PCR Protocol

Reagents you will need

- [ ] Ice
- [ ] Molecular Biology Grade Water
- [ ] Freshly Prepared 80% Ethanol (400 uL/reaction)
- [ ] PCR Master Mix (2x HiFi Master Mix)
- [ ] Singular Conversion Primers (Cat #.)
- [ ] Purification Beads (AMPure or SparQ)
- [ ] Qubit™ 1X dsDNA HS Assay (Q33230)
- [ ] Agilent High Sensitivity D1000 Screen Tape (5067- 5584) and Reagents (5067- 5585)

1. Preparation

- [ ] 1.1 Program a thermal cycler with the PCR conditions outlined in Table 1. Ensure the Lid is heated to 105°C.

Table 1

PCR Conditions			
Step	**Temp**	**Time**	**Cycle**
Initialization	98°C	2 min	1
Denaturation	98°C	20 sec	**7 cycles**
Annealing	57°C	30 sec	
Extension	72°C	30 sec	
Final Extension	72°C	1 min	1
Final Hold	4°C	Hold	

*DO NOT exceed 7 cycles.

- [ ] 1.2 Equilibrate DNA Purification beads (i.e. AMPure beads or SparQ beads) to room temperature for at least 30 min.
- [ ] 1.3 Measure the concentration of your sample using a Qubit.
- [ ] 1.4 With your tubes on ice, dilute all libraries down to 1ng/uL with water. If library concentration is less than 1 ng/uL, adjust the library volume in the green box in Table 1 (excel file) so that a total of 1ng of library is added. The water volume will adjust accordingly.
- [ ] 1.5 Calculate the number of reactions that you will need.

2. PCR

- [ ] 2.1 Vortex and spin down the primers. Gently mix the PCR Master mix by flicking and spin down.
- [ ] 2.2 Prepare the Master Mix (MM) as outlined in Table 2 on ice.
  Note: Use the pre-calculated Master mix for reaction volumes (10% additional volume is added to account for dead volume).

Table 2

		Number of reactions (*10% additional volume is added)				
		1	2	4	8	28
Reagent	**Volume for 1 Rxn (uL)**	**Master Mix**				
Water	20	23.1	46.2	92.4	184.8	646.8
Library (1 ng/uL)	2	-	-	-	-	-
Forward Primer (10 uM)	1.5	1.65	3.3	6.6	13.2	46.2
Reverse Primer (10 uM)	1.5	1.65	3.3	6.6	13.2	46.2
PCR Master Mix	25	27.5	55	110	220	770
Total	50	53.9	107.8	215.6	431.2	1509.2

- [ ] 2.3 Pre-heat the thermocycler. Press Pause once the plate reaches 98°C.
- [ ] 2.4 On ice, aliquot 48uL of Master Mix to PCR tubes for each reaction.
- [ ] 2.5 Add 2uL of 1ng/uL library to each reaction tube. Gently pipette up and down to mix. Note: Do not use more than 2ng of library per reaction.
- [ ] 2.6 Spin down the reaction tubes.
- [ ] 2.7 Once the thermocycler has reached 98°C, place the reaction tubes in the thermocycler and run the program.
- [ ] 2.8 Remove samples from block when thermal cycler program is complete.
- [ ] 2.9 Spin down the reaction tubes. Keep at room temperature for the following bead purification step.

3. Bead Purification

- 3.1 Confirm beads are fully equilibrated to room temperature.
- 3.2 Vortex DNA Purification Beads until fully resuspended.
- 3.3 Add 45µL (0.9x) of resuspended DNA Purification Beads to the final PCR sample and mix well by vortexing and briefly spin down the tubes.
- 3.4 Incubate the samples for 5 minutes at room temperature.
- 3.5 Place the samples on a magnetic plate for 3 minutes. The DNA Purification Beads will form a pellet, leaving a clear supernatant.
- 3.6 Do not remove the plate or tubes from the magnetic plate. Remove and discard the clear supernatant.
- 3.7 While the sample remains on the magnetic plate, wash the bead pellet with 200 µL of freshly prepared 80% ethanol, incubate for 1 minute, then remove and discard the ethanol. Repeat this wash once, for a total of two washes. Keep the sample on the magnetic plate throughout both of the wash steps.
- 3.8 Spin the tubes down and place them back on the magnet. Carefully remove and discard all remaining ethanol with a 10 µL pipette, making sure not to disturb the bead pellet. Do NOT dry pellet.
- 3.9 Remove the tubes from the magnet and add 17uL of water to the beads and mix throughly by vortexing and then briefly spin down the tubes.
- 3.10 Allow beads to incubate for 2 minutes off the magnet for DNA to elute.
- 3.11 Place the plate or tubes on a magnetic plate and allow the beads to settle for 3 minutes or until the beads fully pellet.
- 3.12 Transfer 15 µL of clear supernatant containing the libraries to a clean thin-walled PCR 0.2-mL strip-tube or well of a 96-well thermal cycling plate. Be sure not to carry over any beads with the supernatant.

4. Quantitate

- 4.1 Quantify PCR Product using a Qubit HS DNA Assay, or other high sensitivity fluorometer. The expected concentration is between 2-10ng/ul (10-50nM).
- 4.2 Run Tapestation, Bioanalyzer or fragment analyzer in order to determine the size distribution of your library. The average library size should be between ~300 - 500bp.
- 4.3 Fill in green boxes on Excel file to calculate nM concentration.

Samples	ng/uL	Average Length	[nM]
Sample Name:			#DIV/0!
Sample Name:			#DIV/0!
Sample Name:			#DIV/0!
Sample Name:			#DIV/0!