Think positive!

Designing synthetic positive controls for the HMAS AR Panel

Jasmine Hensley & Jessica Rowell

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Let’s review!

Purpose for the AR Panel

- Initially the AR Panel started as a proof-of-concept to test the Juno and HMAS
  - Help towards the goal of HMAS for *Salmonella* subtyping
- There is a huge need for culture-independent AR detection
- There is a lot of interest from other groups
  - National Antimicrobial Resistance Surveillance Team (NARST)
  - Waterborne Disease Prevention Branch (WDPB)
  - Division of Healthcare Quality Promotion (DHQP)
  - Division of Sexually Transmitted Disease Prevention (DSTDP)

Summary of the AR Panel

- Original panel was designed by the Lawrence Livermore National Lab (Tom Slezak and co.) for Ion Torrent
- A selection of targets were chosen for relevance to enterics
- Current panel: 749 amplicons targeting 111 genes
- Future panel: Adding primers for additional genes relevant to NARST, WDPB, DHQP, and DSTDP

Genes on current panel*:

Antimicrobial Class	Number of Genes
Aminoglycosides	5
Beta-lactams	46
Macrolides	32
Phenicols	3
Quinolones	14
Tetracyclines	2
Trimethoprims	6

*Includes one representative amplicon from each gene

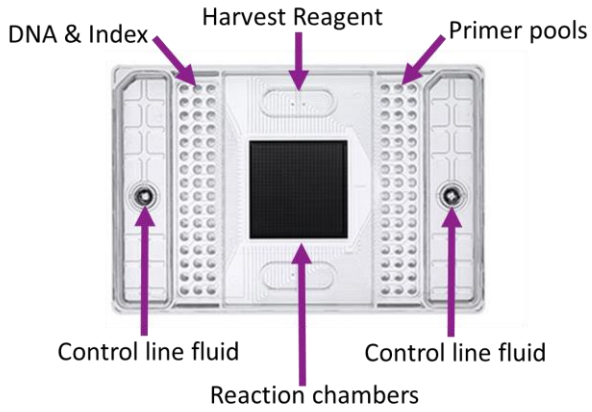
Genes on future panel**:

Antimicrobial Class	Number of Genes
Aminoglycosides	61
Beta-lactams	602
Fosfomycins	1
Glycopeptides	1
Macrolides	31
Oxazolidinones	2
Phenicols	10
Polymyxins (colistin)	52
Quinolones	14
Rifampicins	3
Sulphonamides	53
Tetracyclines	33
Trimethoprims	61

**Includes all priority genes (rated high and medium)

Prepare Samples

Load IFC



Run Juno

Prepare Libraries

Sequence

Analyze Data

What have we been working on?

How do we validate every primer pair?

- For traditional multi-plex assays, you use a positive control to verify function of each primer pair on every run

- This principle should still apply for HMAS, but it's complicated
  - 749 primer pairs covering 111 AR genes
  - Limited space on the IFC

- Furthermore, we want this panel to be useful for PHLs
  - Need uniform controls across all labs

Working with AR genes complicates things

- Option 1:
  - Select dozens of multidrug resistant live bacteria, enough to cover 111 genes
  - EDLB and all partner labs maintain stocks of these MDR bacteria
  - Use a large number of wells on the IFC every run
- Option 2:
  - Design and order a plasmid containing all of our 111 AR gene sequences
  - EDLB and all partner labs now have a super plasmid in their labs
- Option 1 and 2 are both risky, but what is a safer Option 3?

Option 3: Synthetic Positive Controls

- To address our positive control conundrum, we have decided to assess synthetic positive controls
  - Design targets for each primer pair
  - Order as oligo pool
  - Receive uniform, QC'd "positive control"

- If the synthetic positive control works well, each SPHL would be able to order standardized and QC'd pools from the manufacturer as needed

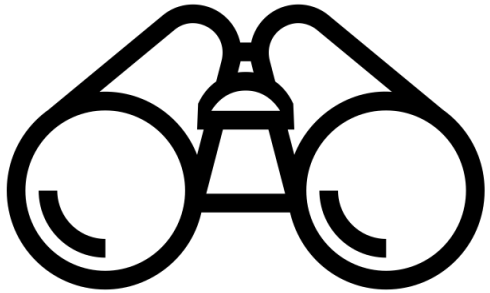
Dry Lab: Synthetic Control Design

Design considerations

✓ The synthetic control should behave like our target in PCR reactions

Same length

Similar GC % (range: 5% less to 10% more)



✓ It look different than our target, so we can distinguish them

✓ It should not destroy the world

We need 749 matching positive controls

749 target amplicons

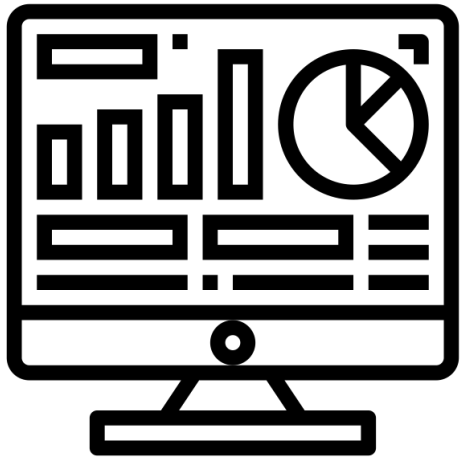
749 positive controls
Matching amplicon length and % GC content

What do our targets look like?

749 primer pairs

Median length = 220 bp (180 bp – 240 bp)

Median % GC content = 47% (23% - 72%)



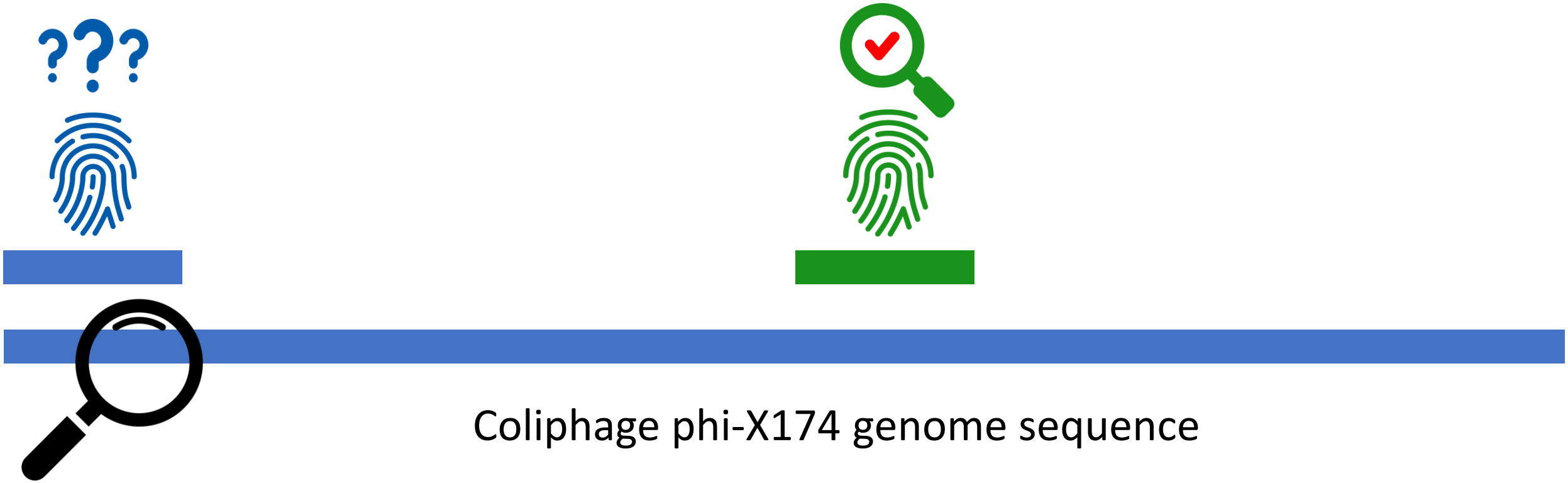
First pass at finding synthetic controls

Amplicon target #1

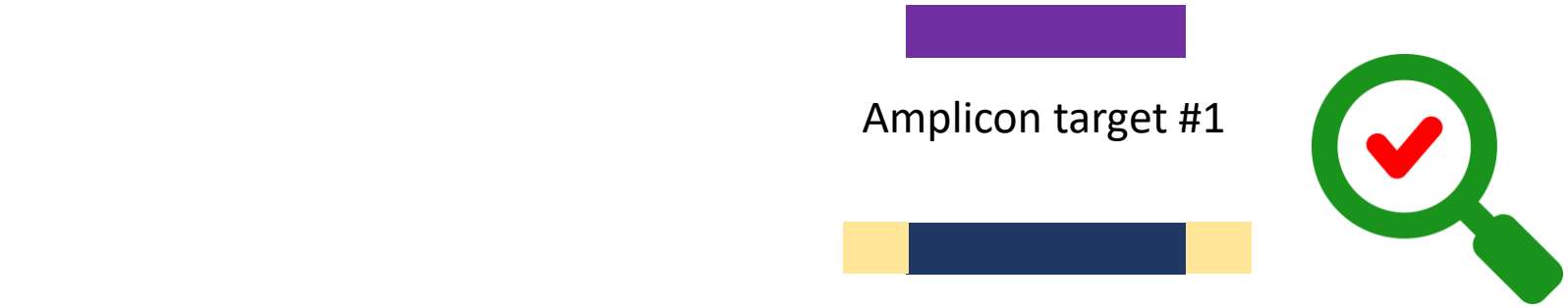
Length
% GC

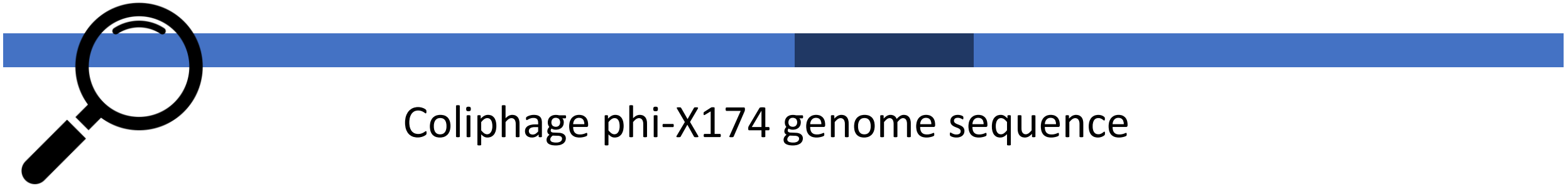
Coliphage phi-X174 genome sequence

Search until we find a matching chunk

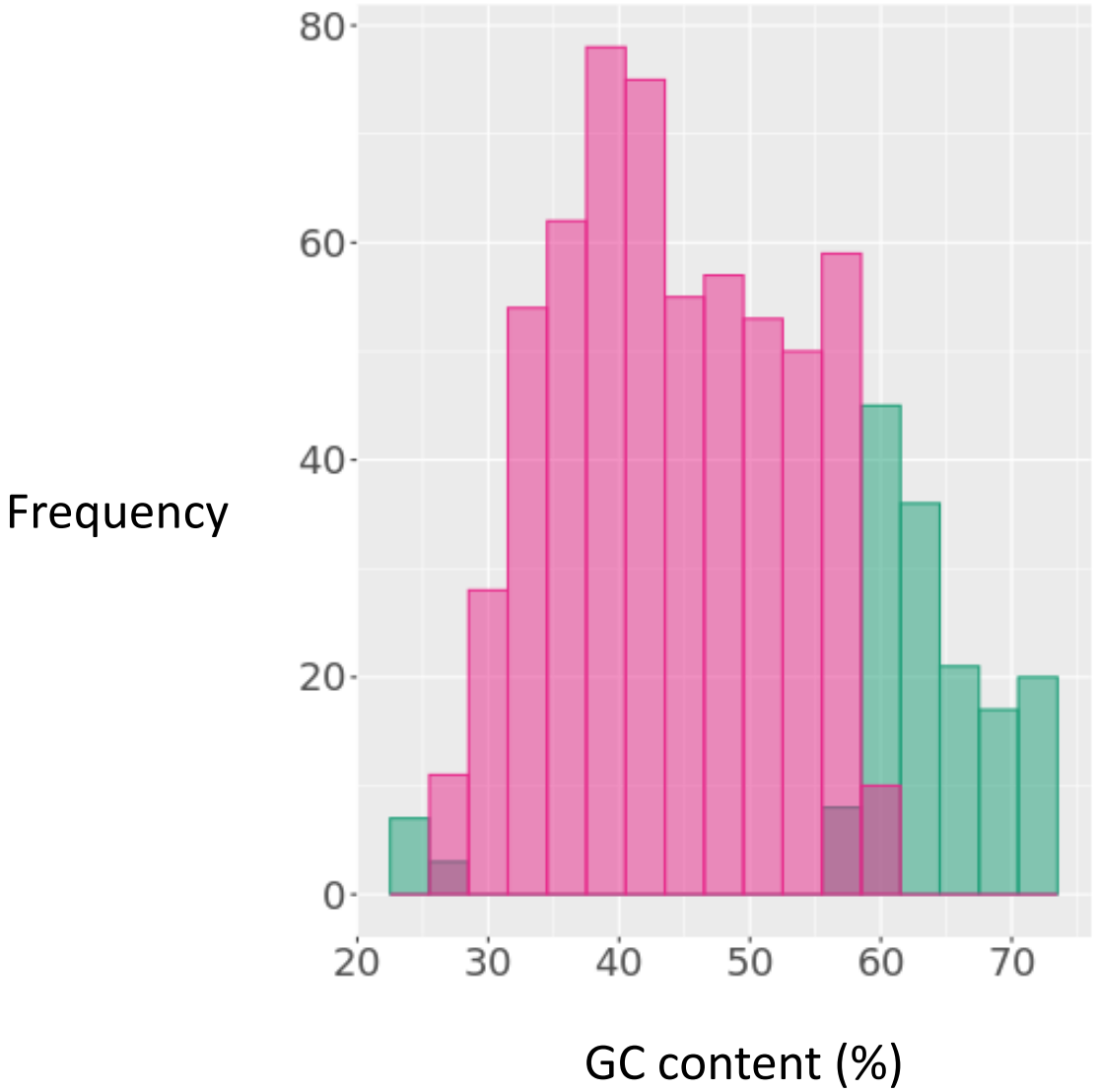


Search until we find a matching chunk





GC content in our targets was problematic



Positive control found

Positive control not found

???

How do we find synthetic controls for those targets with high or low GC content?

Repeat with 2 other reference genomes

Coliphage phi-X174

GC: 45%

Streptomyces coelicolor

GC: 72%

Wolbachia pipientis

GC: 32%

Repeat with 2 other reference genomes

Coliphage phi-X174

592 targets

Streptomyces coelicolor

147 targets

Wolbachia pipientis

10 targets

Wet Lab: Plans for Testing

Plans for testing synthetic controls

- Dilute synthetic control pool to 5 concentrations for testing initially
  - 5 ng/µL
  - 2 ng/µL
  - 0.2 ng/µL
  - 0.02 ng/µL

- Test alongside known real bacterial isolates from the CDC/FDA AR Bank
  - *Enterobacterales* Carbapenemase Diversity Panel
  - Gram Negative Carbapenemase Detection Panel

- Timeline on-hold because of COVID-19, but everything has been received