Sequencing

Modified by Zhaoyang Liu, April 2016

1. PCR reaction
   Aliquot 50ul Big Dye into 1.5ml Eppendorf tube and store at -20C.
   Set up the PCR reaction with Big Dye as follows:
   Sequencing primer: 2ul (2uM)
   5x Big Bye buffer: 2ul
   DNA template: ~200-400ng
   Big Dye: 1ul
   Add H2O to 10ul

   PCR program: 96 C 1min
   96 C 10s
   50 C 5s 25 cycles
   60 C 10s
   12 C ∞

2. PCR product purification
   Aliquot 500ul HiDi buffer into 1.5ml Eppendorf tube and store at -20C.
   -Transfer the 10ul of PCR product into a 1.5ml Eppendorf tube.
   -Add 1ul 125mM EDTA and 2 volume (20ul) 100% EtOH.
   -Centrifuge at >1200g for 15min at room temperature. (Note: 4C works better)
   -Carefully remove supernatant. Add 100ul 70% EtOH. (Note: fresh made 70% EtOH works better)
   -Centrifuge at >1200g for 5min at room temperature. (Note: 4C works better)
   -Carefully remove supernatant. Air dry in dark for at least 20min. (Note: tube must be completely dry!)
   -(Note: Samples can be covered with aluminum foil and store at 4C if not use immediately.)
   -Add 10ul HiDi buffer and vertex.
   -Transfer samples to 96 well PCR plate.
   (Note: loading order: A1-H1, A3-H3, A5-H5, A7-H7, A9 –H9, A11-H11; A2-H2, A4-H4…….)

A1		A3		A5		A7		A9		A11	
B1		B3		B5		B7		B9		B11	
C1		C3		C5		C7		C9		C11	
D1		D3		D5		D7		D9		D11	
E1		E3		E5		E7		E9		E11	
F1		F3		F5		F7		F9		F11	
G1		G3		G5		G7		G9		G11	
H1		H3		H5		H7		H9		H11	

3. Sequencing
   -Log into the computer with username: HSCADMINSTRATOR, password: Password1
   -Turn on 3730 DNA analyzer instrument
   -Launch Run3730 Data Collection V2.0 on desktop. (Note: a service console will open. It contains 4 icons that will change from red/yellow to green. Don't close the console window when the software is running.) Login Name: 3730user; Password: 3730users
   -In the GA Instruments tab click "plate manager" and fill in as follows:
   a. click "new" at the bottom left of the window
   b. enter plate ID (barcode) and plate name. (Note: No space. These two ID can be the same.)
   c. choose application. For sequencing, choose "sequencing analysis".

d. choose plate type: 96 well plate
e. choose plate sealing: septa
f. enter names of owner and operator
g. click OK. A sample sheet will show up. Fill in sample names, as well as:
h. Result Group: SeqScape
i. instrument protocol: BigDye 3.1
j. Analysis protocol: Bigdye 3.1
(Note: for column h to j, choose the whole column and click Ctrl+D. The whole column will be filled)

-Click Run Scheduler
-Search your plate ID to find your plate.
-Once finding your plate, click add samples, done.
-Put your plate into the stackers.
-Click the Green arrow below the file tab to start.

4. Sequencing data analysis
   -Click Sequencing Analysis software on the desk top. Username: finnell, Password: finnell01
   -Click file, add samples. Find your samples in E/3730_Runs/Seq_Result_Group.
   -Add all samples. Click the green arrow to start analysis.
   -A new data file will be generated after the analysis. Copy all files into your USB drive.
   -Open the trace file with the software SeqScanner (Applied Biosystem)