



Southern Gardens Diagnostic Lab

Understanding PCR

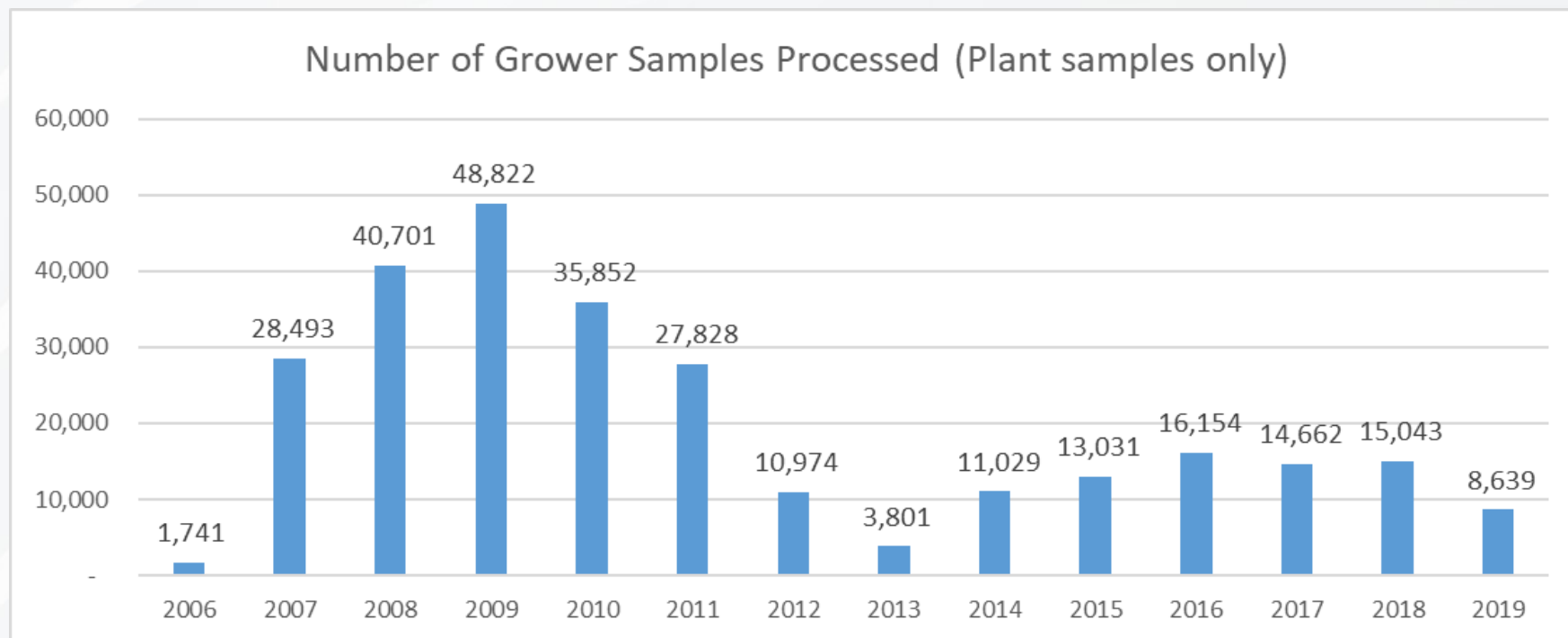
Southern Gardens Diagnostic Lab

- Has been in operation since 2006
- Over 350,000 samples run at no cost to the grower/researcher
- Initially all of the samples were diagnostic samples: either positive or negative
- Now, virtually all are samples that come from research trials
 - Grower, industry, University and Federal
 - Validation of results from other labs

Southern Gardens Diagnostic Lab

- Initially, all costs were covered by Southern Gardens
- Beginning in 2008, FCPRAC and later CRDF, began partial funding of the laboratory
 - Covers 2/3 of the direct costs of the assays
 - SGC pays the other 1/3
 - SGC samples account for about 1/3 of the samples
 - SGC covers all of the indirect costs
- Current budget based on ~25,000 samples per year
 - Grower, ACP, SGC groves, SGC research

Sample volume



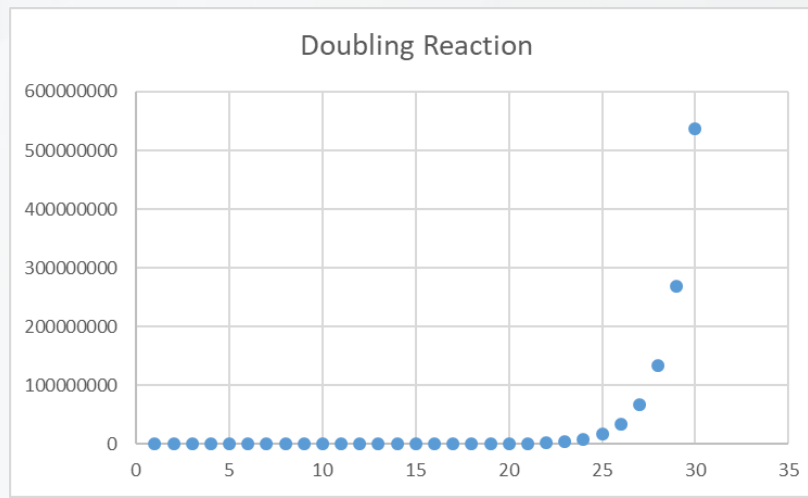
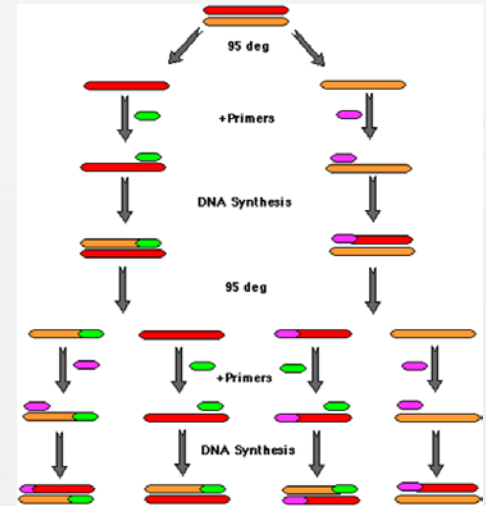
- Only includes outside grower samples (does not include ACP samples or SGC samples)

Results

- Results are reported only to the person that submitted the samples
 - Exception was when HLB was a select agent (reported positives to state and federal agencies)
- Results reported as Ct value and a diagnosis of:
 - HLB Positive
 - HLB Questionable
 - No HLB found
- Verification test (conventional PCR typically only is positive at a Ct of 30-32 or lower)

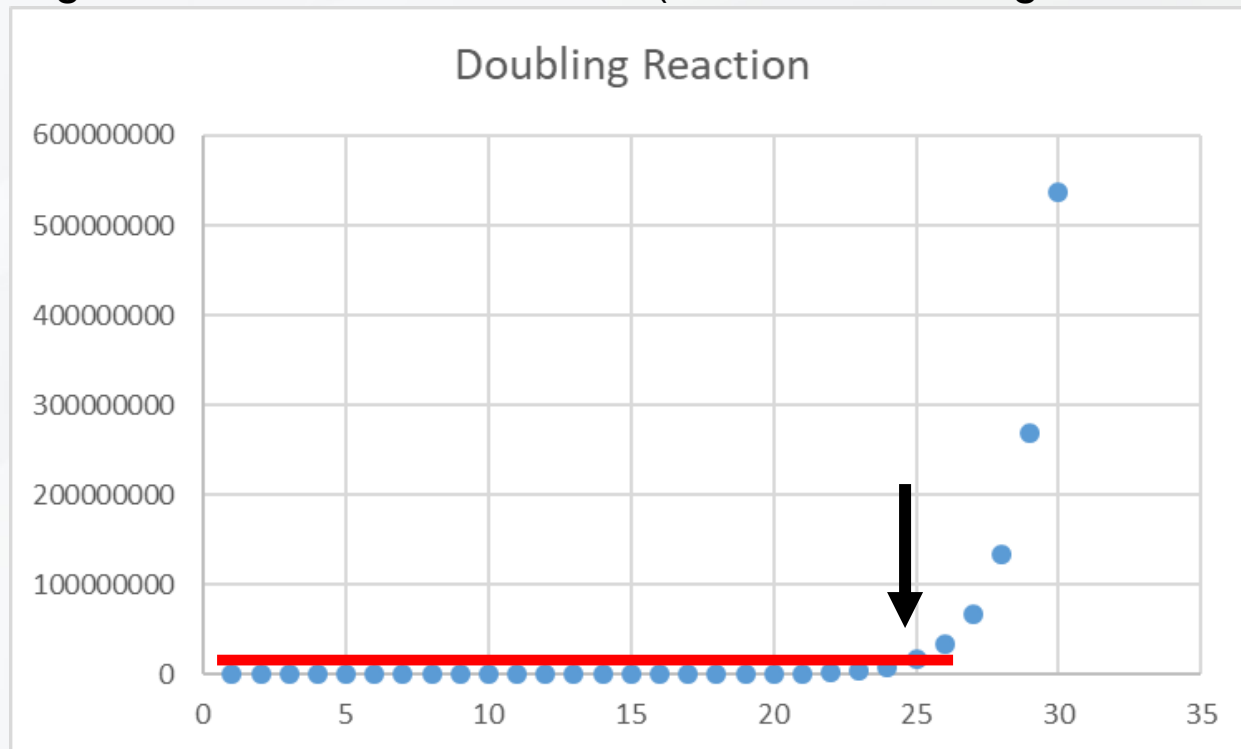
Polymerase chain reaction (real time)

- PCR is an amplification of DNA
 - Exponential doubling
 - qRT-PCR: quantitative
 - Ct value indicative of how much DNA is present in the initial RX
 - Low number means a lot of DNA
 - High number means less DNA

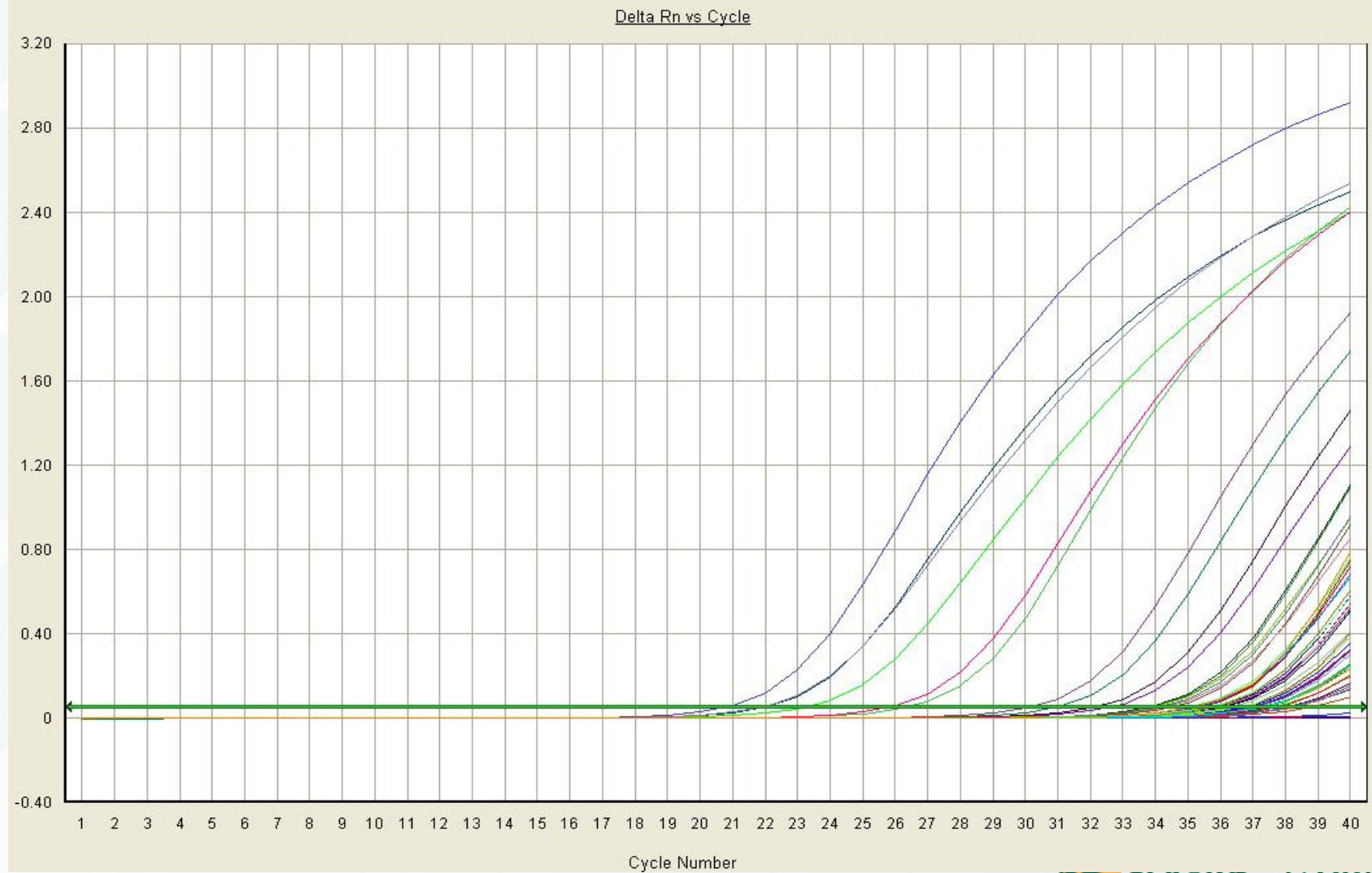


Calculation of Ct value

- Real Time **PCR Ct Values**. What does **Ct** mean? In a real time **PCR** assay a positive reaction is detected by accumulation of a fluorescent signal. The **Ct** (cycle **threshold**) is **defined** as the number of cycles required for the fluorescent signal to cross the **threshold** (ie exceeds background level)



Real Time PCR



Lab Report

HLB Testing Report

Fax:
Email:

Assay Date Range:

4/11/2007 to 4/11/2007

USSC/Southern Gardens Diagnostic Lab

County

| Diagnosis | | HLB Positive | | | | | | | |
|-----------|-----|--------------|-------|-----------|--------|-----------|----------|-----------|----------------------|
| Block | Row | Tree | CT | Local Id: | Lab Id | Collected | Received | Assayed | Sample submitted by: |
| | 5 | 70 | 22.50 | 03-29-63 | 7741 | 3/27/2007 | 4/4/2007 | 4/11/2007 | |
| | 8 | 56 | 25.36 | 03-29-63 | 7746 | 3/27/2007 | 4/4/2007 | 4/11/2007 | |
| | 8 | 58 | 23.14 | 03-26-07 | 7745 | 3/27/2007 | 4/4/2007 | 4/11/2007 | |
| | 11 | 71 | 26.66 | 03-29-63 | 7742 | 3/27/2007 | 4/4/2007 | 4/11/2007 | |

| Diagnosis | | No HLB Found | | | | | | | |
|-----------|-----|--------------|-------|-----------|--------|-----------|----------|-----------|----------------------|
| Block | Row | Tree | CT | Local Id: | Lab Id | Collected | Received | Assayed | Sample submitted by: |
| | 1 | 82 | 33.83 | 03-09-29 | 7743 | 3/27/2007 | 4/4/2007 | 4/11/2007 | |
| | 12 | 74 | 39.87 | 03-29-24 | 7740 | 3/27/2007 | 4/4/2007 | 4/11/2007 | |

| Diagnosis | | Questionable | | | | | | | |
|-----------|-----|--------------|-------|-----------|--------|-----------|----------|-----------|----------------------|
| Block | Row | Tree | CT | Local Id: | Lab Id | Collected | Received | Assayed | Sample submitted by: |
| | 3 | 19 | 31.33 | 03-29-37 | 7744 | 3/27/2007 | 4/4/2007 | 4/11/2007 | |

Number Positive 4

Number Questionable 1

Total samples 7

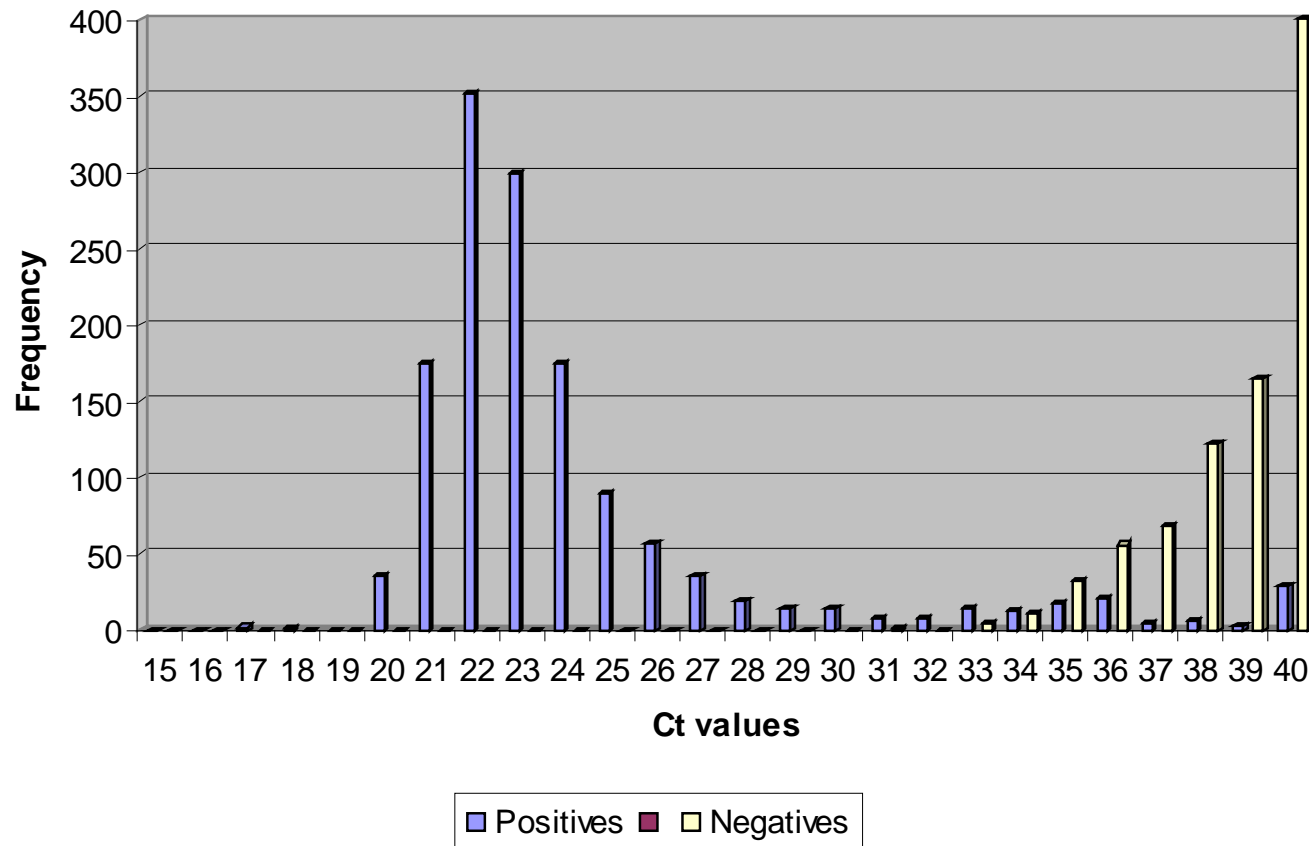
Total Positive (+ and questionable) 71.4%

CT < 30 Positive
CT between 30-32 Questionable
CT > 32 No HLB Found

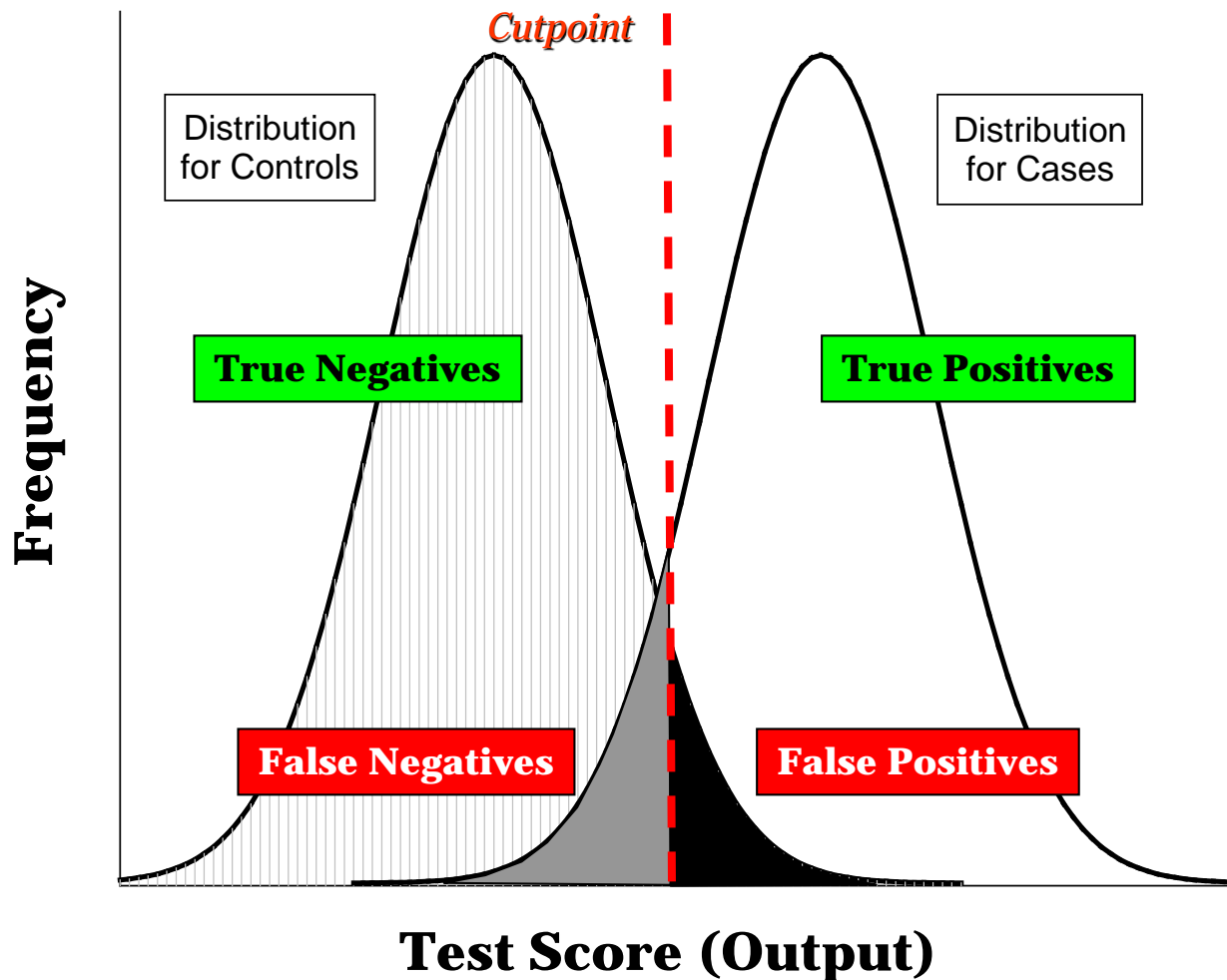


Threshold Determination

Distribution of CT values

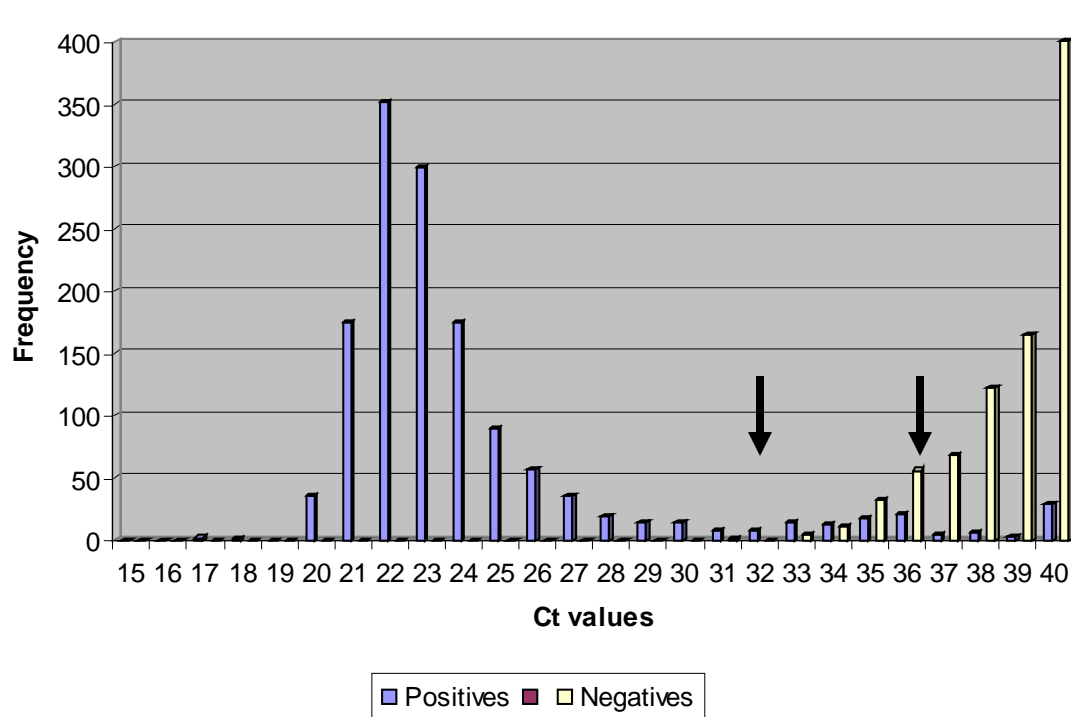


Cases vs. Controls



Threshold Determination

Distribution of CT values



| CT | Specificity | 1-Specificity | Sensitivity | J |
|----|-------------|---------------|-------------|--------|
| 23 | 1.0000 | 0.0000 | 0.62347 | 0.6235 |
| 24 | 1.0000 | 0.0000 | 0.7473 | 0.7473 |
| 25 | 1.0000 | 0.0000 | 0.810655 | 0.8107 |
| 26 | 1.0000 | 0.0000 | 0.852412 | 0.8524 |
| 27 | 1.0000 | 0.0000 | 0.87761 | 0.8776 |
| 28 | 1.0000 | 0.0000 | 0.891289 | 0.8913 |
| 29 | 1.0000 | 0.0000 | 0.901368 | 0.9014 |
| 30 | 1.0000 | 0.0000 | 0.912167 | 0.9122 |
| 31 | 0.9998 | 0.0002 | 0.917207 | 0.9170 |
| 32 | 0.9998 | 0.0002 | 0.922246 | 0.9220 |
| 33 | 0.9989 | 0.0011 | 0.933045 | 0.9319 |
| 34 | 0.9964 | 0.0036 | 0.942405 | 0.9388 |
| 35 | 0.9891 | 0.0109 | 0.954644 | 0.9437 |
| 36 | 0.9763 | 0.0237 | 0.969762 | 0.9461 |
| 37 | 0.9606 | 0.0394 | 0.972642 | 0.9333 |
| 38 | 0.9329 | 0.0671 | 0.976962 | 0.9098 |
| 39 | 0.8953 | 0.1047 | 0.979122 | 0.8745 |

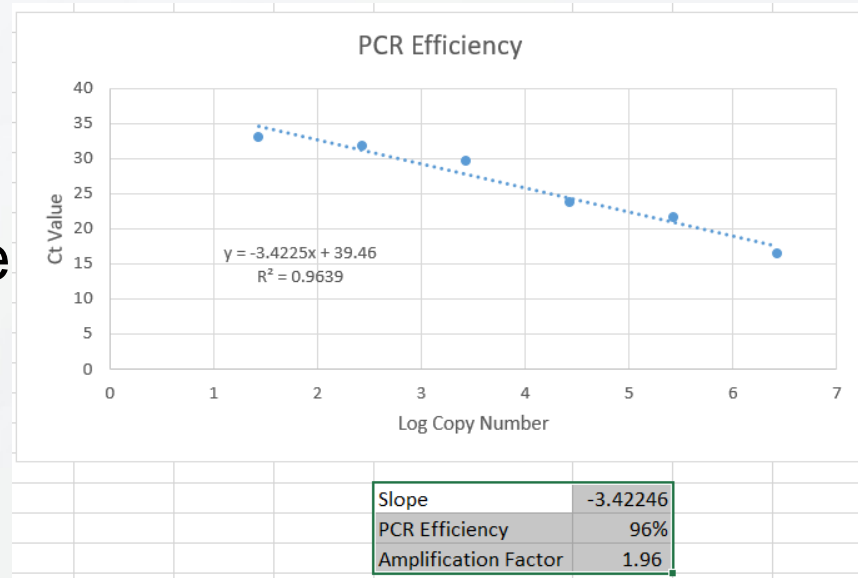
ROC Analysis
Sensitivity vs
Specificity

Copy number

- Starting about 2 years ago, SGCL started offering another means of reporting:
 - Copy number
 - Estimation of the actual number of starting copies of the gene of interest
 - The gene we are amplifying has three copies in CLas (to determine cells, divide by 3)
 - The process takes more time and is more involved
- Must ask for this service

Copy number

- Results reported as:
 - Copies per 100ng of DNA
 - Copies per 100mg of tissue
 - Divide by 3 to get cells
- Does not indicate live or dead



| Lab Id | Date Sampled Collected | Date received | Assay date | Ca_ct | UnDil_DNA_Conc | DNA Conc_ng/ul | Copy Num/2ul | Copy Num/100ng DN | Copy #/100mg of Tissue |
|--------|------------------------|---------------|------------|-------|----------------|----------------|--------------|-------------------|------------------------|
| 268502 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 30.35 | 29.455 | 10 | 3.39E+02 | 1.70E+03 | 3.00E+05 |
| 268503 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 29.13 | 38.657 | 10 | 8.34E+02 | 4.17E+03 | 9.68E+05 |
| 268504 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 26.07 | 30.72 | 10 | 8.03E+03 | 4.01E+04 | 7.40E+06 |
| 268505 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 26.23 | 25.686 | 10 | 7.12E+03 | 3.56E+04 | 5.49E+06 |
| 268506 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 29.65 | 29.018 | 10 | 5.67E+02 | 2.83E+03 | 4.93E+05 |
| 268507 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 29.47 | 41.298 | 10 | 6.49E+02 | 3.24E+03 | 8.04E+05 |
| 268508 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 34.44 | 46.659 | 10 | 1.63E+01 | 8.17E+01 | 2.29E+04 |
| 268509 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 27.34 | 41.203 | 10 | 3.15E+03 | 1.57E+04 | 3.89E+06 |
| 268510 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 28.87 | 39.003 | 10 | 8.47E+02 | 4.24E+03 | 9.92E+05 |
| 268511 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 28.00 | 48.862 | 10 | 1.61E+03 | 8.03E+03 | 2.36E+06 |
| 268512 | 1/9/2019 | 1/11/2019 | 29-Jan-19 | 28.60 | 19.471 | 10 | 1.03E+03 | 5.15E+03 | 6.02E+05 |

New services

- Willing to run other assays
 - Other assays have been reported to be “more sensitive”
 - The assay that we are running has been extensively validated around the world
 - SGDL is agnostic to which assay we use
 - We are willing to run other assays but it will be up to the user to interpret (will run comparison assays if requested)
 - Willing to run other assays (endophytes) if primer sequences, PCR conditions/parameters provided
 - Live vs Dead (if/when available)

Questions?

- Dr. Jamie Duffer
- Paula Gadea
- Lynn Briefman
- Jose Paniagua
- Pearl Mai
- Dr. Claudia Kaye
- Orlanda Cutino