What are SNPs?

- Single Nucleotide Polymorphisms
  - Differences between individuals (isolates) of a species
  - EuPathDB: differences between strains / isolates (if clonal)
    - Some organisms are diploid so will also have allelic SNPs within strain
  - Genes that are different due to SNPs are alleles.
  - Our model does not include insertions/deletions (indels) currently

```
tgondii_gt1_chr      ATTCGATGCGCAGAGGAGGAACTACAGAGACGGAGCGGCACTGAAGCTTTTGCCAAAGAC
tgondii_veg_chr      ATTCGATGCGCAGAGGAGGAACTACAGAGACGGAGCGGCACTGAAGCTTTTGCCAAAGAC
tgondii_me49_chr     ATTCGATGCGCAGAGGAGGAACTACAGAGACGGAGCGGTACTGAAGCTTTTGCCAAAGAC 1129631
neospora_chr         ATTCGCTGCGCAGAAGAAGAGCTGCAAAGACGCAGCGGCACCGAGGCGTTCGCCAAAGAC
tgondii_rh_chr       ------------------------------------------------------------

tgondii_gt1_chr      TTACTTCTCCTCCTTGTCGGGGCTGAGGCCTCTTCCGCTGCGAAACAGGCTGGTAAGGCG
tgondii_veg_chr      TTGCTTCTCCTCCTCGTCGGGGCTGAGGCCTCTTCCGCTGCGAAACAGGCTGGTAAGGCG
tgondii_me49_chr     TTGCTTCTCCTCCTCGTCGGGGCTGAGGCCTCTTCCGCTGCGAAACAGGCTGGTAAGGCG 1129571
neospora_chr         CTTCTCCTCCTCCTCGTCGGGGCAGACGCGTCGCCTGCTGCGAAACAGGCTGGTAAGCCA
tgondii_rh_chr       ------------------------------------------------------------

tgondii_gt1_chr      GCGGCGACGA---AGGGTGGCTCTGAA----------------------------GAGC
tgondii_veg_chr      GCGGCGACGA---AGGGTGGCTCTGAA----------------------------GAGC
tgondii_me49_chr     GCGGCGGCGACGAAGGGTGGCTCTGAA----------------------------GAGC 1129540
neospora_chr         CCCGCGGCGGACGGACGTCGCGCGCCACGCGAAGGCGAGAAAAAGGGGAAGCGTTTGAGC
tgondii_rh_chr       ------------------------------------------------------------
```

SNPs in EuPathDB are derived from two sources

- Chip based assays
  - Arrays are designed that allow identification of SNP alleles given a DNA sample. There are multiple different arrays in PlasmoDB. Also Barcode assays (24 SNPs) assayed by PCR
  - Isolate DNA is then assayed on these arrays.
- Direct deep sequencing of DNA from isolates.
  - Reads are aligned to a reference genome and SNPs called.
- What are Isolates – Session this afternoon where we look at isolates.

How are resequencing SNPs called in EuPathDB

- Reads (hopefully paired end) are received from provider (best if via SRA)
- Reads are aligned to the reference using Bowtie2 (end-to-end)
- Reads realigned around indels using GATK
- SNPs, indels and consensus sequence generated using VarScan (min read depth 5, min read frequency 20%)
- SNPs based on this reference comparison are stored in the DB
- Every isolate alignment is checked for every SNP position and if sufficient evidence to make “like reference” call then this is indicated in the DB along with evidence for call.
- In the end, have allele(s) for every isolate at every position if the evidence from the alignment warrants it.
- http://tinyurl.com/pebcdlz (SNP record page - link to alignment)

Homozygous / Heterozygous SNPs

- Ploidy of organism is critical
  - Apicomplexans are haploid for majority of cycle
  - Trypanosomatids, Amoebas, etc are diploid (or worse)
- Why does this matter for SNP calling/queries?
  - Read frequency is the defining parameter
- What does a heterzygous SNP look like?
  - http://tinyurl.com/o23wndt – record page
  - http://tinyurl.com/nh5jbxj

What can we do with SNPs?

- SNPs are genetic markers
  - Distinguish specific strains / isolates.
  - Enable fine structure mapping of phenotypes in genetic crosses or association studies.
  - Enable population studies etc.
- Identify SNPs based on a variety of characteristics.
  - Within a group of isolates (includes allele frequency and confidence parameters)
    - Restrict to location (on chromosome or within genes)
  - Compare two groups of isolates to identify SNPs that distinguish the two groups.
- Identify Genes
  - Identify genes that are appear to be under selection based on SNP characteristics.
    - Number of SNPs (coding, non-coding, synonymous etc)
    - Ratio of non-synonymous / synonymous provides an indication of whether genes are under purifying or diversifying (balancing) selection.

Purifying vs. Diversifying selection

- Purifying selection (gene is evolutionarily constrained to maintain the primary amino acid sequence)
  - Genes that have a low Non-synonymous / Synonymous ratio
  - Tend to be genes critical for basic metabolic processes such as enzymes, cell cycle related etc.
  - Due to very high A/T bias in *falciparum*, the ratio of non-synonymous/ synonymous can be skewed due to severe codon bias.
    - *P. reichenowi* (closely related species infecting chimps) is less A/T rich and makes a good "strain" for identifying genes under purifying selection.
- Diversifying (balancing) selection (it is evolutionarily advantageous to quickly change the amino acid sequence)
  - Genes that have a high Non-synonymous / Synonymous ratio.
  - Tend to be things like surface antigens that the organisms use to escape immune detection.

Read frequency threshold 80%

Minor allele frequency >= 0

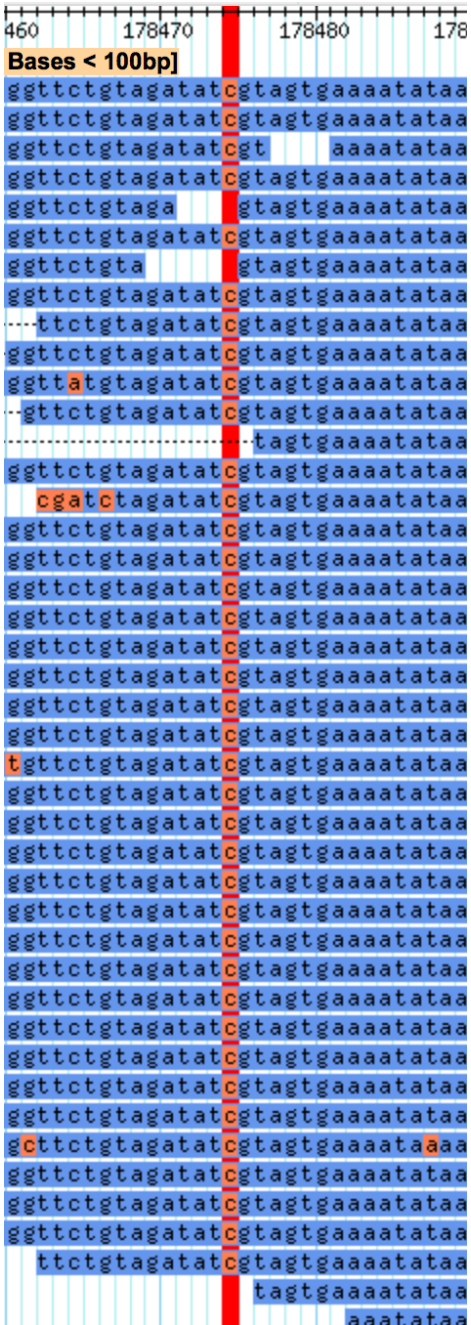
Percent isolates with a base call >= 80

Aligned Reads …. 1 isolate

Aligned Strains (Isolates)

```
Pf3D7_01_v3 158913 ACATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTT CTTTACAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
         7G8 158913 .CATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTC CTTTACAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
       Dd2-1 158913 .CATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTT CTTTACAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
        G224 158913 .CATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTC CTTTGCAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
         GB4 158913 .CATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTC CTTTACAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
        H209 158913 .CATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTC CTTTGCAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
         HB3 158913 .CATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTT CTTTACAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
    RV_3675 158913 .CATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTT CTTTACAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
    RV_3735 158913 .CATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTC CTTTACAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
    RV_3736 158913 .CATACTACA TCTCACATTT TTCATTTTAC GAAAGGTTTC CTTTACAACG AAAAGTAAGA TTAATATGGA TAAAAATGAA
```

Homozygous SNP



Heterozygous SNP