

# De-MetaST

## version 1.0

- To make the executable "De-MetaST", compile with g++ by going into the De-MetaST directory and typing

```
$ make
```

### **Input:**

- 1-Forward nucleotide primer (5' to 3')
- 2-Reverse nucleotide primer (5' to 3')
- 3-Database file(s)

### **Output:**

- 1-"De-MetaST\_hits.fa" - FASTA formatted file - containing all hit/amplicons ≤5000 bp
- 2-"non-blasted\_De-MetaST\_hits.fa" - FASTA formatted file - containing all hit/amplicons >5000 bp

### **Additional Features:**

- Pressing Enter during forward primer prompt will use file named "Primers.txt" as input rather than being prompted for each.
- The Primers.txt file must be located in the same directory as the executable "De-MetaST" file.
- Hit/amplicons >5000 bp by default will not be passed onto BLAST. All retrieved hits >5000 bp are binned into a separate FASTA formatted file "non-blasted\_De-MetaST\_hits.txt". To change this value, open the "Search.h" file in a simple text editor and locate the statement "int max\_length = 5000);" where the 5000 can be changed. The 5000 max length was chosen to avoid excessive computation spent on querying large individual read lines (e.g., each representing a full genome) in BLAST where the feasibility of generating a PCR amplicon is low. Be sure to re-make the De-MetaST executable file if this value is changed.

## **Re-Making the Executable:**

### **1-Enter into the directory**

```
$ cd /home/user/De-MetaST
```

### **2-Remove the object files and executable**

```
$ make clean
```

### **3-Re-make the executable**

```
$ make
```

## **Example Execution (basic):**

- To execute De-MetaST, be sure to navigate to the directory containing the executable before executing below
- In the same directory as the De-MetaST executable, place both "CAM\_PROJ\_FarmSoil.read.fa" and "Primers.txt"

```
$ ./De-MetaST CAM_PROJ_FarmSoil.read.fa
Input Filename for Primers (press Enter to use Primers.txt)
<PRESS ENTER>
Opening: Primers.txt
Forward Primer: CARGGNGAYACNGARCC
Reverse Primer: YTTNCCRTCNCCKRTCNGT

Searching /home/user/CAMERA_Databases/CAM_PROJ_FarmSoil.read.fa
$
```

### **Example Execution (advanced):**

- If your query databases are located in a different directory, you can call to search several of them, each with multiple primer pairs.
- To execute De-MetaST, be sure to navigate to the directory containing the executable before executing below
- Provide the full path to each database queried if they are not located in the same directory as the De-MetaST executable

```
$ ./De-MetaST /home/user/CAMERA_Metagenomes/CAM_PROJ_FarmSo
il.read.fa /home/user/CAMERA_Metagenomes/CAM_PROJ_GOS.read
.fa /home/user/CAMERA_Metagenomes/CAM_PROJ_Antarctica.read
.fa
Input Filename for Primers (press Enter to use Primers.txt)
<PRESS ENTER>
Opening: Primers.txt
Primer Set: 1
Forward Primer: CARGGNGAYACNGARCC
Reverse Primer: YTTNCCRTCNCCKRTCNGT

Primer Set: 2
Forward Primer: CCTACGGGAGGCAGCAG
Reverse Primer: ATTACCGCGGCTGCTGG

Searching /home/user/CAMERA_Databases/CAM_PROJ_Antarctica.r
ead.fa
$
```

- Which sequences does your degenerate primer set retrieve?
  - Check in the "De-MetaST\_hits.fa"
  - For any >5000 bp amplicons check "non-blasted\_De-MetaST\_hits.fa"