H3ABioNet
Pan African Bioinformatics Network for H3Africa

wellcome connecting science

Next Generation Sequencing Bioinformatics Course 2021

Module 6 – Pathogen variant calling

Session 4: Summary

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	*rpoB*	S450X, D435X
Streptomycin	*rpsL*	K43R
Isoniazid	*katG*	S315T
Fluoroquinolone	*gyrA*	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: