Chromosome Resequencing

Field	Value
Project	Plague Denmark
Date	2021-APR

Objectives

1. Identify Yersinia pestis-positive samples for Resequencing based on:
    - Library complexity
    - Genome coverage, specifically the Chromosome.
    - Informative SNPs.

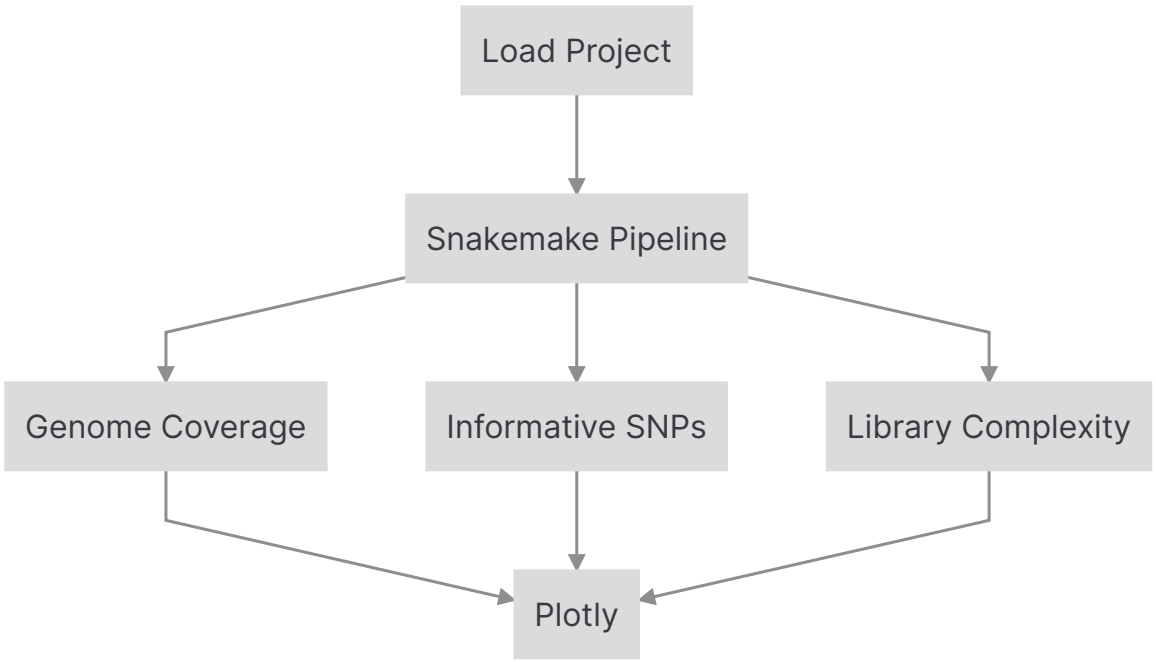
Conclusions

1. The following enriched libraries are good candidates for resequencing:
    - D24, D62, D72, R21
2. The threshold of 50% coverage at 3X is a relatively good predictor of reaching the minimum number of informative SNPs for phylogenetic applications.
3. All of the libraries that Ravneet has enriched have drastically better complexity than libraries enriched by Katherine. Some factors could be:
    - Different samples, with different preservation and infectious load.
    - Different baitsets, Ravneet was the first to use the newly synthesized core baits.
    - Different technique, Ravneet may have better laboratory technique somewhere in the workflow :)

Overview

1. Run the plague-phylogeography snakemake pipeline for Medieval Denmark samples.
2. Calculate library complexity: Preseq.
3. Calculate the number of informative SNPs: Snippy.
4. Calculate genome coverage: Qualimap.

5. Create charts: Plotly.
6. Identify samples for Resequencing.



Results

Genome Coverage and SNPs

This stats table reflects the Genome coverage and SNPs merged across all libraries for a sample. The Black Death sample 8291 is included for context as a high coverage genome, and setting the minimum expected number of SNPs.

`Coverage Status` is set to 0 if the sample is below 50% and will be colored red on the following chart.

`SNP Status` is set to 0 if the sample is below 90 SNPs and will be colored red on the following chart.

- The Coverage of the reference and the Number of SNPs is highly related.
- The threshold of 50% coverage at 3X is a relatively good predictor of reaching the minimum number of SNPs (relative to Black Death 8291).
- Three samples have high coverage (70%+) and do not need resequencing.
    - D51, D71, D75, R36

- Five samples have moderate coverage (50-70%) which could be improved by resequencing.
    - D62, D72, P187, P212, P387
- Three samples have low coverage and moderate SNPs. Depending on the complexity curves, they may be good candidates for resequencing.
    - D24, P384, R21
- Two samples have extremely low coverage and few SNPs. These are NOT good candidates for resequencing.
    - D25, P246, R44

Library Complexity

Based on the Genome Coverage and SNPs, these plots are being used to evaluate whether medium and low coverage samples should be resequenced.

Category	Sample	Description	Action
Medium	D62	Still has an upward trajectory.	Resequence
Medium	D72	Starting to plateau.	Resequence
Medium	P187	Plateaued with few unique molecules.	None
Medium	P212	Plateaued with few unique molecules.	None
Low	P384	Plateaued with few unique molecules.	None
Low	P387	Plateaued with few unique molecules.	None
Low	R21	Similar to D72.	Resequence
Low	D24	Marginally worse than D72.	Resequence

Methods

Data Preparation

1. Clone the plague-phylogeography projects repository.

```
git clone https://github.com/ktmeaton/plague-phylogeography-proje
```

2. Load the Plague Denmark into the plague-phylogeography snakemake pipeline.

```
workflow/scripts/project_load.sh results ../plague-phylogeography
```

Analysis

1. Run `eager local` to generate Preseq output.

```
snakemake eager_local --profile profiles/infoserv --configfile re
```

2. Create a MultiQC report.

```bash
snakemake multiqc_local --profile profiles/infoserv --configfile
results/config/snakemake.yaml
```

*Note: This output was very messy and complicated. I used Plotly instead to make individual charts.

3. Prep tables for plotly.

```
cd plague-phylogeography-projects/denmark/multiqc/local/multiqc_d
mkdir -p plotly
cd plotly
../../../../../scripts/plotly_preseq.sh ../mqc_preseq_plot_1.txt
```

The following samples had multiple libraries:

- P187
- P212
- P246
- P384

- P387

There appeared to be little significant variation between the libraries. I chose the M4 experiment on library a to be representative of each.

*Note: I just made the "Perfect" one manually.

4. Create a snakemake report.

```
mkdir -p results/report/local/
snakemake multiqc_local --report results/report/local/report.html
```

Upload Results

1. Unload project results.

```
workflow/scripts/project_unload.sh results ../plague-phylogeograp
```

2. Upload project results.

```
cd ../plague-phylogeography-projects/
git add -A
git commit -m ""
git push origin
```

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tags: Experiment