## Step 13: Test PCR

1. Make Test PCR Master Mix with the following table:
2. Mix PCR reaction in a strip tube and run the following programs:
   1. Herculase taq
      1. 98 C, 2 min
      2. 16 cycles [98 C, 20sec; 65 C, 20 sec; 72 C, 30 sec]
      3. 72 C, 3 min
      4. 4 C, hold
   2. Phusion taq
      1. 98 C, 3 min
      2. 16 cycles [98 C, 10sec; 65 C, 30 sec; 72 C, 30 sec]
      3. 72 C, 5 min
      4. 4 C, hold
3. Run 20uL PCR product and 1:20 dilution of sublibrary on a gel

|  |  |  |  |
| --- | --- | --- | --- |
| **Herculase Taq:** |  |  |  |
| **Reagent** | **Stock Concentration** | **uL per rxn** | **uL for Master Mix** |
| **MilliQ Water** | NA | 17.775 | 136.8675 |
| **Herculase II Buffer** | 5X | 5 | 38.5 |
| **dNTPs** | 100mM | 0.25 | 1.925 |
| **Primer** | 10uM | 0.625 | 4.8125 |
| **Herculase Taq** | NA | 0.35 | 2.695 |
| **Template** | NA | 1 |  |
|  |  |  |  |
| **Total** |  | 25 | 184.8 |

## Step 14: Final PCR

1. Adjust the following table according to desired amount of template.
2. Create a Final PCR Master Mix using the following table, and find appropriate template volume and cycle numbers given template concentration and test PCR results.

|  |  |  |  |
| --- | --- | --- | --- |
| **STEP 14 - FINAL PCR** |  |  |  |
|  |  |  |  |
| **Herculase Taq:** |  |  |  |
| **Reagent** | **Stock Concentration** | **uL per rxn** | **uL for Master Mix** |
| **MilliQ Water** | NA | 25.55 | 196.735 |
| **Herculase II Buffer** | 5X | 10 | 77 |
| **dNTPs** | 100mM | 0.5 | 3.85 |
| **Primer** | 10uM | 1.25 | 9.625 |
| **Herculase Taq** | NA | 0.7 | 5.39 |
| **Template** | NA | 12 | 92.4 |
|  |  |  |  |
| **Total** |  | 50 | 385 |

1. Create PCR reactions and run on the following program:
   1. 98 C, 3 min
   2. number of cycles X [98C, 20 sec; 65 C, 30 sec; 72 C, 30 sec]
   3. 72 C, 5 min
   4. 4 C, hold

## Step 15: Bead Clean-up, .9X

1. Ensure beads are at room temperature & homogenized before use and prepare fresh 80% ethanol
2. Add the volume of beads from the table below to each library, and mix by inversion

|  |  |
| --- | --- |
| **STEP 15 - BEAD CLEAN-UP** | |
|  |  |
| **Library Volume** | 50 |
| **.9X Bead Volume** | 45 |

1. Incubate at room temperature for 5 minutes
2. Place tubes on magnetic plate and incubate for 2 minutes or until the supernatant clears
3. Aspirate and discard supernatant
4. Wash beads with 200 uL 80% ethanol, and rinse up and down by pipetting
5. Incubate for 30 seconds
6. Aspirate ethanol and discard
7. Repeat steps 6,7, & 8 for a second wash
8. Using a P10 to remove any remaining drops of ethanol Air dry for ~5 minutes, until all ethanol has disappeared
9. Add 42 uL elution buffer and mix by inversion until beads resuspended
10. Incubate for 2 minutes in a tube rack
11. Place back on magnetic rack for 2 minutes or until supernatant clears
12. Transfer supernatant to clean labeled tubes

## Step 16: Bioanalyzer/Gel, Picogreen

## Step 17: Pooling