TREASURE Pilot

**Notes**

* Ensure PBS is molecular grade in a new bottle (not home-brewed)
* Ensure all liquid media is not home-brewed or expired

**Swab collection:**

* Morning collection, prior to EVS daily cleaning. Important that collection occurs at approximately the same time each day to prevent some specimens from being before and others after daily cleaning
* Check with EVS to determine if room has been cleaned. If room has been cleaned, return the following day.

**Processing:**

Sponge Swab Processing

The following steps are to be performed in a Class II Biosafety Cabinet:

1. Ensure that one negative control is processed on each day (total of three negative controls). Negative controls will be sponge swabs that are removed from the bag, but with no surfaces are sampled.
2. Place sample bags with environmental sponges in Stomacher bag rack
3. Add a 45mL aliquot of PBS (no tween) to each bag.
4. Aseptically orient the long side of the sponge with the bottom of the Stomacher bag.
5. Squeeze the sponge until it is fully saturated in PBS.
6. Process each swab in the Stomacher 400C Circulator for 1 minute at 200 RPM.
7. Allow foam to reduce in homogenate for about 5-10 minutes.
8. Carefully hold and squeeze the sponge on one side of the bag and from the opposite side removing all the homogenate from the stomacher bag by pouring into a conical tube.
9. Divide PBS into 5 vials:
10. 8ml sample into 40ml enrichment broth (BHI, LB, TSB, THIO)
11. Freeze residual PBS ~10-20ml @ -80°C
12. Vortex each mixture of sample (PBS) and enrichment broth

Culture Enrichment

1. Incubate each of the 4 vials of enrichment media for 24 hours at 37°C
2. Record if specimens are turbid
3. Vortex briefly to homogenize specimens
4. Pipette 12ml from each of the vials into a new 50ml conical tube
5. Freeze residual from enrichment vials at -80°C
6. Centrifuge combined specimen at 4000g for 30 min at 4 °C
7. Remove and discard supernatant
8. Resuspend pellet in 1ml of PBS

DNA Extraction

1. DNA extraction – DNeasy PowerSoil Pro
2. Measure DNA concentration from all specimens
3. Qubit (DSDNA) - record results
4. Freeze extracted DNA @ -80°C

Delivery of Specimens & Shotgun Metagenomic Sequencing

1. Delivered using cold packs (not dry ice)
2. SeqCenter 1Gbp Shotgun Metagenomic Sequencing