# Sea Cucumber RAD Library Prep Protocol, Part 1Macintosh HD:Users:natalielowell:Desktop:Screen Shot 2016-11-29 at 3.51.20 PM.png

## Before you begin:

1. All DNA extracts normalized to 500 ng in 20 uL of water
2. Fill in the following tables

|  |  |
| --- | --- |
| **DATE** | 20181206 |
| **# Individuals** | 72 |
| **# Sublibraries** | 6 |
| **# Individuals per sublibrary** | 12 |

## Step 1: Digest with SbfI-HF

1. Make Digestion Master Mix according to the following table[[1]](#footnote-0)
2. Add 5 uL of master mix to each sample and mix by pipetting and add new lid
3. Run the thermocycler program 37INC 80KILL and verify steps:
   1. 37 C for 1hr to 1hr 30 min
   2. 80 C for 20 min
4. Cool to room temperature
5. Store in freezer or continue to next step

|  |  |  |  |
| --- | --- | --- | --- |
| **STEP 1 - DIGESTION** |  |  |  |
|  |  |  |  |
| **Reagent** | **Stock Concentration** | **uL per rxn** | **uL for Master Mix** |
| Cutsmart Buffer | 10X | 2.5 | 198 |
| MilliQ Water | NA | 2 | 158.4 |
| Sbf1-HF | 20000 U/mL | 0.5 | 39.6 |
| RNAse A\* | 100mg/mL | 0.1 | 7.92 |
|  |  |  |  |
| Total |  | 5 | 396 |
| Total w RNAse\* |  | 5.1 | 403.92 |

## Step 2: P1 Adapter Ligation

1. Make P1 Adapter Ligation Master Mix with the following table
2. Add 3.4 uL of master mix to each sample and mix by pipetting
3. Add 1.8 uL P1 Adapter to each sample and mix by pipetting, add new lid to your plate and to P1 Adapter plate
4. Incubate at room temperature for 1-2 hours
5. Heat kill enzyme by incubating at 65 C for 10 min
6. Cool to room temperature

|  |  |  |  |
| --- | --- | --- | --- |
| **STEP 2 - LIGATION** |  |  |  |
|  |  |  |  |
| **Reagent** | **Stock Concentration** | **uL per rxn** | **uL for Master Mix** |
| MilliQ Water | NA | 1.95 | 154.44 |
| NEBuffer 2 | 10X | 1 | 79.2 |
| rATP | 100mM | 0.3 | 23.76 |
| T4 DNA ligase | 2000000 u/mL | 0.25 | 19.8 |
|  |  |  |  |
|  |  |  |  |
| Total |  | 3.5 | 277.2 |

## Step 3: Pool Samples into Sublibraries

1. Pool samples evenly among individuals in each sublibrary for a total of 120-180 uL
   1. E.g., for 12 individuals in a sublibrary, 14 uL per individual, 168 uL total

|  |  |
| --- | --- |
| **STEP 3 - POOLING INTO SUBLIBRARIES** |  |
|  |  |
| **uL per sublibrary** | 14 |
| **total volume of sublibrary** | 168 |



1. [↑](#footnote-ref-0)