

1. Turn on Main Zymark power switch
2. Turn on Sciclone
3. Turn on Computer
4. Turn on Twister II
5. Close the air compressor tank valve and turn the compressor on
6. Turn on Z8 syringe unit
7. Arrange magnets, chilling station, ethanol reservoir, and waste reservoir on Sciclone deck as shown in Figure 1. Remove subclone isolation solution reservoirs if present

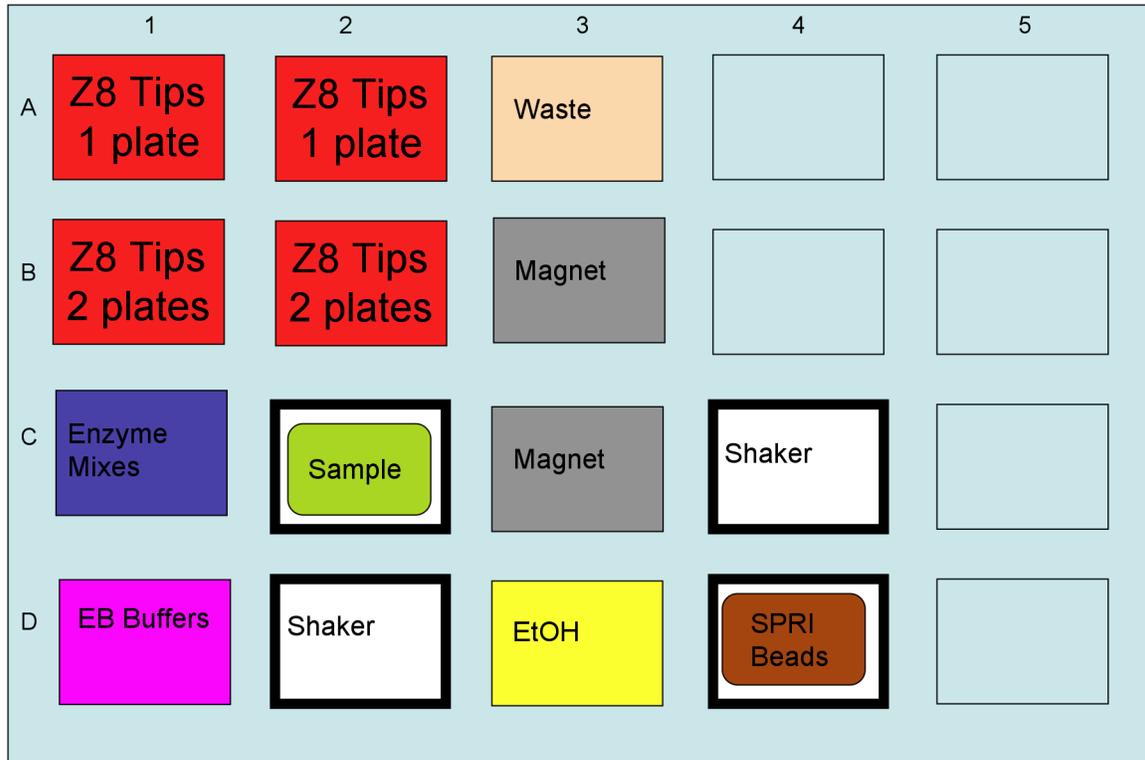
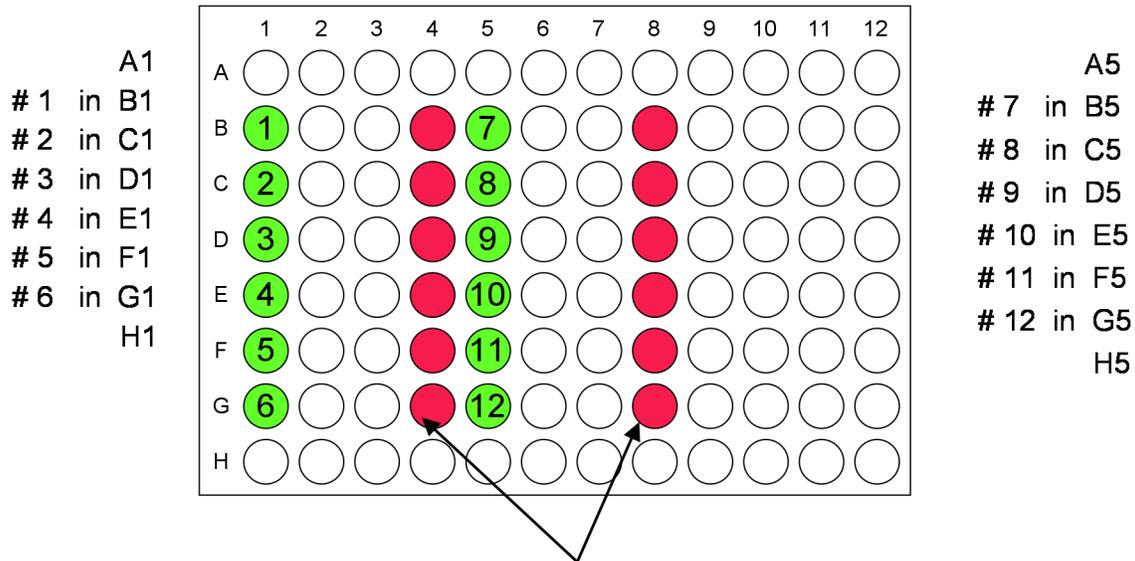


Figure 1. Sciclone deck arrangement

8. Plug in enzyme chiller pump
9. Log into computer (this will take some time as the computers is slow)
10. Fill enzyme chiller pump reservoir (Igloo cooler) with a bucket of ice while waiting for the computer to log in
11. Open "Shortcut to Sciclone ALH" on the computer desktop
12. Open the "Protocols" tab in the lower left of the Sciclone Pipettor window
13. Open and run "Z8_prime.ptc" This primes the Z8 syringes and will take ~30 minutes.
14. Prepare (or have someone prepare) the appropriate enzyme mixes according to the published protocol. (see Enzyme Mix Preparation note below)
15. Place enzyme mixes in the appropriate wells of the enzyme chiller
16. Retrieve the SPRI and EB deepwells from the refrigerator
17. Verify the volumes for SPRI and EB are >300 ul. Vortex the SPRI beads until they are resuspended.

18. Place the SPRI and EB blocks on the Sciclone deck
19. Place 2 or 4 boxes of Z8 tips on the Sciclone deck depending on one or two sample plates, respectively.
20. Place sample DNA in 96 well V-bottom plates, columns 1 and 5, as shown in Figure 2.



Finished libraries will be located in these columns

Figure 2. Sample plate layout.

21. Place the sample plates in Rack 1 of the Twister II such that well A1 is the closest, left-most well to you as you face the rack.
22. Close the Sciclone Pipettor window on the computer desktop
23. Open “Editor” on the computer desktop
24. Click “Open” icon in the upper left of the Clara editor window
25. Open either “454xPrep24x1Plate” or “454xPrep24x2Plates” depending on the number of samples plates
26. Click the “Schedule” icon in the upper right of the Clara editor
27. In the pop-up window click on the application to schedule (from step 25) and type “1” in the box to the right of “Run”. Hit the “Schedule” button and the pop-up will disappear and a Gantt chart will appear. Minimize the Gantt chart.
28. Click the “Sequence” icon in the upper right of the Clara editor
29. In the pop-up window click on the application to sequence (from step 25) and type “1” in the box to the right of “Run”. Hit the “Sequence” button and the pop-up will disappear and another Gantt chart will appear. Minimize the Gantt chart.
30. Click the “Run” icon in the upper right of the Clara editor. This will open the Clara Execution Manager.
31. Click the “Open” icon in the upper left of the Clara Execution Manager. A window will pop-up.

32. Select the application to run in the pop-up window and click the “down arrow” button to the right of the pop-up window. Click OK and the window will disappear and the Material Initialization window will pop up..
33. Click OK in the Material Initialization window and it will disappear.
34. Click the “Create” icon in the upper left of the Clara Execution Manager. This will open windows for both the Sciclone Pipettor as well as the Twister II.
35. In the Twister II window click “Initialize” and then “Home all Axes”. Watch the Twister as it homes to ensure it is working properly.
36. In the Sciclone Pipettor window click on red buttons 2-5 (from the left) to change them to green. This activates the Sciclone shakers.
37. In the Clara Execution Manager click the “Initialize” icon in the upper middle of the window. The Sciclone robot will home. Watch the Sciclone robot to ensure it is functioning properly.
38. Double check the placement of all materials on the Sciclone deck.
39. Click “Run” in the upper middle of the Clara Execution Manager.
40. What is next??

Enzyme Mix Preparations

1. In a strip well, add the following reagents from the GS DNA Library preparation kit, in the order indicated to generate the polishing reaction mix:
 - 5 µl 10x Polynucleotide Kinase Buffer
 - 5 µl BSA
 - 5 µl ATP
 - 2 µl dNTPs
 - 5 µl T4 DNA polymerase
 - 5 µl T4 Polynucleotide Kinase
 - 27 µl final volume
2. In a microcentrifuge tube, add the following to generate the adaptor reaction mix:
 - 10 µl 2X Ligase Buffer
 - 5 µl Library Adaptors (These may be either the general adaptors or individual MID tagged adaptors)
 - 15 µl total
3. In a strip well , add the following to generate the ligation reaction mix:
 - 10 ul 2x ligation buffer
 - 5 ul Ligase

15 ul total

4. In a strip well, add the following to generate the fill-in reaction mix:
 - 40 μ l Molecular Biology Grade water (ddH₂O)
 - 5 μ l 10x Fill-In Thermo-Pol Polymerase buffer
 - 2 μ l dNTP mix
 - 3 μ l Fill-In BST Polymerase Large Fragment
 - 50 ul total volume
5. Place all strip wells in their respective places on the enzyme chilling station on the SciClone ALH
6. Place the DNA in a 96 well v-bottom plate into the input stack of the Twister II robot