# WGS Implementation & Lessons Learned WA-PHL

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# Agenda

- Background
- Getting started
- FDA vs. CDC protocol
- Challenges
- WA-PHL process
- Lessons learned

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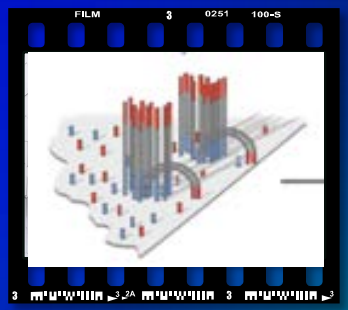
# Timeline

FDA-CFSAN-WAPHL start *Salmonella* sequencing collaborative project



Formal IT support request

Started sequencing

Server established

Analysis software installed


>600 isolates sequenced for FDA, regular clinical isolate testing ongoing

January '13 | March '13 | July '13 | October '13 | April '14 | August '14 | Feb '15

MiSeq arrives

BaseSpace connection


Foodborne outbreak clinical testing begins

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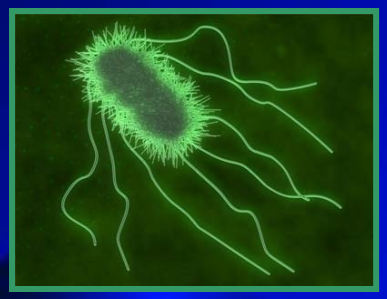

# WGS at WA-PHL

-FDA-directed Genome Trakr Project

- FDA protocol
- Environmental isolates/food (NARMS)

-PulseNet-directed foodborne outbreak collaboration

- AMD resources
- CDC protocol
- Clinical isolates
  - *Listeria*
  - Non-O157 STEC and *E. coli O157*
  - *Salmonella*
  - *Campylobacter*

- WA State PHL WGS Protocol

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# Considerations

- Platform
- IT
  - Communication
  - Ensure IT and the manufacturer know your needs
    - Amount of data transfer
    - Data storage
    - Equipment access/administrator rights
  - Data analysis
    - BioNumerics and/or other software
- Resources
  - People (dedicated personnel vs. scheduled testing)
- Location of equipment
  - Temp control, amplicon contamination, vibrations

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# Getting Started

- Equipment
  - USP surge protector for the sequencer
  - Freezer/refrigerator space
  - Nanodrop for DNA quality
  - Qubit for DNA quantification
  - Magnetic stand-96 by Ambion
  - 96-well shaker (FDA protocol)
  - Centrifuge for 96-well plate (or strip tubes)
  - Phone next to machine (tech support)
- Plan for data storage (e.g. external hard drive)
- Reagents and consumables
- Staff training
- Hazardous waste

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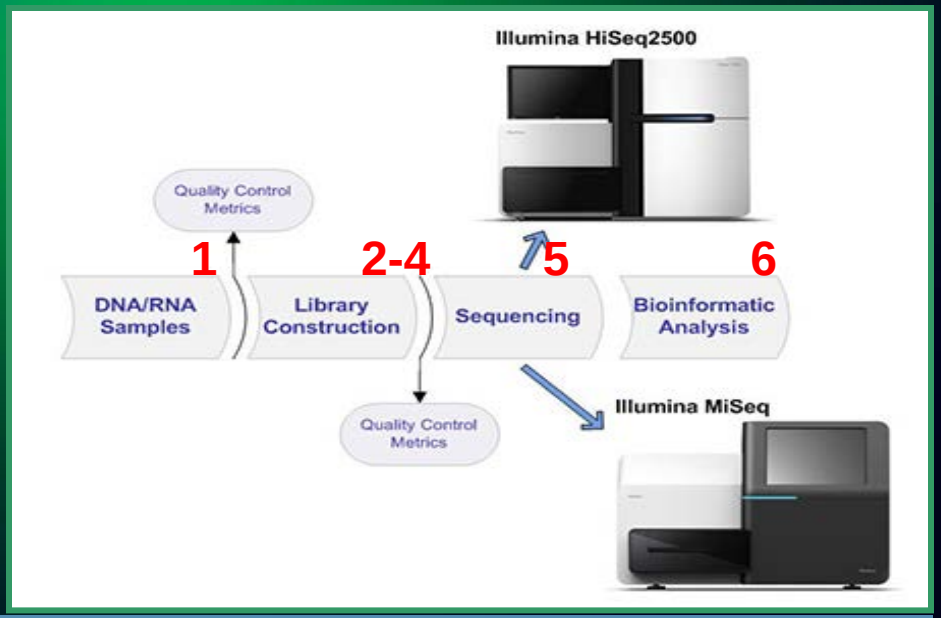
# Overview of WGS

Main steps:

1. Extraction
2. Library Preparation
3. Library normalization
4. Pooling
5. Sequencing
6. Analysis



# Method FDA vs CDC

## Overall goal is quality data

| | FDA | CDC |
|---|---|---|
| **Extraction:** | Gram (+) ~110 min<br>Gram (-) ~105 min | Gram (+) ~120 min<br>Gram (-) ~3-4 hrs |
| **Library prep:** | Similar, ~3 hrs | Similar, ~3 hrs |
| **Library normalization:** | Bead-based, ~2.0 hrs | Dilution-based, ~60 min |
| **Pooling:** | 5 µL ea sample | Based on genome sizes |
| **Sequencing:** | Same, ~40 hrs | Same, ~40 hrs |
| **Analysis:** | hqSNP analysis<br>Kmer | BioNumerics<br>wgMLST |

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# Challenges

- Data Organization
  - Keeping track of WGS identifiers from multiply agencies
  - Duplication of isolates
- Technical Issues
  - MiSeq, BaseSpace, Reagents, Varying results

# Keys to Our Process

- Run scheduling
  - Collaborate with research projects
  - Fill cartridge with historical isolates if not enough currently available
- WA State PHL SOP
  - Clinical and environmental
  - Add PhiX to every run for troubleshooting
- Worksheets
- Preventing Contamination
  - Indices rotation schedule, bleach wash

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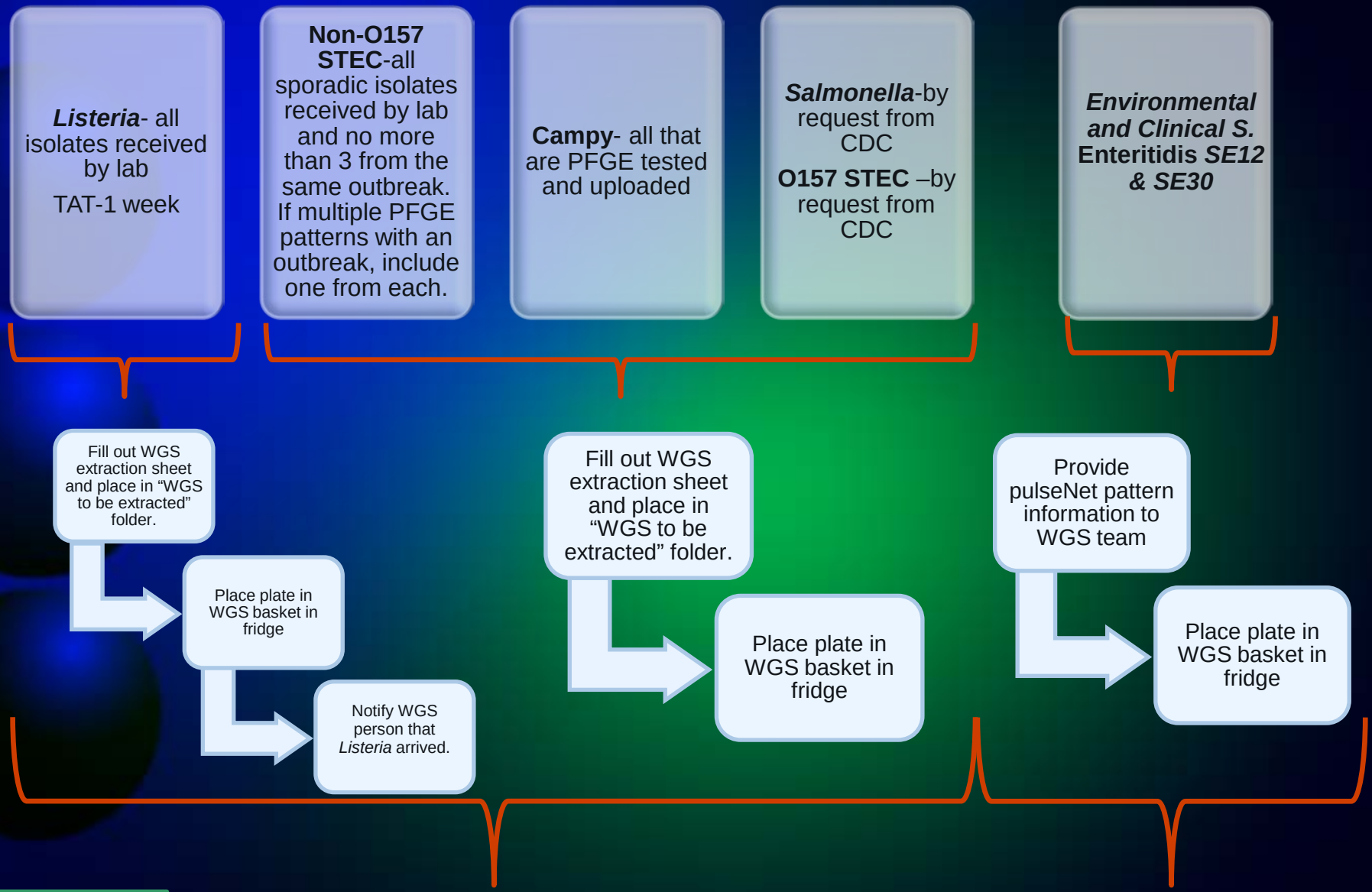
# Submitting WGS Isolates – PFGE Lab Steps

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# WGS – Molecular lab Steps

## CDC Pipeline

**Email PulseNet # to CDC to obtain WGS ID**

## FDA Pipeline

**Assign WAPHL #, provide metadata, request SAMN#**

**Prepare overnight cultures * Perform WGS * Create Report *Verify QC passes* Share BaseSpace project folder**

- Request SAMN# from NCBI with biosample submission form
- PFGE Lab - Upload SAMN#s to BioNumerics & PFGE@CDC.gov
- Data feed back upon request or if issues

- FDA uploads sequences to NCBI

For Clinical samples(SE04 and SE05).

- Share run with NYS DOH Wadsworth.
- Receive analysis information from NY
- Upload accession info (SAMN#/WAPHL) through BioNumerics.
- Email CDC

# Analysis Flow

- Data uploaded to BaseSpace
- Run and/or project shared on BaseSpace
- CDC or FDA picks up from BaseSpace
- CDC or FDA completes the analysis
- WA requests the data analysis from the CDC
- FDA analysis by Wadsworth(clinical Salmonella)
- Wadsworth sends WA updated tree with data analysis
- WAPHL shares data with Epis

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# Lessons Learned

1. Communicate: IT, manufacturer, lab teams…get everyone on board
2. Lay out a plan for isolate flow & tracking

Remember the main goal =

**QUALITY DATA**

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# Acknowledgements


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- NCBI
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- NYS DoH Wadsworth Center
  - Dr. Wolfang and his team


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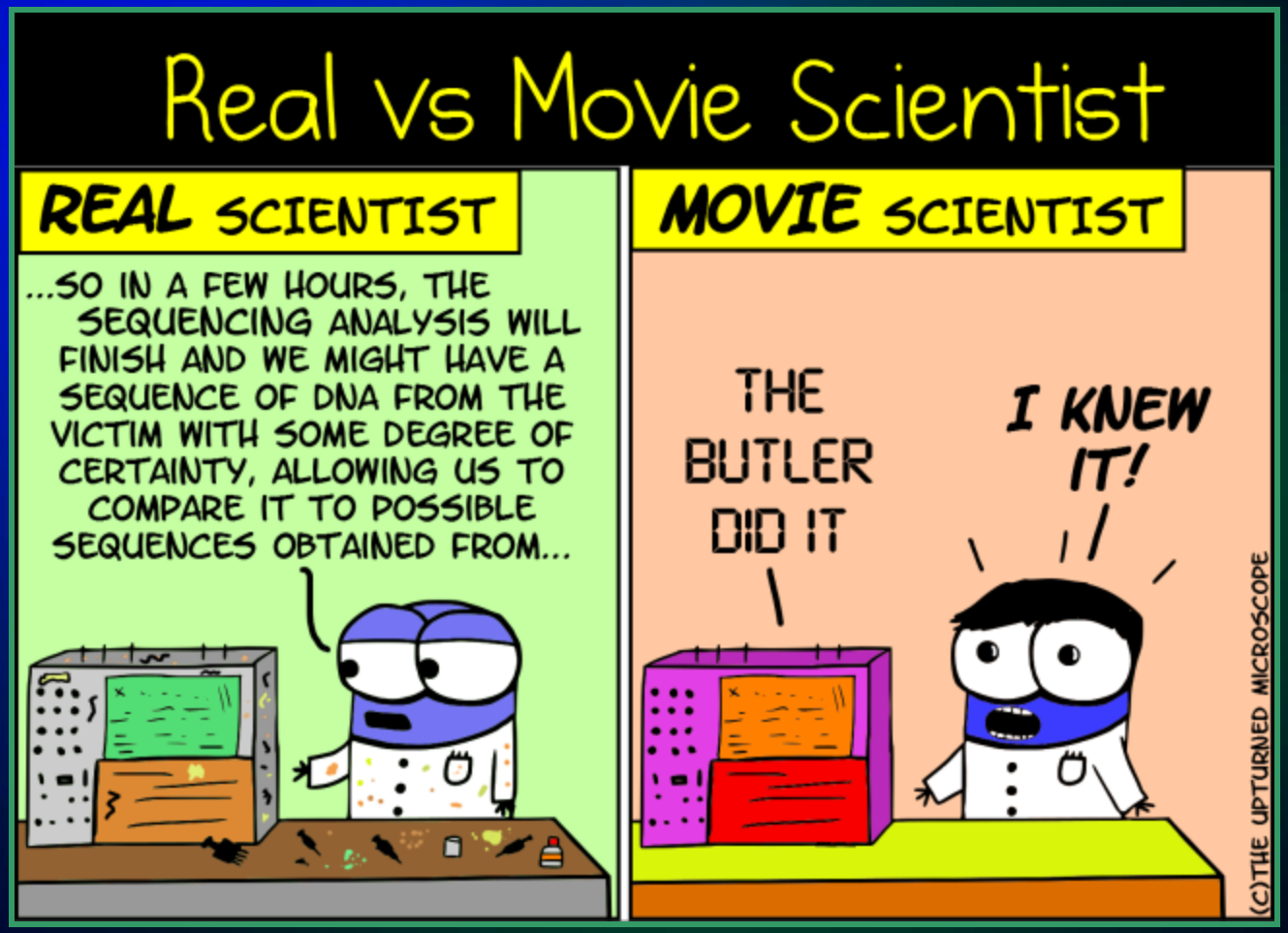
# Questions?

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