Script P9: Phage-Bacteria Interaction Network

HANNIGAN GD, GRICE EA, ET AL.

Abstract

This protocol provides a method to study phage-bacteria interactions by generating a Network in Cytoscape using the application CoNet. To CoNet tutorial can help with analysis. Based on the methods from the following publication:

Hannigan, Geoffrey D., et al. "The Human Skin Double-Stranded DNA Virome: Topographical and Temporal Diversity, Genetic Enrichment, and Dynamic Associations with the Host Microbiome." *mBio* 6.5 (2015): e01578-15.

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Guidelines

Required Software

- Cytoscape v3.1.1
- CoNet

Relevent Files

Output:

- CoNet/skinmet_metaphlan_merged_output_genera.txt
- CoNet/taxonomy_species_rel_abund_table.tsv
- CoNet/org_skinmet_for_conet.txt
- CoNet/org_phage_for_conet.txt
- CoNet/org_perm.txt
- CoNet/org_boot.txt

R script: R15

Before start

Perl scripts and other supplementary information is available at:

https://figshare.com/articles/The_Human_Skin_dsDNA_Virome_Topographical_and_Temporal_Diversity_Genetic_Enrichment_and_Dynamic_Associations_with_the_Host_Microbiome/1281248

Protocol

CoNet Analysis

Step 1.

First format input tables for downstream analysis. The phage relative abundance table is generated from the UniProt species level taxonomic classifications.

cmd COMMAND

```
mkdir ./uniprot_taxonomy_format_for_conet
cat ./uniprot_contig_virome_trembl_rel_abund/trembl_species_rel_abund/rel_abund_* | cut -
f 1 | sort | uniq > ./uniprot_taxonomy_format_for_conet/trembl_species_master_list.tsv
```

CoNet Analysis

Step 2.

Match each sample taxonomy rel abund list to corresponding master list.

cmd COMMAND

```
mkdir ./uniprot_taxonomy_format_for_conet/samples_mapped_to_master_list_species
for file in $(ls ./uniprot_contig_virome_trembl_rel_abund/trembl_species_rel_abund | grep '
rel'); do
    awk 'FNR==NR {a[$1]=$2;next}{ print $1"\t"a[$1] }' ./uniprot_contig_virome_trembl_rel_a
bund/trembl_species_rel_abund/${file} ./uniprot_taxonomy_format_for_conet/trembl_species_ma
ster_list.tsv | sed "1 s/^/Taxa_ID\t${file}\n/" | cut -
f 2 | sed 's/rel_abund_//' | sed 's/_R1\.txt//' > ./uniprot_taxonomy_format_for_conet/sampl
es_mapped_to_master_list_species/${file}
done
```

CoNet Analysis

Step 3.

Add title to top of master list.

cmd COMMAND

```
sed '1 s/^/Taxa_ID\n/' ./uniprot_taxonomy_format_for_conet/trembl_species_master_list.tsv >
 ./uniprot_taxonomy_format_for_conet/trembl_species_master_list_with_header.tsv
```

CoNet Analysis

Step 4.

Paste together the relative abundance information files with the master taxonomy list.

cmd COMMAND

```
paste ./uniprot_taxonomy_format_for_conet/trembl_species_master_list_with_header.tsv ./unip
rot_taxonomy_format_for_conet/samples_mapped_to_master_list_species/* > ./uniprot_taxonomy_
format_for_conet/taxonomy_species_rel_abund_table.tsv
```

CoNet Analysis

Step 5.

The output file, taxonomy_species_rel_abund_table.tsv contains all viral classifications. Since we are only interested in the phage, we ran the following commands:

cmd COMMAND

```
head -n 1 taxonomy_species_rel_abund_table.tsv >> phage_species.txt
grep "phage"  taxonomy_species_rel_abund_table.tsv >> phage_species.txt
grep "Phage" taxonomy_species_rel_abund_table.tsv >> phage_species.txt
```

CoNet Analysis

Step 6.

The file phage_species.txt contains counts, not relative abundances. For the metagenome samples, we are going to use the general level relative abundances generated by MetaPhlAn. But first, we are going to fix the header from the MetaPhlAn output:

cmd COMMAND

```
sed 's/_R1_trimmed_metaphlan_genera//g' skinmet_metaphlan_merged_output_genera.txt  > skinmet_taxa.txt
```

CoNet Analysis

Step 7.

Now we are going to use the script R13 to organize the input files so that the columns of the virome and microbiome file match (i.e. the Ra sample from Subject 1 at visit 2 is the same column number for both the virome and metagenome files).

CoNet Analysis

Step 8.

Additionally, we are going to convert the counts in phage_species.txt to relative abundance of the phages. This R script generates the files org_phage_for_conet.txt and org_skinmet_for_conet.txt.

CoNet Analysis

Step 9.

In Cytoscape, we opened CoNet and then in the "Data" menu we loaded the org_skinmet_for_conet.txt and org_phage_for_conet.txt and followed the CoNet tutorials.

LINK:

http://psbweb05.psb.ugent.be/conet/tutorial5.php

SOFTWARE PACKAGE (Unix)

Cytoscape, 3.1.1

Shannon P.

CoNet Analysis

Step 10.

Following the matrix infor recommendation, we opened the "Preprocessing and filter menu" and selected row_minocc, setting it to 84. We also checked the box that says "Keep sum of filtered rows"

CoNet Analysis

Step 11.

To perform permutations, in the "Methods menu", we checked the "Pearson", "Spearman", "Mutual information", "Bray Curtis" and "Kullback Leibler" boxes.

CoNet Analysis

Step 12.

We selected the "Automatic threshold setting" button, selected "edgeNumber", entered 250 for the edge selection parameter and enabled "Top and bottom".

CoNet Analysis

Step 13.

In the "Randomization menu" under "Randomization routine", we selected "edgeScores" as the routine and "shuffle_rows" as the resampling method.

CoNet Analysis

Step 14.

Under "Randomization options", we checked the "Renormalize (edgeScores routine only)" box.

CoNet Analysis

Step 15.

Under "Save" we saved the output file as "org_perm.txt"

CoNet Analysis

Step 16.

We ran the permutations and removed the intermediate network from Cytoscape.

CoNet Analysis

Step 17.
To perform bootstraps in the "Methods menu", we removed the 250 edge selection parameter.

CoNet Analysis

Step 18.
In the "Randomization menu" we disabled "Renormalize (edgeScores routine only)" , we changed the resampling method to "bootstrap" we selected "benjaminihochber" multiple test correction with a p value threshold of 0.05. Under "Load null distributions", we selected "org_perm.txt", we selected "sime" as the p-value merge options, and we saved the new output file as "org_boot.txt".

CoNet Analysis

Step 19.
In Cytoscape, under Layout, we selected Edge-weighted Spring Embedded-pval-simes-merge. Under the "Style" section of the control panel, we changed the node color to reflect taxon type (blue for bacteria and yellow for phage) and relative abundance (the darker the color, the more abundant the taxon).

CoNet Analysis

Step 20.
To visualize network properties, in the Cytoscape toolbar, we selected "Tools"->"NetworkAnalyzer"->"Network Analysis" and treated the network as undirected.

CoNet Analysis

Step 21.
The network has 21 nodes and a heterogeneity score of 0.819. In the results panel, we observed the Node Degree distribution and fit a power-law to it. The characteristic path length for the network was 2.781.