Sequencing Preparation

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:
Experiment Time:

Source: Cornell Sequencing Handbook

Primary Experimenter (contact):
Other Experimenters:

Assembled: 6/27/2012

Reagent	Details	Quantity
ddH2O (nuclease-free)		Up to 18 µL
Primer (8 µM stock)	Conc. labeled on microfuge tubes in primer box	1 µL
DNA template		*Var.
Sequencing tube		

***Plasmid**, ~1 µg; **PCR product**, #base pairs/5.0 = amount of PCR product in ng that we need
Example: 250bp PCR product. 250bp ÷ 5.0 = 50ng of DNA + 8 pmole primer in 18ul (Note: maximum PCR product concentration is 100ng/ul)

Order #	Template	Template conc. (ng/µL)	Template volume (µL)	Primer ID
-	Plasmid ID , Sample name; Ex) p8, Gibson 1			Ex) 0033
1				
2				
3				
4				
5				
6				
7				

8				
9				
10				
11				
12				
13				
14				
15				

NOTE: Please write calculations on back of sheet.

Procedure:

Critical Steps:

- Clearly label all sequencing tubes before beginning
- NEVER add primers directly from blue cap tubes, only the diluted 8 µM stocks

NOTE:
For primers that aren't strictly sequencing primers, stocks are 10µM. These can still be used for sequencing.

☐ **Label microcentrifuge tubes**

- Write the order # on the top of the tube
- Write the Order name on the side of the tube
- Ex) "Gibson 1" on side, "1" on top

☐ **Calculate µL of DNA template to add to reach desired amount**

$$\frac{Desired\ ng\ DNA}{\boldsymbol{X}\ DNA\ template\left(\frac{ng}{\mu L}\right)} = (\boldsymbol{Y}\ \mu L\ to\ add\ for\ desired\ ng\ DNA)$$

- Aim for ~1 µg for plasmid DNA
- Please write calculations on backs of the front pages of this protocol

☐ **Calculate µL of ddH2O to add**

- (17 µL) – (**Y** µL to add for desired µg DNA) = (µL H2O to add)
- Please write calculations on backs of the front pages of this protocol

☐ **Add ddH2O, primer, and finally DNA template to tube**

- Add primer from the 8 µM stock