCR Clean-up Kit

1.5ml microcentrifuge tubes

95% ethanol

vacuum adapters

Agarose gel

1X TAE buffer

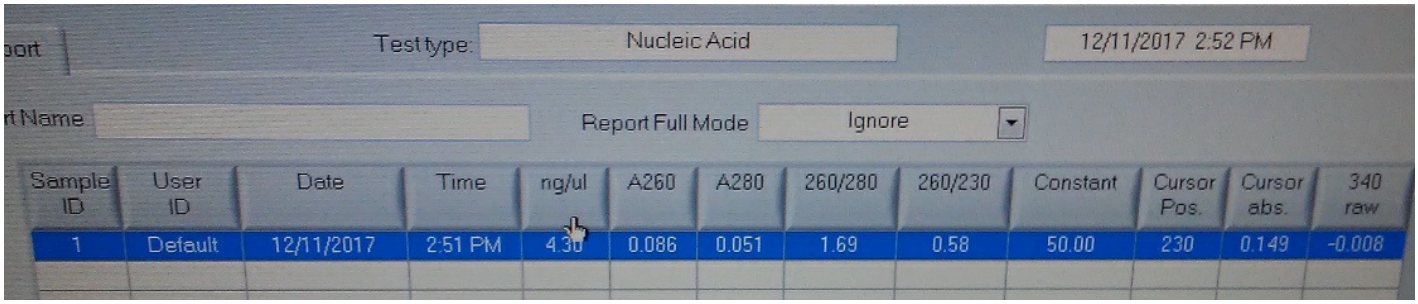
60° C heating block or water bath

Procedure:

1. Prepare a 1.5% agarose gel
    a. 75 mL TAE buffer
    b. 7.5 uL SybrSafe
    c. 1.126 g agarose
2. Run 400ul through the gel
    1. Add 80 ul loading buffer to sample
    2. Load ~53ul sample into each well
    3. **forgot to add DNA ladder to end well**
    4. Run gel at 45mV for 1.5 hours
3. Weigh a labeled 1.5 mL microcentrifuge tube for each fragment and record the weight
    a. Mass of tube:
        1. 1.136
        2. 1.128
        3. 1.151
        4. 1.141
        5. 1.132
        6. 1.138
        7. 1.127
        8. 1.145
        9. 1.137
4. Visualize and photograph the DNA (**did not take a photo**)
5. Use a razorblade to cut out the DNA fragment from the gel- transfer the slice to the microcentrifuge tube, weigh and record the weight. Subtract the weight of the tube to obtain the weight of the agarose slice. (** cut DNA fragment + shearing**)
    a. Mass of tube + gel slice:
        1. 1.551
        2. 1.505
        3. 1.531
        4. 1.523
        5. 1.542
        6. 1.540
        7. 1.543
        8. 1.495
        9. 1.568
6. a. Mass of gel slice:
        1. 0.415 g 415mg
        2. 0.377 g 377mg
        3. 0.380 g 380mg
        4. 0.382 g 382mg
        5. 0.410 g 410mg
        6. 0.402 g 402mg
        7. 0.416 g 416mg
        8. 0.350 g 350mg
        9. 0.431 g 431mg
7. Add **Membrane Binding Solution** at a ratio of 10uL per 10mg agarose slice
    1. Amount added:
        1. 415 ul
        2. 377 ul
        3. 380 ul
        4. 382 ul
        5. 410 ul
        6. 402 ul
        7. 416 ul
        8. 350 ul
        9. 431 ul
8. Vortex the mixture and incubate at 60° C for 10 minutes, or until the gel slice is completely dissolved. Vortex the tube every few minutes to increase the melting rate
9. Purification - note: only 700ul of the dissolved gel mass + binding solution can be passed through the column at one time, but can be done a total of 10 times. Because of this, we used three columns. Each column was able to pass three dissolved gels two times.
    a. Place three **SV Minicolumns** in three **Collection Tubes**
    b. Transfer 700ul of dissolved gel to a **SV Minicolumn** and incubate for 1 minute at room temperature

c. Centrifuge the **SV Minicolumn** assemblies in a microcentrifuge at 16,000 x g for 1 minute
d. *Run the samples from the Collection Tubes through the Minicolumn one more time and centrifuge at 16,000 x g for 1 minute.*
e. Discard flow-through
f. Repeat steps b-e until all the dissolved gel has been ran through the columns twice
g. Remove the Minicolumns and discard the liquid from the collection tubes; return the columns to the collection tubes.
h. Wash the column by adding 700ul of **Membrane Wash Solution** to each **SV Minicolumn.** Centrifuge the assemblies for 1 minute at 16,000 x g and empty the collection tubes. Repeat the wash with 500ul of **Membrane Wash Solution** and centrifuge the SV Minicolumn assemblies for 5 minutes at 16,000 x g
i. Remove the SV Minicolumn assemblies from the centrifuge, empty the collection tubes, and re-centrifuge the columns for 1 minute to allow evaporation of residual ethanol
j. Transfer the minicolumns to clean 1.5 ml microcentrifuge tubes.
k. *Add 30 uL Nuclease-free water to the center of each column- incubate at room temperature for 1 minute and centrifuge at 16,000 x g for 1 minute.*
l. *Resuspend flow-through via flicking the tube, and add 20uL of Nuclease-free water to the center of the column. Incubate and centrifuge for 1 minute*
m. Pool the samples

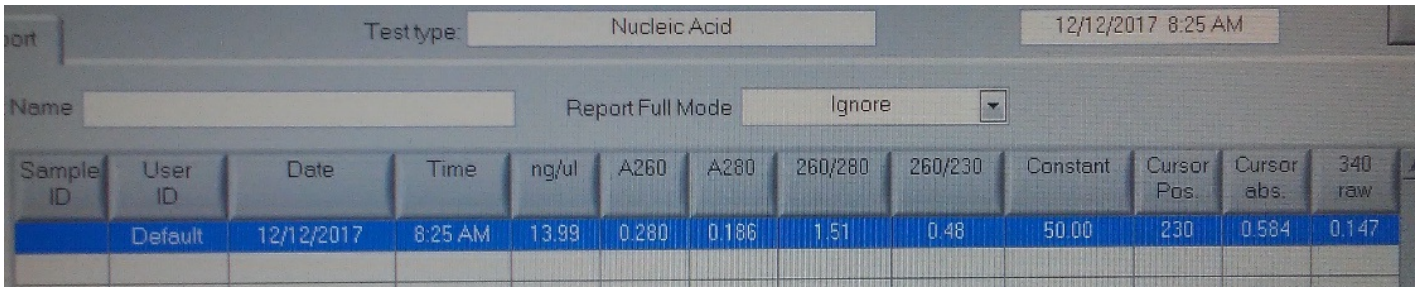
Nanodrop:



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
1	Default	12/11/2017	2:51 PM	4.30	0.086	0.051	1.69	0.58	50.00	230	0.149	-0.008

Concentration of sample is too low. Concentrated the sample using the Pedersen SpeedVac (starting volume ~139ul; ending volume ~25ul)

Nanodrop:



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
	Default	12/12/2017	8:25 AM	13.99	0.280	0.186	1.51	0.48	50.00	230	0.584	0.147

260/280 and 260/230 values are kind-of low.. I think that I took too much of the noise from the band when doing the gel extraction. Consulted with Thea, and we decided to re-do the Wizard.

12 Dec 2017

1. Prepare a 1.5% agarose gel and run 150uL sample
    a. 75 mL TAE buffer
    b. 7.5 uL SybrSafe
    c. 1.126 g agarose
2. Run Wizard product from 11 Dec 2017 (~20ul) + 200ul of the SequalPlate sample through gel
    1. Add 44 ul loading buffer to sample
    2. Load ~53ul sample into each well
    3. Run gel at 45mV for 1.5 hours
3. Weigh a labeled 1.5 mL microcentrifuge tube for each fragment and record the weight
    a. Mass of tube:
        1. 0.987
        2. 0.986
        3. 0.988
        4. 0.986
        5. 0.987
4. Visualize and photograph the DNA
5.

6. Use a razorblade to cut out the DNA fragment from the gel- transfer the slice to the microcentrifuge tube, weigh and record the weight. Subtract the weight of the tube to obtain the weight of the agarose slice
    a. Mass of tube + gel slice:
        1. 1.190
        2. 1.125
        3. 1.185
        4. 1.150
        5. 1.189
7. a. Mass of gel slice:
        1. 0.203 g 203mg
        2. 0.139 g 139mg
        3. 0.197 g 197mg
        4. 0.164 g 164mg
        5. 0.202 g 202mg
8. Add **Membrane Binding Solution** at a ratio of 10uL per 10mg agarose slice
    1. Amount added:
        1. 203 ul
        2. 139 ul
        3. 197 ul
        4. 164 ul
        5. 202 ul
9. Vortex the mixture and incubate at 60° C for 10 minutes, or until the gel slice is completely dissolved. Vortex the tube every few minutes to increase the melting rate
10. Purification
    a. Place a **SV Minicolumn** in a **Collection Tube**
    b. Transfer dissolved gel from tube 1 to a **SV Minicolumn** and incubate for 1 minute at room temperature
    c. Centrifuge the **SV Minicolumn** assembly in a microcentrifuge at 16,000 x g for 1 minute
    d. *Run the samples from the Collection Tubes through the Minicolumn one more time and centrifuge at 16,000 x g for 1 minute.*
    e. Discard flow-through
    f. Repeat steps b-e for tubes 2-5
    g. Remove the Minicolumn and discard the liquid from the collection tube; return the column to the collection tube.
    h. Wash the column by adding 700ul of **Membrane Wash Solution.** Centrifuge the assembly for 1 minute at 16,000 x g and empty the collection tube. Repeat the wash with 500ul of **Membrane Wash Solution** and centrifuge the SV Minicolumn assembly for 5 minutes at 16,000 x g
    i. Remove the SV Minicolumn assembly from the centrifuge, empty the collection tube, and re-centrifuge the column for 1 minute to allow evaporation of residual ethanol
    j. Transfer the minicolumn to a clean 1.5 ml microcentrifuge tube.
    k. *Add 30 uL Nuclease-free water to the center of the column- incubate at room temperature for 1 minute and centrifuge at 16,000 x g for 1 minute.*
    l. *Resuspend flow-through via flicking the tube, and add 20uL of Nuclease-free water to the center of the column. Incubate and centrifuge for 1 minute*
    m. Concentrate sample using Pedersen SpeedVac (starting volume ~48ul; ending volume ~20ul)

Nanodrop results:

5ul of sample submitted to UW-Madison Biotech center for sequencing on 13 Dec 2017. The remainder of sample put in -20 freezer for storage.