The Basics of Understanding Whole Genome Next Generation Sequence Data

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NCEZID/DFWED/EDLB

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50 state Training call

National Center for Emerging and Zoonotic Infectious Diseases

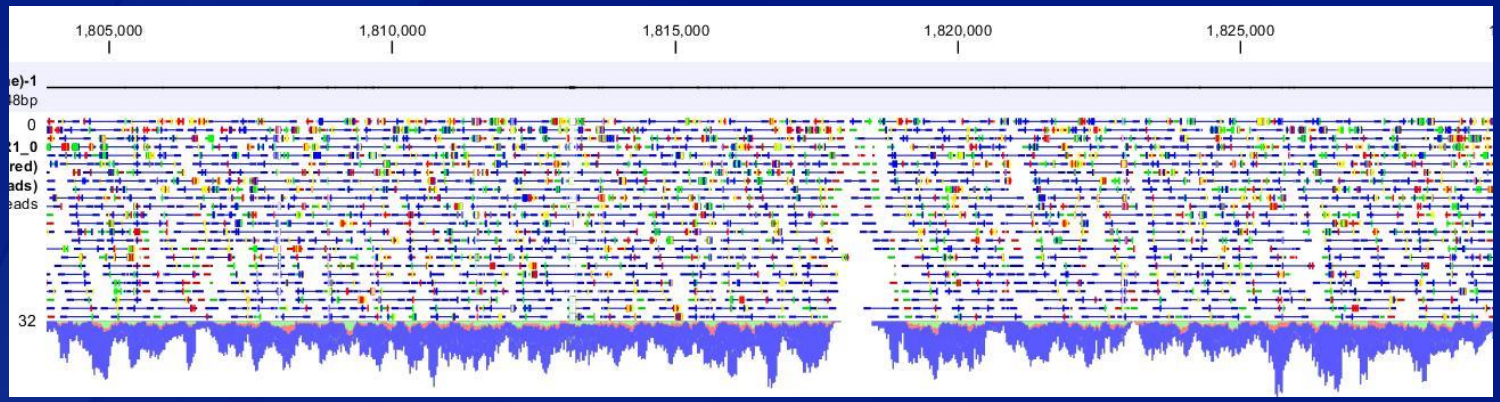
Division of Foodborne, Waterborne and Environmental Diseases

Objectives

- Provide a basic overview of the terminology surrounding whole genome sequence (WGS) data
- Explain ways to analyze WGS data to characterize isolates

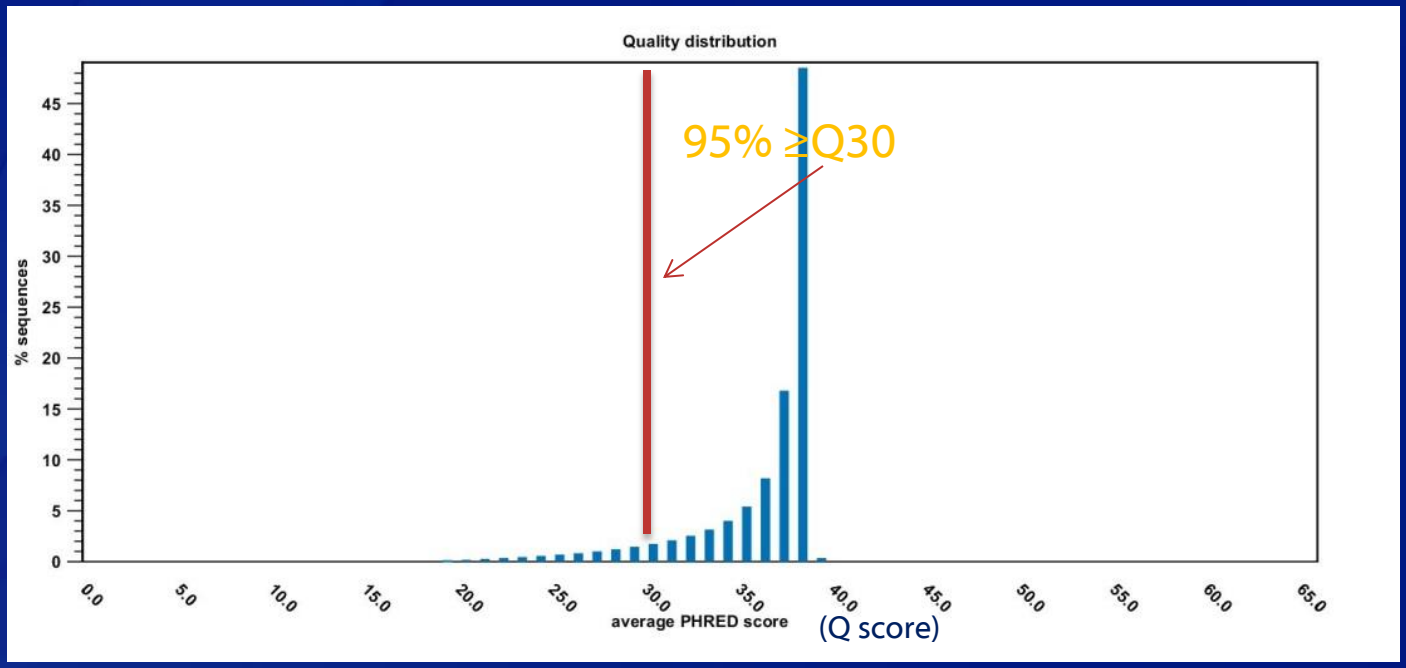
WGS terms: Raw Read

- **Raw Read**
  - Single sequencing output from your NGS machine; length depends on sequencing chemistry
  - Generally 100 thousand – millions of raw reads are generated per isolate sequenced using NGS



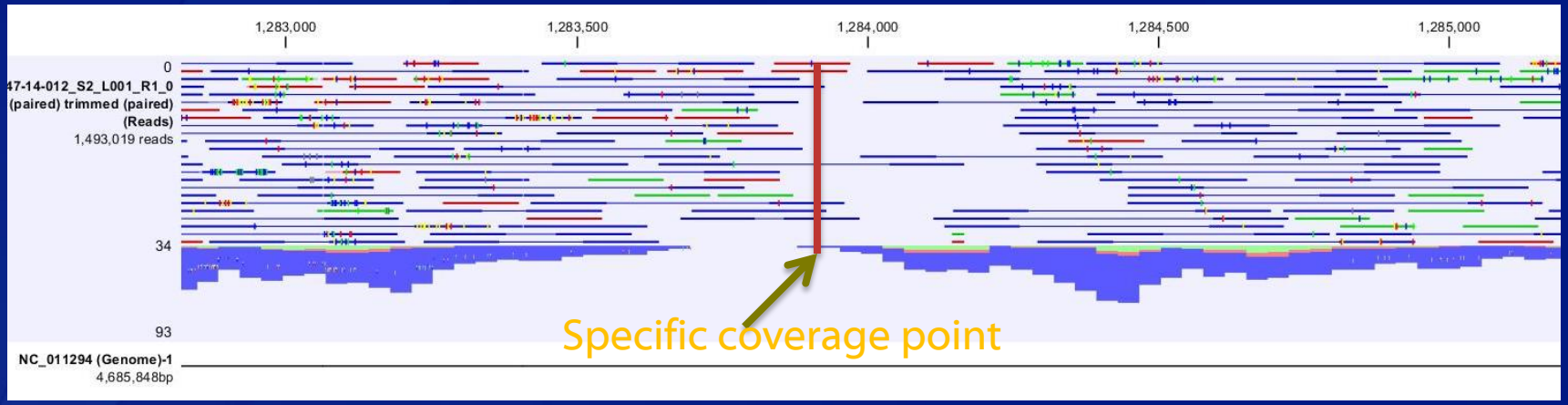
WGS terms: Quality Scores

- **Quality scores**
  - Likelihood the base call is correct
    - Phred – part of fastq file generated from sequencer that scores base call quality
    - Q30 – the percentage of base calls that have a 1 in 1000 chance or less of being incorrect (Q20 – 1 incorrect in 100 base calls)
      - indicates how much usable data you have from a run



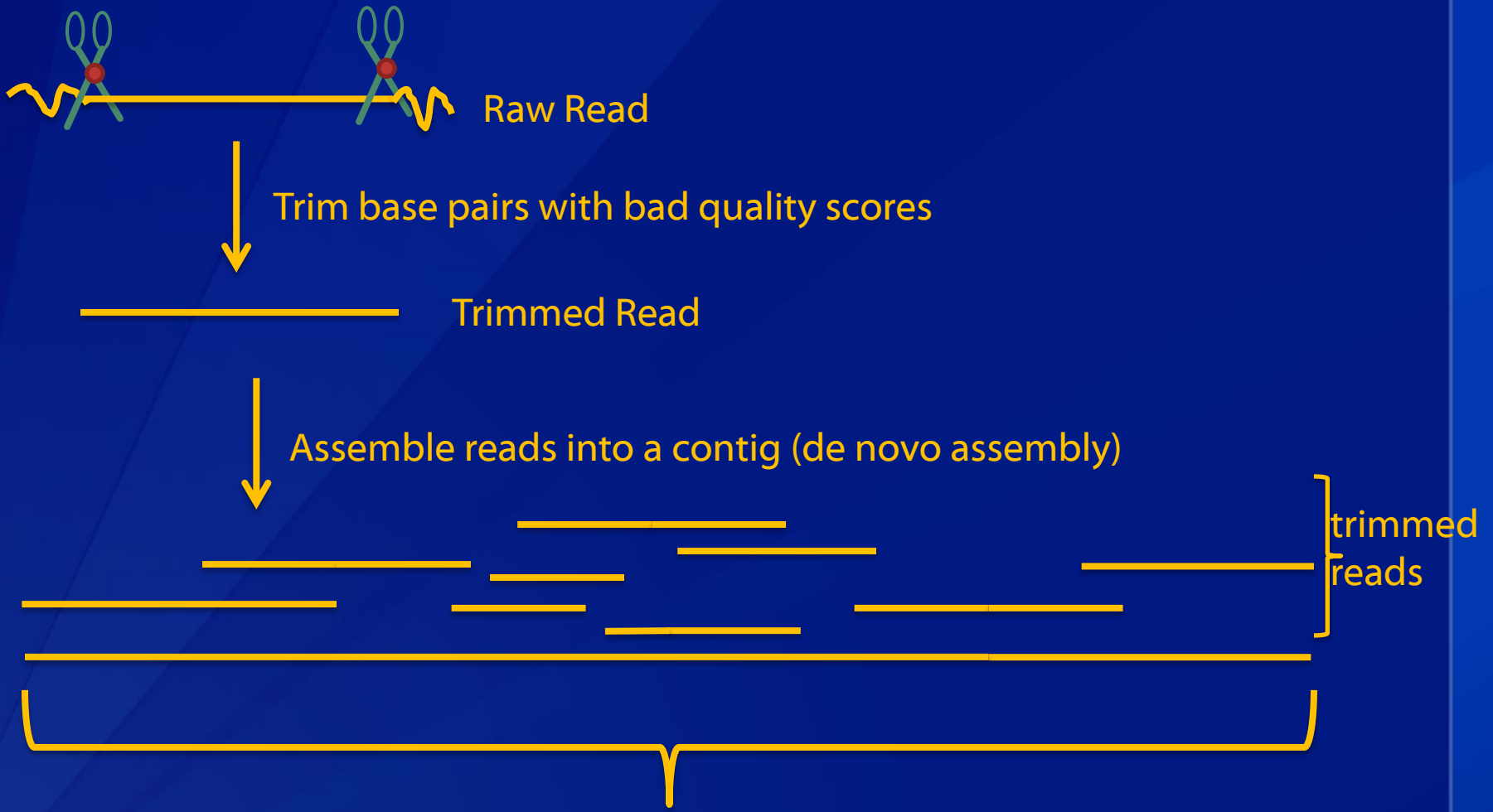
WGS terms: Coverage

- **Coverage**
  - Average – divide the total # of bases by the genome size (i.e. 156,000,000 (total bases from sequencer)/ 3,000,000 (size of genome = 52x coverage))
  - Specific – how many reads span the 1 base you are looking at



-Average genome coverage of genome example is 41x – coverage at specific coverage point is 7x

WGS terms: de Novo Assembly

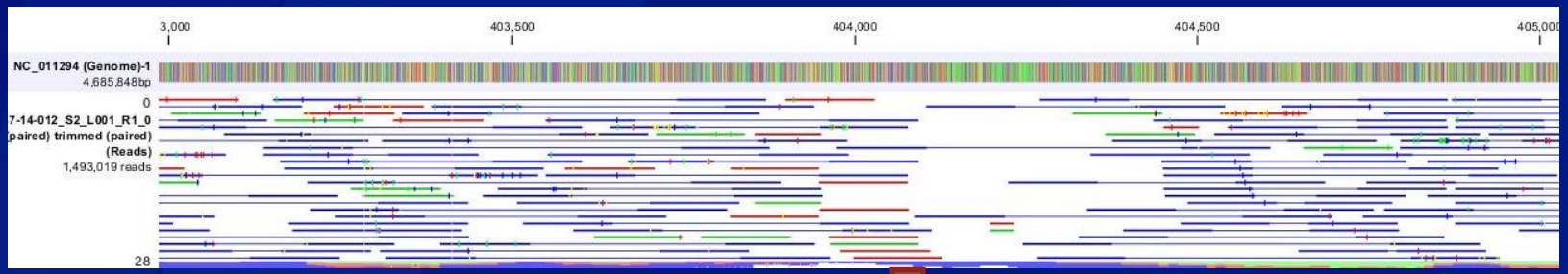


Contig:

Assembly of overlapping reads into a single longer piece of DNA

WGS Terms: Reference-guided Assembly

- **Map raw reads to a closely related reference genome**



Contigs extracted from read mapping of raw reads
(can set quality and coverage thresholds)

Contig 1

Contig 2

Choosing de Novo versus Reference-guided Assembly

de Novo

-Computationally costly

-Difficult if there are repeat regions

-Assembles genome and plasmids

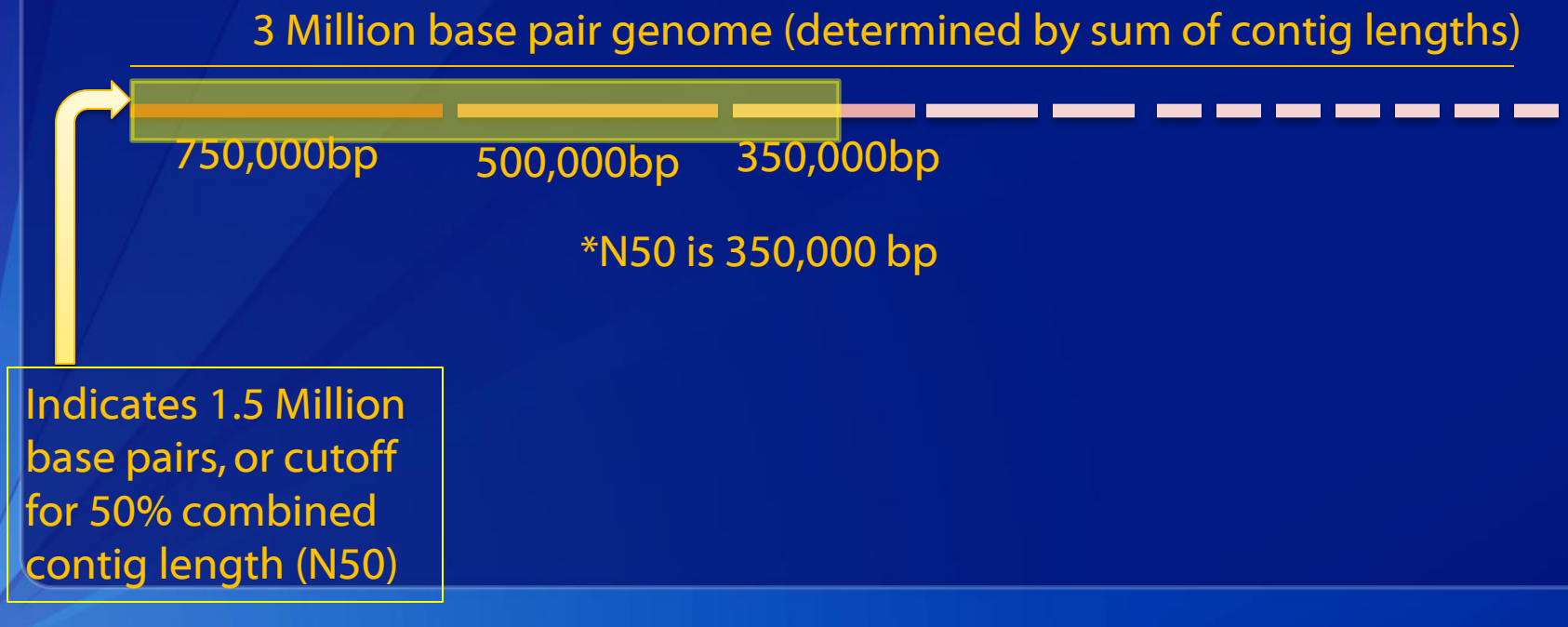
Reference – guided

-Requires closely related good reference genome

-Only assembles reads that match the reference – does not assembly plasmids or insertion elements if there is no reference

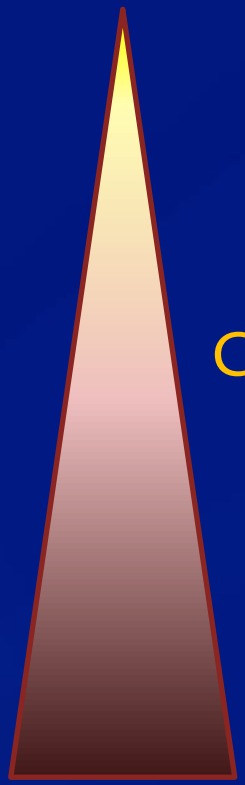
Assessing Assembly Quality

- **Assembly metrics can indicate sequence quality**
  - Number of contigs raw reads assembles into
    - Good: *E. coli* <200, *Salmonella* < 100, *Listeria* < 30
  - N50 statistic– Calculated by summarizing the lengths of the biggest contigs until you reach 50% of total combined contig length
    - Good: >200,000 bp



Ways to Analyze WGS data

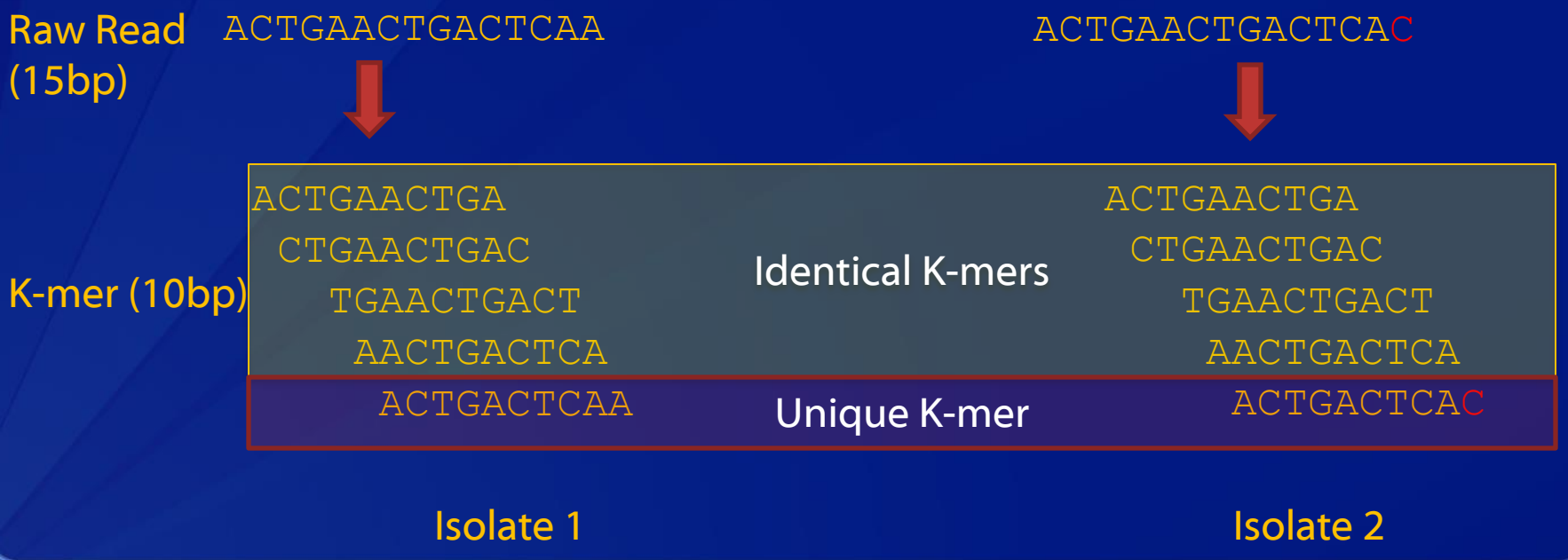
- Kmer analysis
- Whole genome multilocus sequence typing (wgMLST)
- High quality Single Nucleotide Polymorphism (hqSNP) analysis



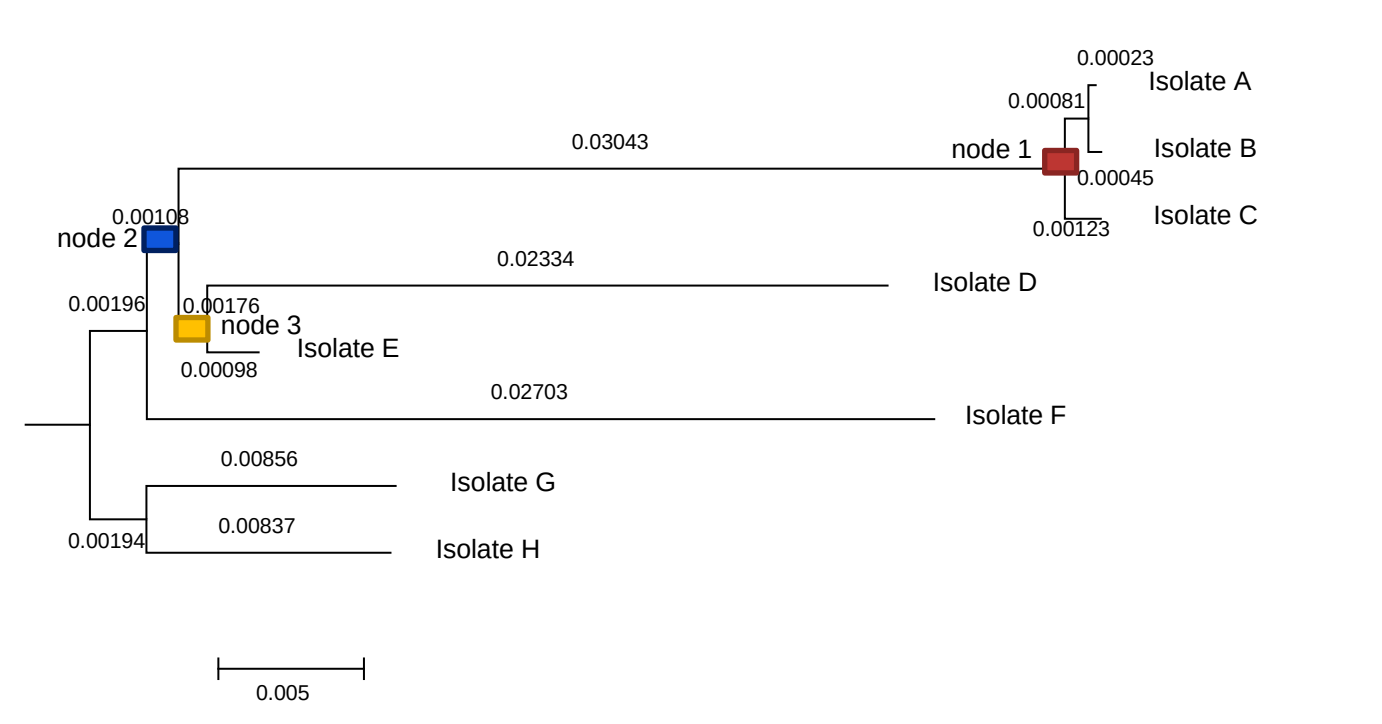
Computational demands

K-mer analysis

- **K-mer :**
  - Computer algorithms use a sliding window to chop up raw reads into shorter lengths (k) of DNA
  - k is determined by which length gives you the best specificity and most adequate resolution
  - Comparing similar and unique kmers gives you a measure of relatedness



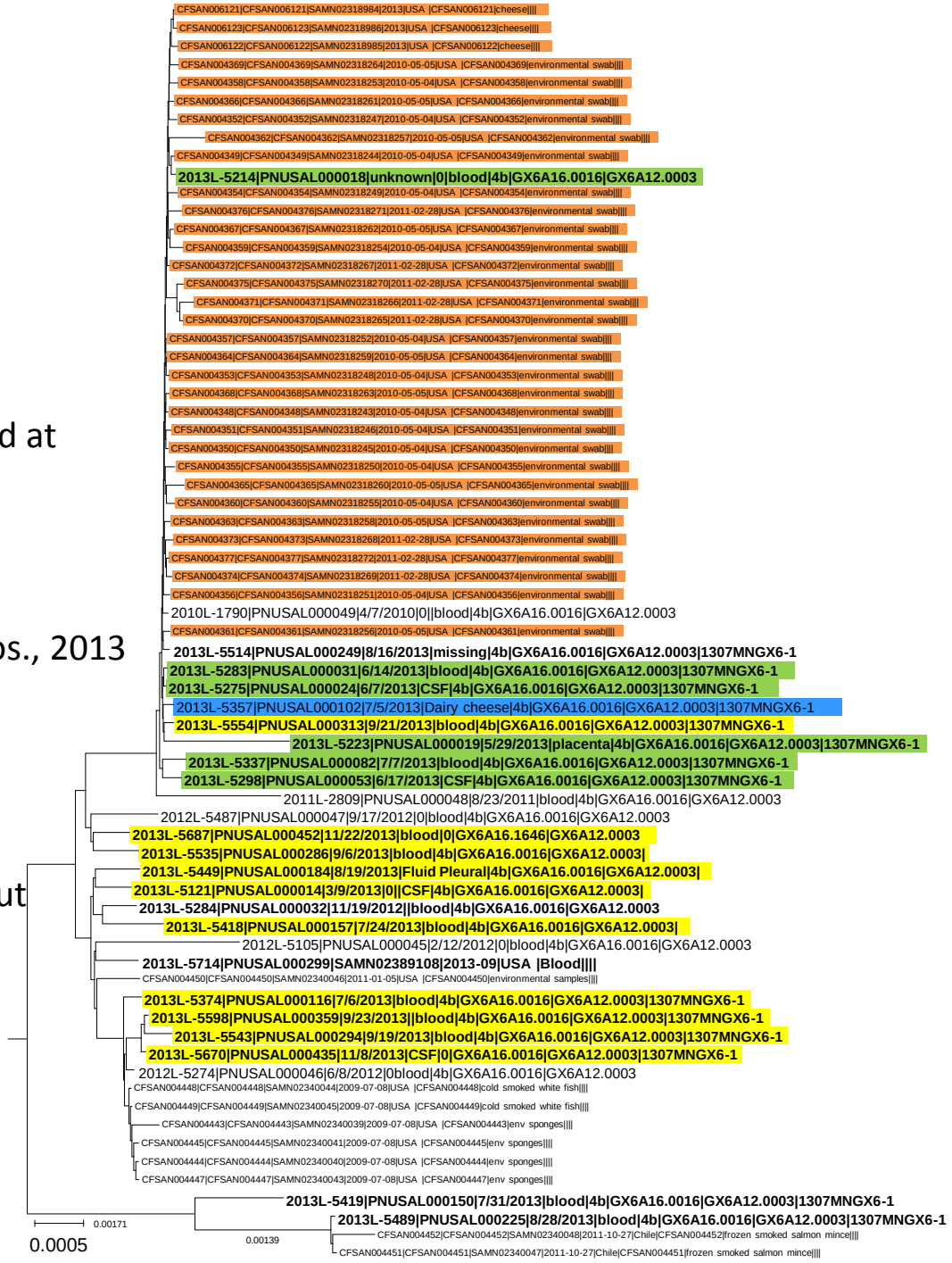
Understanding WGS Data Analysis: Phylogenetic Trees



- Branch length indicates relatedness, shorter horizontal branch length = highly related (isolates in red node 1); longer branch length = less related (yellow node 3)
- Branch length is affected by # of isolates you are comparing as well as relatedness
- Where branches join is referred to as a node, the node indicates a common ancestor (blue node 2), could indicate common transmission source

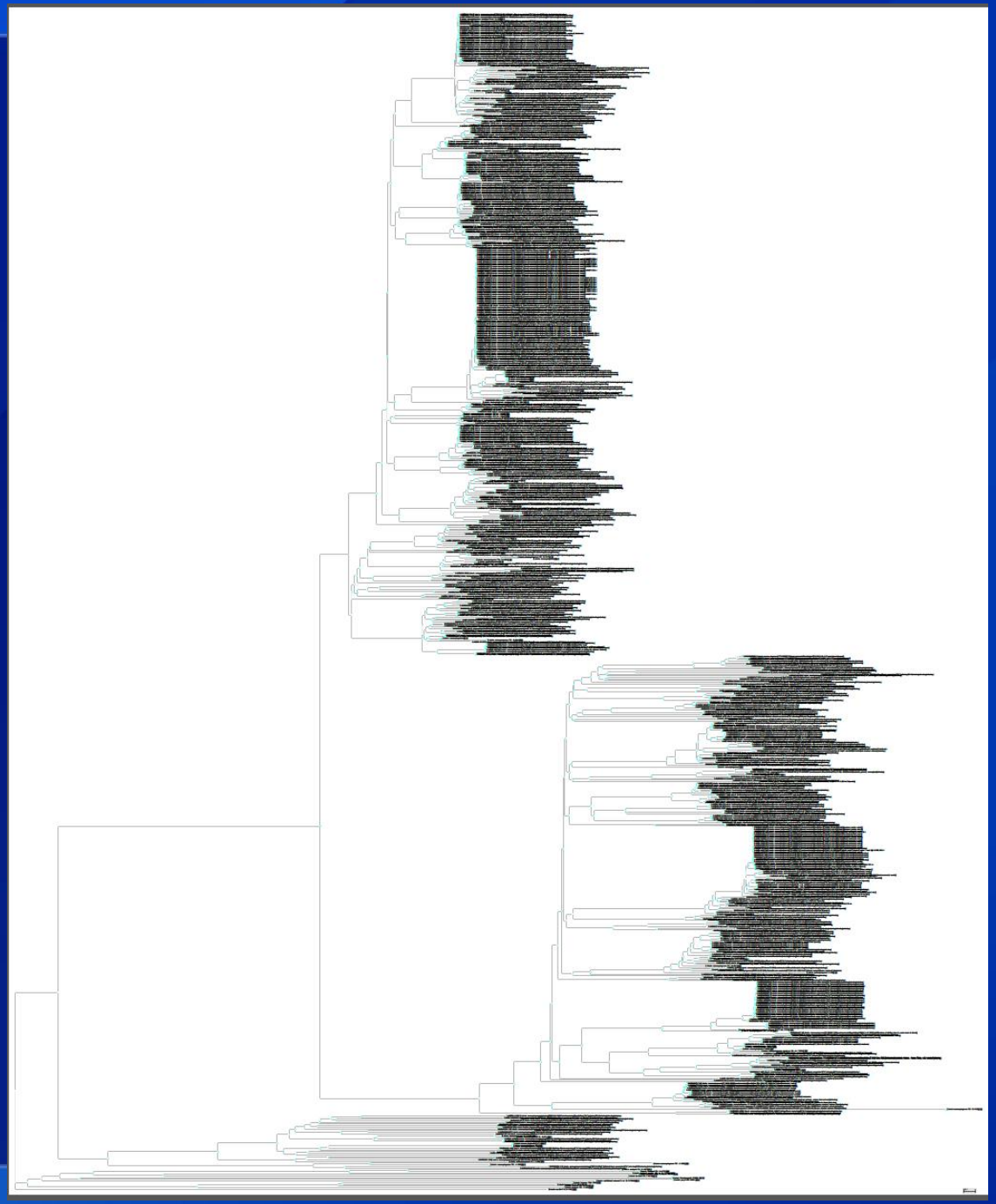
Kmer Tree

- Environmental and food samples that FDA collected at Crave Bros., 2010-2013
- Clinical, Crave Bros. 2013
- Implicated food, Crave Bros., 2013
- New clinical isolates sequenced after closing out the Crave Bros. outbreak



Kmer Tree from NCBI

- As more isolates added to the tree it becomes more difficult to identify clusters



Caveats to K-mer analysis

Advantages:

- Does not require a reference or multiple sequence alignment
- Relatively fast analysis
- Does not require assembly

Disadvantages:

- K-mer analysis does not provide information about where in the genome the differences are
- Does not consider sequence quality*
- Does not provide a true phylogenetic relationship
- Does not lead to strain type nomenclature

SNP Analysis Terms

- **Single Nucleotide Polymorphism (SNP)**

  ATGTTCCTC sequence

  ATGTTGCTC reference

  *phylogentically informative differences

- **Insertion or Deletion (Indel)**

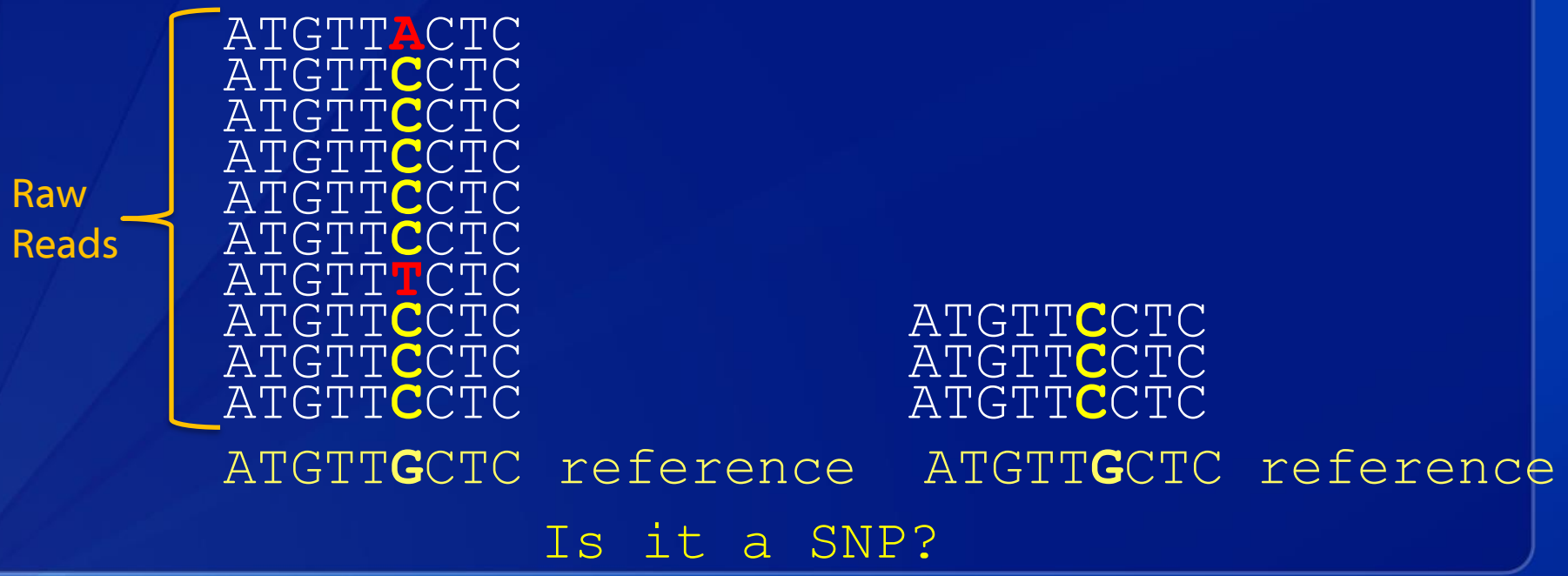
  ATGTTCCCTC sequence

  ATGTTC-CTC reference

  *differences not used in hqSNP analysis

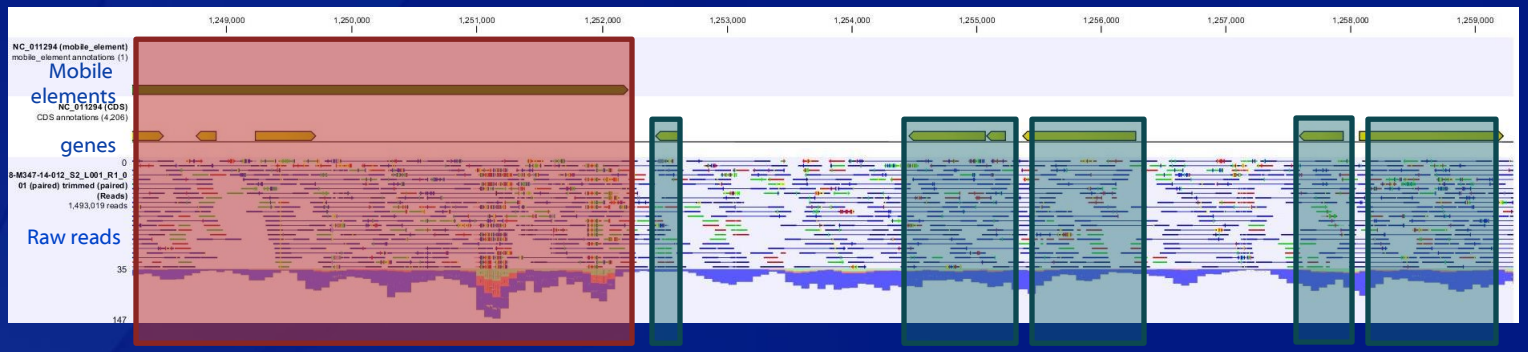
Ways to perform SNP Analysis

- **Reference-based SNP calling**
  - High quality SNP (hqSNP)
  - Raw reads are mapped to a highly related reference
  - Called based on coverage and read frequency at SNP location
  - Shows the phylogenetic relationship



Where to call SNPs

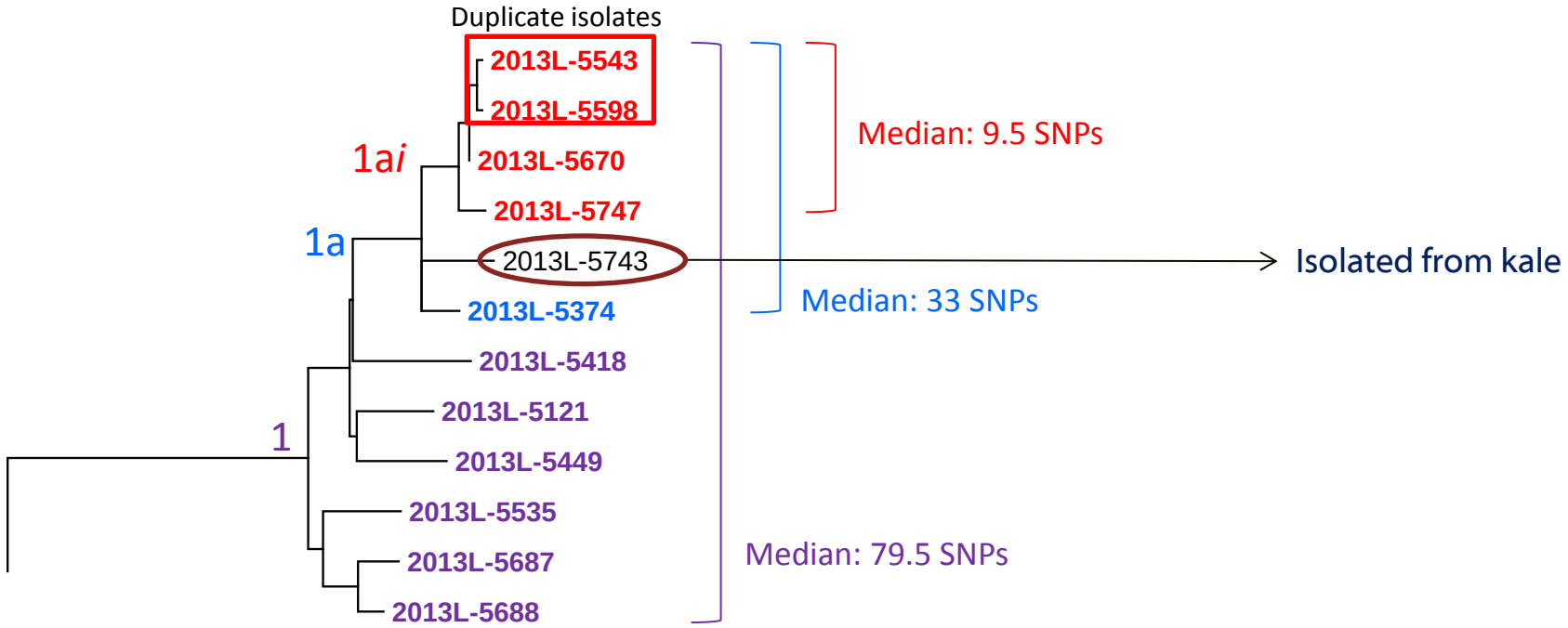
- **Focusing on different part of the genome will give you different SNP counts**
  - Can look at SNPs in whole genome, in core genes only, or even mask part of the genome and not consider any SNPs found there.
- **Different SNP calling pipelines will give you different SNP counts based on thresholds and where they call SNPs**

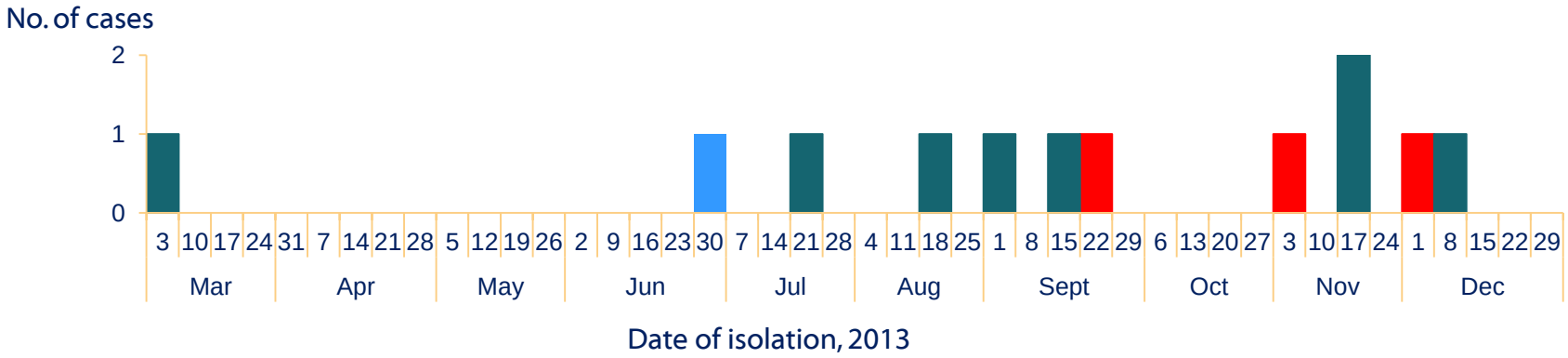


Mask mobile elements
-do no consider SNPs in this location

Only call SNPs in genes

Cluster 1 (1312MLGX6-1): Discriminatory Power





Caveats to hqSNP Analysis

Advantages:

- **Phylogenetically informative**
- **SNP position can be identified on genome to determine what gene or intragenic region contains the SNP**

Disadvantages:

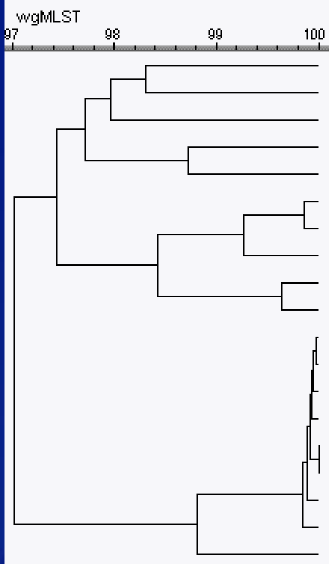
- **Requires a closed reference or good draft genome**
  - Recent closed references from all serotypes are not available
- **Computationally costly**
  - Requires multiple sequence alignment to a reference
- **Does not lead to strain type nomenclature**

Whole Genome MLST (wgMLST)

- Compare gene content between different isolates (can compare over 3000 genes in *Listeria*)
- 1 or more differences (SNP or indel) equal to a new allele name
- Can categorize genes into subgroups: virulence profiles, serotypes, antimicrobial resistance determinants, housekeeping gene MLST, ribosomal MLST, core genome MLST, etc.
- Software like BIGSdb and BioNumerics 7.5 can run these analyses

Locus 1	ACTAGAGGGAAA	ACTAGAGGCTAA	ACT-GAGGGTAA
	allele 1	allele 2	allele 3

wgMLST Tree



wgMLST

LMO_1	LMO_2	LMO_3	LMO_4	LMO_5	LMO_6	LMO_7	LMO_8	LMO_9	LMO_10	LMO_11	LMO_12	LMO_13	LMO_14	LMO_15	LMO_16	LMO_17		Outbreak
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1312MLGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1312MLGX6-1
11	1		17	9	2	10	24	10	16	13	19	20	15	1	10	1	✓	1312MLGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1312MLGX6-1
11	1		17	9	2	10	14	5	8	13	3	4	5	1	10	1	✓	1312MLGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1312MLGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1312MLGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1312MLGX6-1
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11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1307MNGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1307MNGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1307MNGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1307MNGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1307MNGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1307MNGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1307MNGX6-1
11	1		17	9	2	10	14	10	16	13	19	20	15	1	10	1	✓	1307MNGX6-1
11	1		17	9	2	10	14	5	8	13	3	4	5	1	10	1	✓	1307MNGX6-1

*wgMLST tree made for Crave Bros and 1312MLGX6-1 cluster highlighting what the dendogram looks like and the different allele calls

Caveats to wgMLST Analysis

Advantages:

- **Phylogenetically informative**
- **All subtyping genes, virulence genes, and antibiotic resistance genes are pulled out as part of the analysis**
- **Can create a standardized nomenclature based on allele calls**

Disadvantages:

- **Computationally costly to initially assign alleles**
- **Comparing character data not genetic data**
  - SNPs and indels treated equally
  - No difference between 1 or more SNP or indel differences in naming an allele

Concluding remarks for WGS

- **Opportunities**
  - Universal high resolution subtyping method
  - All information currently obtained by traditional methods contained in the sequence data
    - Can use to identify serotype, virulence genes, resistance genes, etc
    - Huge savings opportunity by replacing traditional methods with NGS
- **Challenges**
  - Large amounts of data presents storage and analysis issues
  - Currently no standardization for quality metrics or analysis pipelines
  - Backwards comparability of WGS data with PFGE difficult to establish
  - Interpretation of data – how to define clusters?

Questions?

***Next Seminar series in April will cover the different next generation sequencing platforms**

For more information please contact Centers for Disease Control and Prevention
PulseNet/CDC
1600 Clifton Road NE, Atlanta, GA 30333

E-mail: pfge@cdc.gov Web: http://www.cdc.gov/pulsenet

- Resources:

Program	What for?	Where to find it	Cost?	Platform
BioNumerics 7.x	Assembly, wgMLST, SNP analysis	http://www.applied-maths.com/	Yes	Windows
CLC Bio Genomics Workbench	Workflows, read metrics, assemblies, etc, SNP analyses	http://www.clcbio.com/products/clc-genomics-workbench/	Yes	Windows/ Linux
Geneious	Assemblies, trees, SNP analysis	http://geneious.com/	Yes	Windows
MEGA5	Phylogenies	megasoftware.net/ SRT	No	Windows
Lasergene	Assemblies, read metrics, analysis	http://www.dnastar.com/	Yes	Windows
Genome Workbench	Viewing trees, analysis	http://www.ncbi.nlm.nih.gov/tools/gbench/	No	Windows/ Linux
CG-Pipeline	Assembly, read metrics, assembly metrics, read cleaning, etc	sourceforge.net/projects/cg-pipeline	No	Linux
Snp Extraction Tool	Creating Phylogenies	github.com/lskatz/lyve-SET	No	Linux

* List of some of the tools we have experience with and are available to use at CDC