Using De-MetaST-BLAST

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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-silco 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

Protocol

Choosing Degenerate Primers

Step 1.

Use MEGA to perform an alignment of the gene of interest

Choosing Degenerate Primers
Step 2.

Identify conserved primer regions (18-30nt)

Choosing Degenerate Primers
Step 3.

The predicted amplicon (70-200bp for RT-qPCR) must have enough variability that sequencing these amplicons can distinguish taxonomy

Choosing Degenerate Primers
Step 4.

Check for G4 complexes and other secondary structures that the primers may form

Choosing Degenerate Primers
Step 5.

Check for non-specific amplification and thermal stability for PCR protocols

Choosing Degenerate Primers
Step 6.

Validate primers using De-MetaST-BLAST

Installation of De-MetaST-BLAST
Step 7.

Install the De-MetaST-BLAST program

🔗 LINK:
http://sourceforge.net/p/de-metast-blast/

Execution of De-MetaST-BLAST
Step 8.

Open the terminal

Execution of De-MetaST-BLAST
Step 9.

$ cd/Desktop/Sam/De-MetaST-BLAST/

(or cd to wherever the program is)

Execution of De-MetaST-BLAST

Step 10.

$ ./De-MetaST-BLAST CAM_PROJ_X.read.fa

(execute program, choose database)

Step 11.

Input filename for primers (press Enter to use Primers.txt)

(Custom file.txt or hit enter)

Step 12.

<Press Enter>

Opening: Primers.txt

Forward Primer: CARGGNGAYACNCARCC

Reverse Primer: YTTNCCRTCNCKRYCNGT

Step 13.

Searching /home/user/CAMERA_Databases/CAM_PROJ_X.read.fa

Step 14.

Remote BLASTx running...

BLASTx finished

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