WHOLE GENOME SEQUENCING TECHNOLOGY IN PUBLIC HEALTH LABORATORIES

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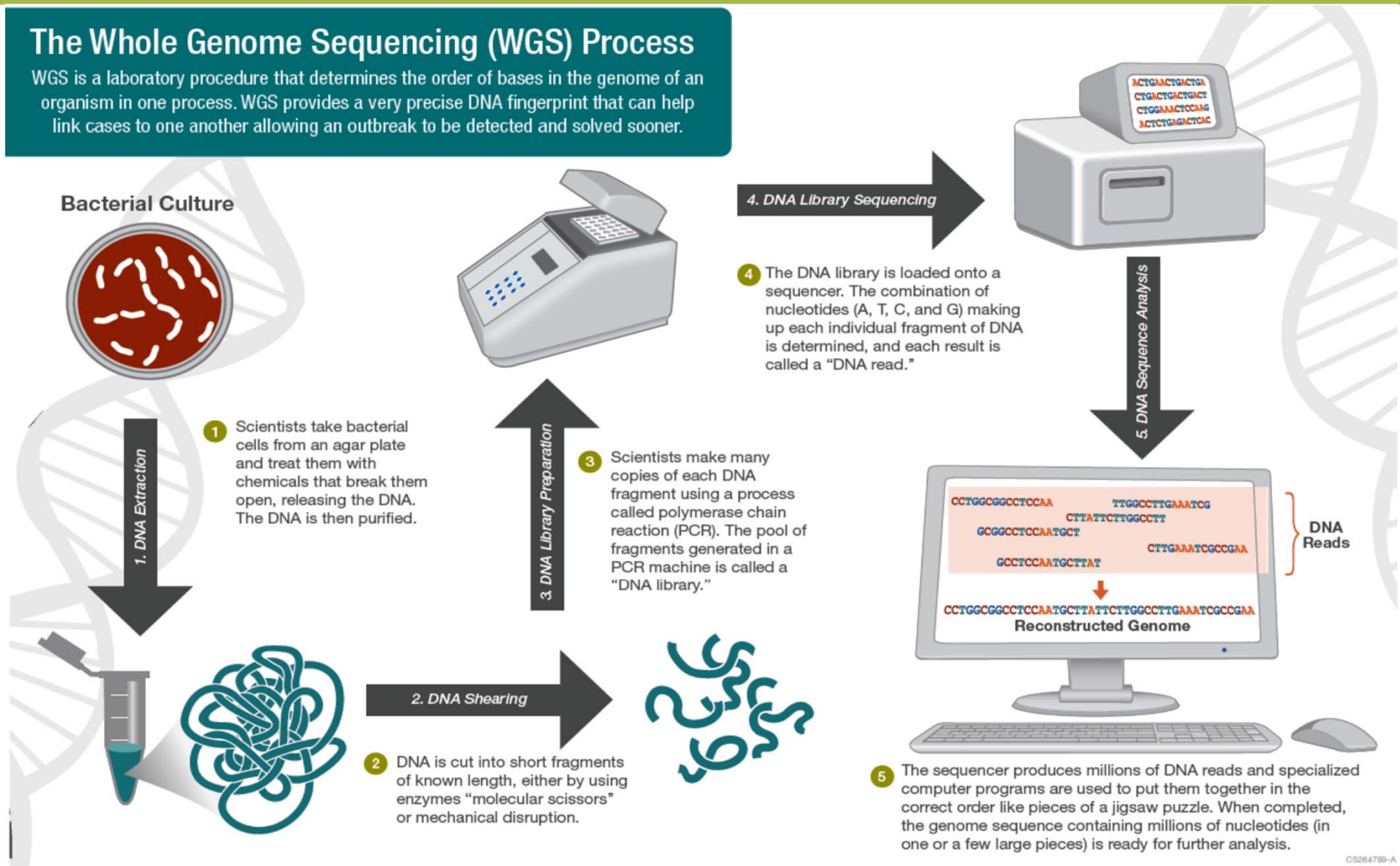
OUTLINE

- Discuss the principles and workflow of whole genome sequencing (WGS)

- Understand how WGS is applied in public health laboratories

- Report how some public health laboratories are utilizing WGS

- Understand the strengths and weakness of WGS

- Understand the opportunities and threats of WGS

- Summarize the WGS capacity in California

LIVE WELL SAN DIEGO

The Whole Genome Sequencing (WGS) Process

WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.

Bacterial Culture

1. DNA Extraction

1 Scientists take bacterial cells from an agar plate and treat them with chemicals that break them open, releasing the DNA. The DNA is then purified.

2. DNA Shearing

2 DNA is cut into short fragments of known length, either by using enzymes "molecular scissors" or mechanical disruption.

3. DNA Library Preparation

3 Scientists make many copies of each DNA fragment using a process called polymerase chain reaction (PCR). The pool of fragments generated in a PCR machine is called a "DNA library."

4. DNA Library Sequencing

4 The DNA library is loaded onto a sequencer. The combination of nucleotides (A, T, C, and G) making up each individual fragment of DNA is determined, and each result is called a "DNA read."

5. DNA Sequence Analysis

CCTGGCGGCCTCCAA   TTGGCCTTGAAATCG
                       CTTATTCTTGGCCTT
GCGGCCTCCAATGCT
                       CTTGAAATCGCCGAA
GCCTCCAATGCTTAT

DNA Reads

CCTGGCGGCCTCCAATGCTTATTCTTGGCCTTGAAATCGCCGAA
Reconstructed Genome

5 The sequencer produces millions of DNA reads and specialized computer programs are used to put them together in the correct order like pieces of a jigsaw puzzle. When completed, the genome sequence containing millions of nucleotides (in one or a few large pieces) is ready for further analysis.

CS264789-A

Picture provided by cdc.gov

WGS APPLICATIONS

- Determine **environmental exposure** from organisms such as *Legionella sp.*, *Coccidiodes sp.*, *Cryptosporidium sp.* (ie. Legionellosis outbreak in New York City)

- Determine transmission pathways of **healthcare associated infections (HAIs)** and use to prevent spread in facilities (ie. CRE outbreak in Los Angeles)

- Predicting how pathogens will become **resistant,** track emerging and spreading resistance (ie. *C.auris*, CRE, CRPA, ESBL, *N. gonorrhoeae*, *S. pneumonia*, *M. tuberculosis*)

cdc.gov

WGS FOR CDC'S PULSENET

- For over 20 years, PulseNet, the national laboratory network that detects foodborne disease outbreaks, has prevented illness and saved money.

- Network has the ability to connect the dots between sick people and sources of infection.

- $500 million saved annually by PulseNet's detection of outbreaks and prevention of foodborne illness

- More outbreaks found and solved after 3 years of using WGS for routine surveillance of Listeria

- CDC has transitioned PulseNet detection method from PFGE to WGS July 2019

cdc.gov

INFECTIOUS DISEASE LABORATORY BRANCH, CALIFORNIA DEPARTMENT OF PUBLIC HEALTH

- Implemented Sequencing in 2015 for MDL and 2016 for VRDL

- Types of organisms or participation of programs

  - PulseNet (*Salmonella sp.*, *Shigella sp.*, *E.coli*, *Yersinia sp.*, *Vibrio sp.*, *Listeria sp.*), C. botulinum, Legionella sp., M. tuberculosis

  - Influenza, norovirus, sapovirus, enterovirus, rhinovirus, adenovirus, hepatitis A, measles, hantavirus

- Challenges with Implementation

  - Financial Support

  - IT infrastructure

  - Bioinformatics and access to pipelines for data analysis

  - Staff training/technical expertise

- Changes to Testing or Organization Structure

  - Additional laboratory space and staff re-aligned to include sequencing in their duties

CALIFORNIA LOCAL PUBLIC HEALTH LABORATORIES

	OCPHL	LAPHL	APHL	SCPHL	SDPHL
Implemented	2016	2017	2016	2016	2017
Organisms	PulseNet	• PulseNet • GAS • HAIs	• All bacteria • HAIs • MTBC	PulseNet	• PulseNet • GHOST (HAV, HCV)
Challenges	• IT • Bioinformatics	• Financial support • Bioinformatics • Training	• Bioinformatics • Training • Validation	• IT • Bioinformatics	• IT • Bioinformatics • Training
Impact/ Change to Laboratory	• Automation • Reporting • Staff is cross-trained	• None	• None	Workflow	None

OCPHL=Orange County PHL, LAPHL=Los Angeles County PHL, APHL= Alameda PHL, SCPHL=Santa Clara PHL, SDPHL= San Diego PHL

- Higher resolution of identification and resistant genes

- Ability to potentially detect pathogens or develop new assays not detectable by current targeted diagnostic assays

- Staff gain high complexity technical expertise

- Staff is empowered to learn new technology

- Analyze data utilizing a universal method

- Capability is not offered in most clinical facilities

- Executive support

- Strong network in California and US

- Many investigations can be eliminated saving money and time

- WGS is a timely method to determine predicted antimicrobial susceptibility saving time and patient health

SWOT-WEAKNESSES

- Expensive

- IT infrastructure hurdles

  - Data management

  - Data sharing and streaming

  - Utilizing other analytical web-based resources

- Staffing constraints and training

- Bioinformatics expertise or support

- Not standardize

- Difficult to analyze and interpret data

- Difficult to transfer knowledge

SWOT-OPPORTUNITIES

- Automation of processing workflow

- PHL network collaborations

- Eliminate traditional tests

- Expand to other organisms

  - Healthcare associated infections

  - Environmental exposures

  - Antimicrobial resistant organisms

- Expand into metagenomics allowing for improved turn around time

- Streamline and improve surveillance and investigations

- Reduce time for genotyping and susceptibilities

- Work with external entities that have resources to bioinformatic experts and can perform analysis on data produced for FREE as an alternative to recruiting bioinformaticians that require high salary to be competitive

SWOT-THREATS

- Difficult to recruit qualified bioinformaticians

- CLIA regulations, no guidance for new technology

- Non-conformity of protocols to compare nationwide

- Can lose classical microbiology expertise

- Lack of support for WGS activities

- Other labs may want to perform sequencing

- Fast turn-over of technology

- Incomplete understanding of genomic content, gene expression, and pathogenesis

SAN DIEGO COUNTY HEPATITIS A OUTBREAK SUCCESS
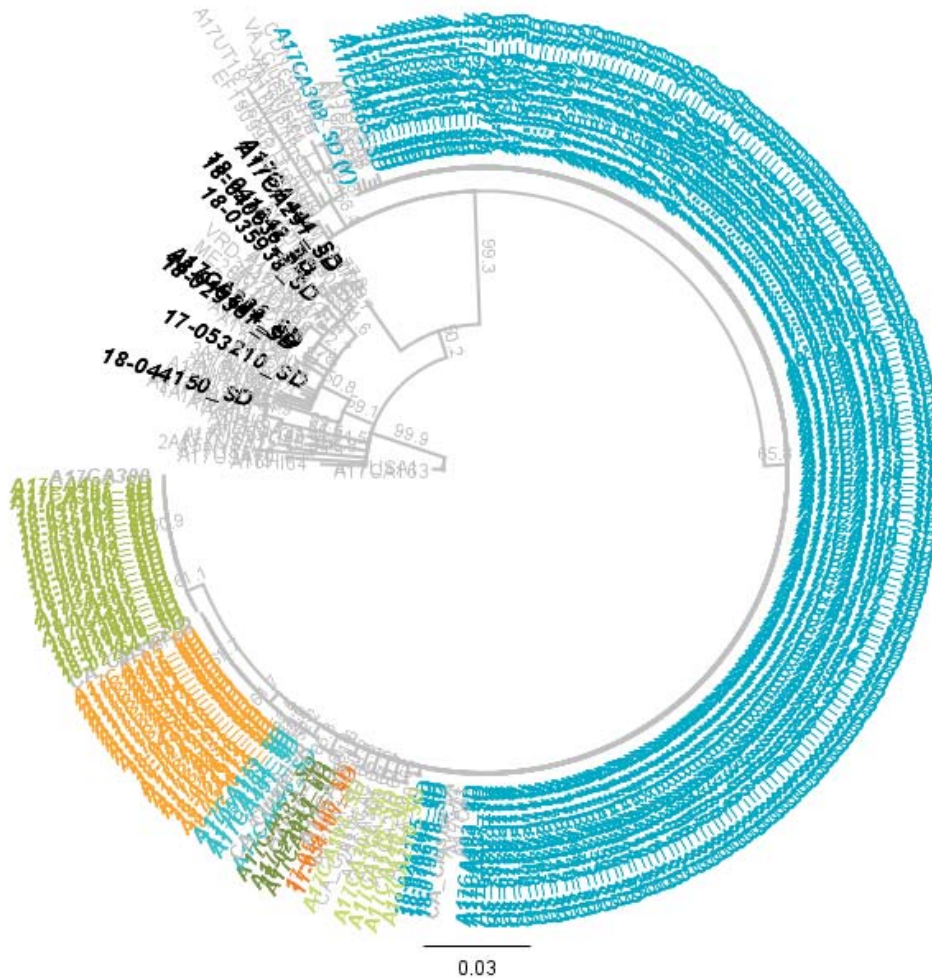
Tree Legend

- IB; CA Cluster A
- IB; Unique Strains
- IB; A17CA79
- IB; A17CA232
- IB; CA Cluster B
- IB; CA Cluster D
- IB; A17CA300
- IA
- Reference Sequences

- The financial investment to utilize WGS can be high. However, if supported well by administration, the return on investment is much higher saving time, money, and increasing the protection of the health in the community

- IT infrastructure and bioinformatics support is immensely helpful and allows public health laboratories improve the output that can be provided

- The strengths and opportunities of WGS has and will continue to propel public health laboratories to level of high functionality

- Utilizing WGS, public health laboratories are no longer behind the curve. We can do as much and more as other leading research institutions

- Public health laboratories with WGS capabilities have access to desirable specimens and data to answer the important and compelling questions in public health

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