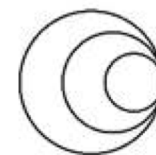




H3ABioNet

Pan African Bioinformatics Network for H3Africa



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Next Generation Sequencing Bioinformatics Course 2021

Module 6 – Pathogen variant calling Session 4: Summary



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NGS Bioinformatics Course Africa 2021
Trainer name: Narender Kumar

Question: Investigating resistance in *M. tuberculosis*

What we know

- 4 isolates from patients with Tuberculosis in a region
- The rapid tests (GeneXpert) have revealed resistance to rifampicin
- WGS performed for all 4 isolates

Isolate	Drug	Resistance
MD001	Rifampicin	Resistant
MD002	Rifampicin	Resistant
MD012	Rifampicin	Resistant
MD024	Rifampicin	Sensitive

Questions we would investigate

- Detect genetic variants for resistance
- resistance to other anti-Tb drugs
- Are these related isolates (pairwise SNP difference)
- Understand their phylogenetic relationship

Summary

- Mapped reads to reference (H37Rv)
- Identify high quality variants (resistance mutations)
- Generate pseudogenomes (with variants)
- Create alignment and extract variant sites
- Calculate pairwise SNP difference
- Generate phylogenetic tree
- Visualization and understanding

Resistance mechanisms: *M. tuberculosis*

Acquired genetic mutations confers resistance

Drug	Gene	Mutations
Rifampicin	<i>rpoB</i>	S450X, D435X
Streptomycin	<i>rpsL</i>	K43R
Isoniazid	<i>katG</i>	S315T
Fluoroquinolone	<i>gyrA</i>	D94X



Other useful resources

- Resistance prediction:

1) ResFinder: <https://cge.cbs.dtu.dk/services/ResFinder/>

Input: Reads or assembled contigs

- 2) Card: <https://card.mcmaster.ca/analyze>

Input: Assembled contigs

- 3) Pathogenwatch: <https://pathogen.watch/>

Input: Reads or assembled contigs (limited for certain species)

- Phylogenetic tree visualization

A) Itol: <https://itol.embl.de/>

B) Microreact: <https://microreact.org/showcase>

c) Phandango: <https://jameshadfield.github.io/phandango/#/>



Thank you

Practical sessions:
