⊘ Phylogenomic analysis of Xanthomonas

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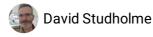[1]University of Exeter

Aug 05, 2022

| 1 Works for me | ⤬ Share |

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Xanthomonas genomics

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ABSTRACT

This is a protocol for using PhaME to generate a phylogenomic tree from a set of *Xanthomonas* spp. genome sequences.

PROTOCOL CITATION

LICENSE

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DISCLAIMER:

1   Create a directory for downloaded genome sequence data:

> Create directory
>
> **mkdir genomes**
>
> Ubuntu 22.04

2   Enter the directory for downloaded genome sequence data:

> Enter directory
>
> **cd genomes**
>
> Ubuntu 22.04

3   Ensure that NCBI Datasets command line tools are installed and executable (or a symbolic link

to the executable) is in the current directory.

NCBI Datasets command line tools 🔗
by NCBI

4 Download the genome assemblies that will be included in the analysis:

xanthomonas_assm_accs.txt

Download assemblies from NCBI

./datasets download genome accession --inputfile xanthomonas_assm_accs.txt --exclude-gff3 --exclude-protein -- exclude-rna --exclude-genomic-cds --filename xanthomonas_genome_assemblies.zip

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📈 You should receive a message something like:
"Downloading: xanthomonas_genome_assemblies.zip197MB done"

Unzip the assemblies download

unzip xanthomonas_genome_assemblies.zip

Ubuntu 22.04 LTS

Make symbolic links to the downloaded assemblies

ln -s ncbi_dataset/data/GCA_*/GCA_*.fna .

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List the symbolic links to assembly sequence files

ls *.fna

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5   Rename the symbolic links to more informative names. We will use the rename_files.pl script
to effect this.

rename_files.pl
source

genomes.list.all.txt

perl rename_files.pl genomes.txt

Ubuntu 22.04 LTS

This should generate a set of .fasta files and .contig symbolic links with informative filenames.

6   Come back out of the directory for downloaded genome sequence data:

Change to previous directory

cd -

Ubuntu LTS 22.04

7   Set-up the reference genome sequence data

Create reference directory

mkdir ref

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Enter the reference directory

cd ref

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Make symbolic link to reference genome assembly

ln -s ../genomes/X._campestris_pv._campestris_ATCC_33913_T.fasta .

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Change back to the root directory

cd -

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8 Set-up the working directory.

Create working directory

mkdir workdir

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cd workdir

Ubuntu 22.04 LTS

Make symbolic links to all the genome assemblies

ln -s ../genomes/*.contig .

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Optionally, at this point, we can delete the symbolic links for any genomes that we want to exclude from the final analysis. It is also an option to add any genome assemblies as .contig files (inf FASTA format).

> Return to previous directory
>
> **cd -**
>
> Ubuntu 22.04 LTS

9   Install PhaME software into a Conda environment called 'phame', following instructions on the software's GitHub page:

> **PhaME**
> source

The PhaME software is described in this paper:

> Shakya M, Ahmed SA, Davenport KW, Flynn MC, Lo CC, Chain PSG (2020). Standardized phylogenetic and molecular evolutionary analysis applied to species across the microbial tree of life.. Scientific reports.
> https://doi.org/10.1038/s41598-020-58356-1

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> Activate Phame Conda environment
>
> **conda activate phame**
>
> Ubuntu 22.04 LTS

11   Create file phame.ctl in current directory.

phame.ctl

12   Execute PhaME:

Execute PhaME

phame ./phame.ctl

Execute PhaME in Conda environment
Ubuntu 22.04 LTS

This will generate output, including tree files, in directory:

./workdir/results/trees/

13   The tree file can now be visualised using any tree-viewing software, for example, iTOL.

Letunic I, Bork P (2021). Interactive Tree Of Life (iTOL) v5: an online
tool for phylogenetic tree display and annotation.. Nucleic acids
research.
https://doi.org/10.1093/nar/gkab301