The Optimization of a Novel qPCR Assay for Brucellosis

UNDERGRADUATE RESEARCH DAY

CORINNE VAUGHAN

VETERINARY SCIENCES

MENTORS: DR. BRANT SCHUMAKER AND NOAH HULL

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Problem Statement

- 1934: State-Federal Cooperative Brucellosis Eradication plan
- Elk and bison in the Greater Yellowstone Area still serve as reservoirs of the disease[1-3]
- 5,000,000 human cases annually[1]
- Two vaccines (RB51- cattle/bison) and S19 (elk)

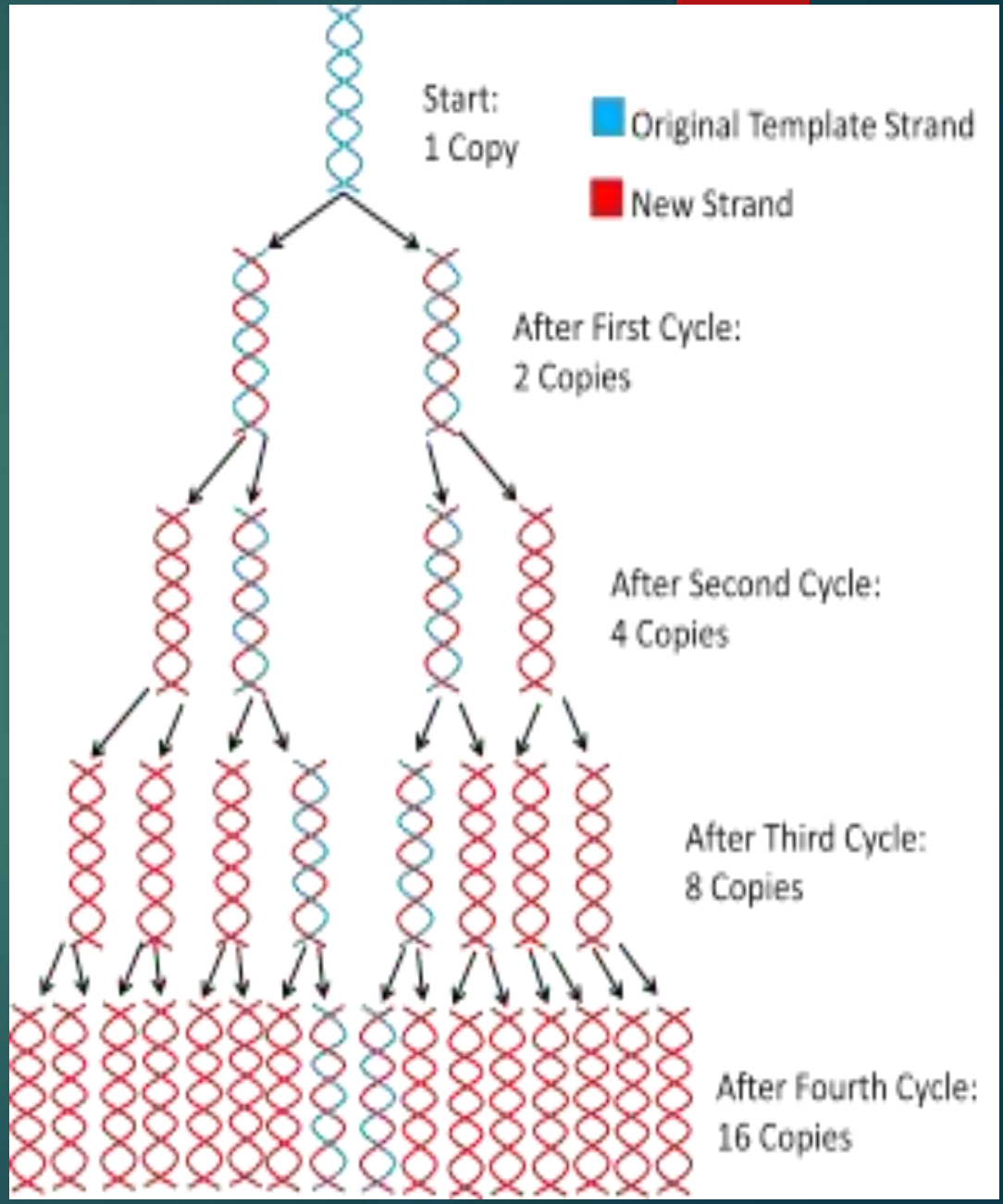
Problem Statement (Continued)

- Current diagnostics:
  - Serologic tests
  - Culture→ "Gold Standard" for diagnosis
- False positives (S19 vaccinates and cross reacting organisms)
- Low bacterial load in samples
- High risk to laboratory personnel **(#1 laboratory acquired infection in world)**

Diagnostic Automation, Inc.

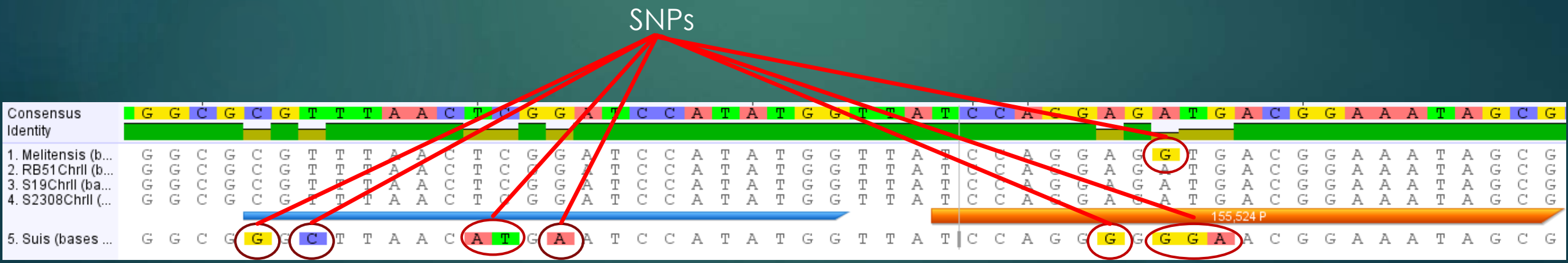
Background

- What is Polymerase Chain Reaction (PCR)?
- Molecular method used in diagnostics
- Steps: DNA Extraction (Tissue/Blood)
  Reaction Mixture
  Run Reaction on PCR Cycler
  Results – *Brucella* DNA is Detected or Not

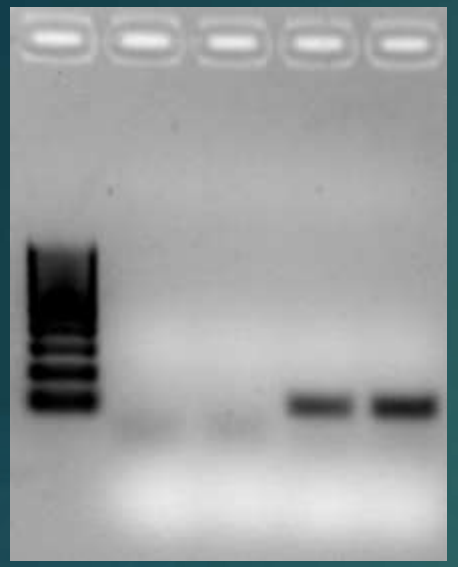


Objectives

- Creation and Validation of a novel qPCR assay including:
  - *In-silico* analysis of 95 whole-genome sequences of *Brucella*
  - Creation of specific primers/probes based on Single Nucleotide Polymorphism (SNP)
  - Optimization of primers/probes
  - Validation on tissues from known culture-positive cattle and bison



Methods



Candidate Primers

Run conventional PCR

Band at expected size?

Yes

Optimize primers on:
- [Primer]
- Template
- Thermal-cycler conditions

Move primer set onto validation

No

Optimize primers on:
- [Primer]
- Template
- Thermal-cycler conditions

Run conventional PCR

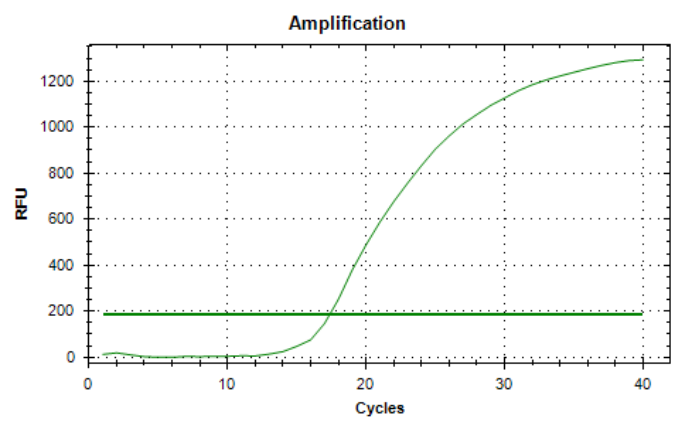
Band at expected size?

Yes

No

Failed Candidate, move to next candidate

Methods (Continued)



Candidate primer/probe

Run conventional PCR with primers only

Band at expected size?

Yes

Optimize primers on:
- [Primer]
- Template
- Thermal-cycler conditions

Run qPCR with primer/probe

Cq Value <40.0?

Yes

Move primer set onto validation

No

Failed candidate, move to next candidate

No

Optimize primers on:
- [Primer]
- Template
- Thermal-cycler conditions

Run conventional PCR

Band at expected size?

Yes

No

Failed candidate, move to next candidate

Results

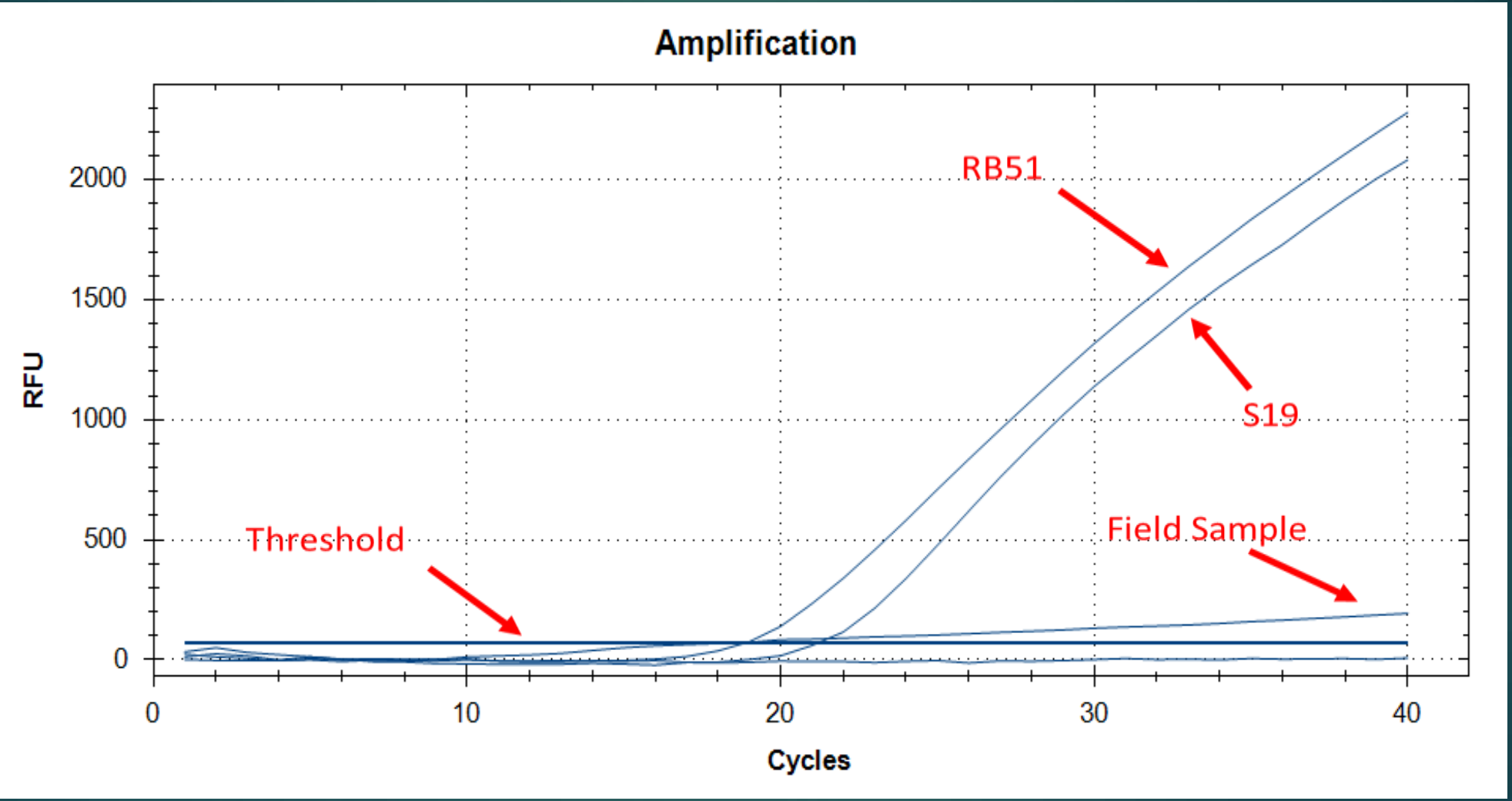
47 Candidate Sets

9 Moved on to validation

38 Eliminated post optimization

- Optimizing nine primer/probe sets
- 38 sets tested and rejected
- Example: 106 base pair set
  - Amplifies field and vaccine strains

Results (Continued)



Results (Continued)

[Primer] (µM)	Sample	Cq Value
10	S19	22.07
20	S19	N/A
30	S19	15.43
40	S19	22.75
50	S19	21
10	RB51	29.07
20	RB51	19.53
30	RB51	18.98
40	RB51	17.35
50	RB51	19.21
10	Field Sample	N/A
20	Field Sample	10.76
30	Field Sample	9.07
40	Field Sample	11.15
50	Field Sample	6.18

N/A = Cq ≥40.0

Results (Continued)

Annealing Temperature (C°)	Sample	Cq Value
64	S19	22.16
64	Field Sample	31.35
64	NTC	39.47
59.4	S19	22.8
59.4	Field Sample	32.18
59.4	NTC	N/A
55.7	S19	22.35
55.7	Field Sample	N/A
55.7	NTC	N/A
53.2	S19	21.41
53.2	Field Sample	N/A
53.2	NTC	N/A
52	S19	21.17
52	Field Sample	N/A
52	NTC	N/A

N/A = Cq ≥40.0

Results (Continued)

Template 63.1 ng/µL	Sample	Cq Value
15.8 ng (0.25 µL)	S19	22.49
15.8 ng (0.25 µL)	Field Sample	N/A
15.8 ng (0.25 µL)	S19	21.74
31.6 ng (0.50 µL)	Field Sample	N/A
31.6 ng (0.50 µL)	S19	21.25
31.6 ng (0.50 µL)	Field Sample	35.38
63.1 ng (1 µL)	S19	20.98
63.1 ng (1 µL)	Field Sample	N/A
63.1 ng (1 µL)	NTC	N/A
126.2 ng (2 µL)	S19	23.72
126.2 ng (2 µL)	Field Sample	14.24
189.3 ng (3 µL)	S19	21.04
189.3 ng (3 µL)	Field Sample	N/A
315.5 ng (5 µL)	S19	18.53
315.5 ng (5 µL)	Field Sample	N/A

N/A = Cq ≥40.0

Discussion

- Primer sets show promise for differentiation→ Field vs. Vaccine strains
- Next step is validation
  - To be conducted on:
    - Known culture-positive tissues
    - Culture-negative but serologically-positive tissues
    - Known cross-reacting organisms
- Potential to replace culture as the "gold-standard" assay

Acknowledgements

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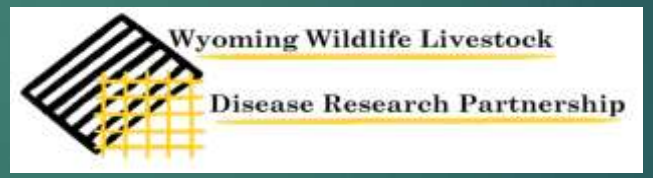

Questions?


Who's the new heifer?
Never seen herbivore.
4.9.12
w/ HARRIS
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References

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