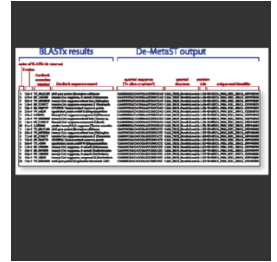


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## Using De-MetaST-BLAST

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**Protocol Integer ID:** 6458



## Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) or Samantha Coy (srose16@utk.edu) for additional information regarding this protocol.

De-MetaST-BLAST, written in C++, is a script that can validate degenerate primers by performing in silico PCR amplification of the primers against metagenomic and metatranscriptomic databases (e.g. CAMERA; MG-RAST). The output is a list of in-silico PCR amplicons that are then blasted against GenBank to determine the most homologous sequence to the amplicon.

Modified from Gulvik, C. A., Effler, T. C., Wilhelm, S. W., & Buchan, A. (2012). De-MetaST-BLAST: A Tool for the Validation of Degenerate Primer Sets and Data Mining of Publicly Available Metagenomes. *PLoS ONE*, 7(11). doi:10.1371/journal.pone.0050362

## Before start

Requirements:

- Potential degenerate primers
- Linux terminal
- Basic knowledge of navigating directories by command line
- De-MetaST-BLAST program installed

## Choosing Degenerate Primers

- 1 Use MEGA to perform an alignment of the gene of interest
- 2 Identify conserved primer regions (18-30nt)
- 3 The predicted amplicon (70-200bp for RT-qPCR) must have enough variability that sequencing these amplicons can distinguish taxonomy
- 4 Check for G4 complexes and other secondary structures that the primers may form
- 5 Check for non-specific amplification and thermal stability for PCR protocols
- 6 Validate primers using De-MetaST-BLAST

## Installation of De-MetaST-BLAST

- 7 Install the De-MetaST-BLAST program  
<http://sourceforge.net/p/de-metast-blast/>

## Execution of De-MetaST-BLAST

- 8 Open the terminal
- 9 `$ cd~/Desktop/Sam/De-MetaST-BLAST/`  
(or cd to wherever the program is)
- 10 `$ ./De-MetaST-BLAST CAM_PROJ_X.read.fa`  
(execute program, choose database)
- 11 Input filename for primers (press Enter to use Primers.txt)



(Custom file.txt or hit enter)

12 <Press Enter>

Opening: Primers.txt

Forward Primer: CARGGNGAYACNCARCC

Reverse Primer: YTTNCCRTCNCCKRYCNGT

13 Searching /home/user/CAMERA\_Databases/CAM\_PROJ\_X.read.fa

14 Remote BLASTx running...

BLASTx finished

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