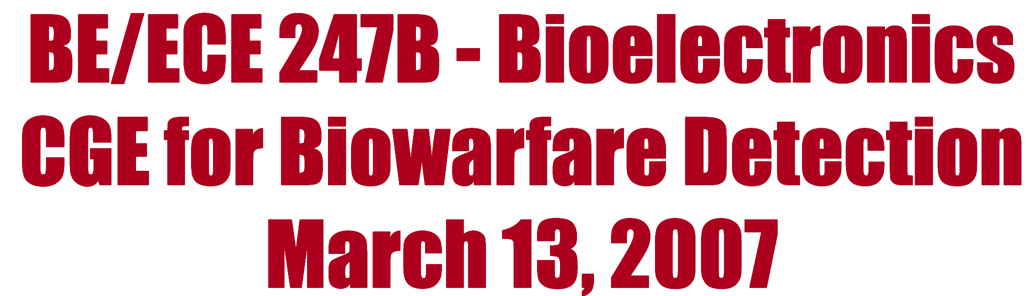
BE/ECE 247B - Bioelectronics
CGE for Biowarfare Detection
March 13, 2007

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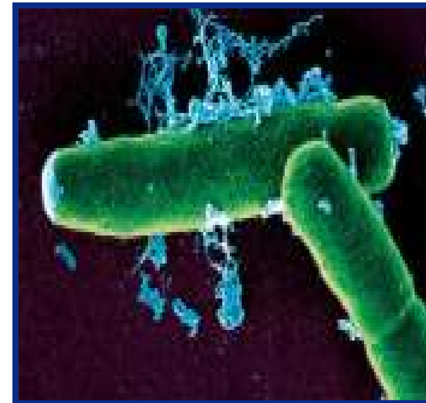
Outline – Overview

- Outline
- Why Biodefense and Why Capillary Gel Electrophoresis
- Biowarfare Detection Procedure
  - Primer Design
  - Sample Prep
  - Capillary Gel Electrophoresis
  - Signal Processing
  - Detection Logic
- Strengths and Limitations of Technology
- Future of the Technology
- Conclusion and Q&A

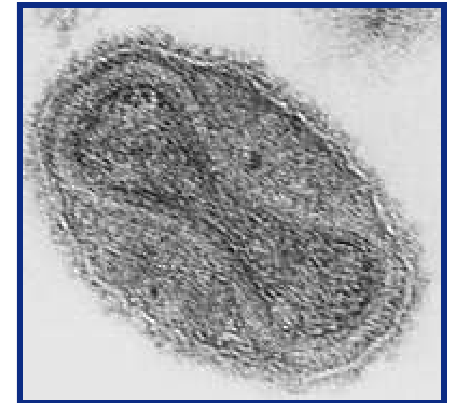
Bio Warfare– Another Frontier of Terror

- Biowarfare agents (BWA): microorganisms or toxic biological products that can cause injury to humans
  - Bacteria: anthrax, plague, tularemia
  - Viruses: smallpox, ebola, marburg
  - Toxins: botulinum, ricin
- Why BWA detection?
  - 2001 anthrax attack made bioterrorism a realistic threat to the US
  - US spent $4.2B for civilian biodefense in 2006 alone[1]

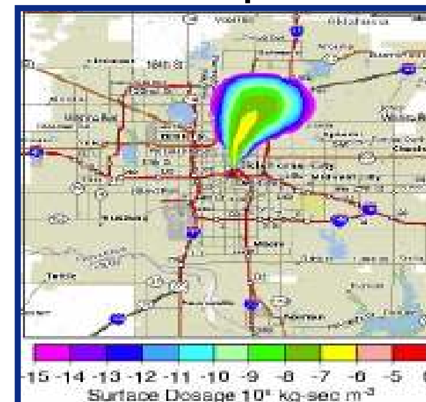
Anthrax Bacteria Spore



Variola Virus (Smallpox)



Plume Exposure[2]



Building Exposure[3]

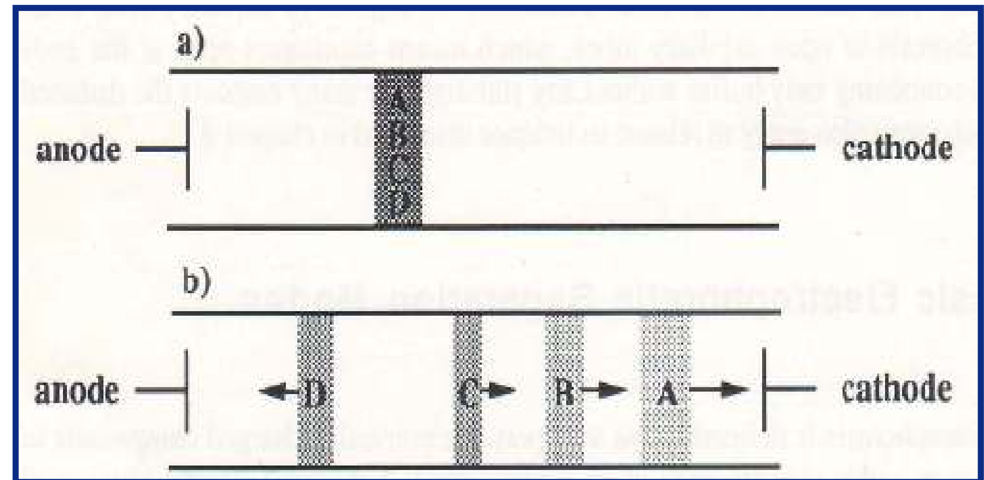
[1] Davis, "FY 06 Biodefense Budget $4.2 B," *Washington Times*, February 10, 2005.

[2] http://www.rap.ucar.edu/projects/shield/technology/mei_2004urb.htm

[3] http://www.eqecat.com/abscorporatesolutions/TRIA.html

Capillary Gel Electrophoresis Technology

- Charged particles in solution can be separated based on charge and size
  - DNA has net negative charge
- DNA length determines effective charge and radius
  - Small DNA fragments have high electrophoretic mobility
  - Large DNA fragments have low electrophoretic mobility
- Gel properties can be manipulated to allow only chosen DNA fragments to migrate



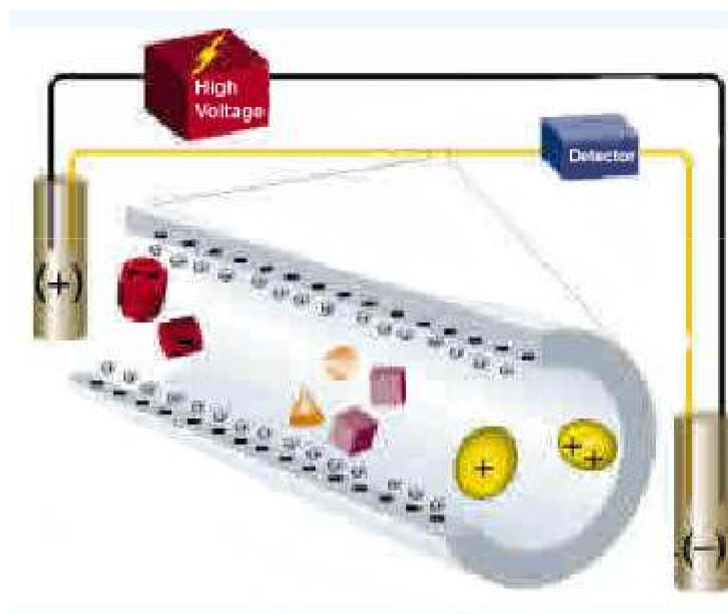
$$\mu_i = \frac{Q_{eff}}{6\pi\eta R}$$

μ_i effective electrophoretic mobility $[cm^2 \cdot s^{-1} \cdot V^{-1}]$
Q_{eff} effective charge of the ion [C]
R total radius of the ion [cm]

Strengths and Limitations of CGE

Strengths

- High efficiency
  - Hundreds of components can be simultaneously separated
- Easily automated
- Low sample requirement
- Low reagent requirement
- Low Joule heating
- Quantitative measurement

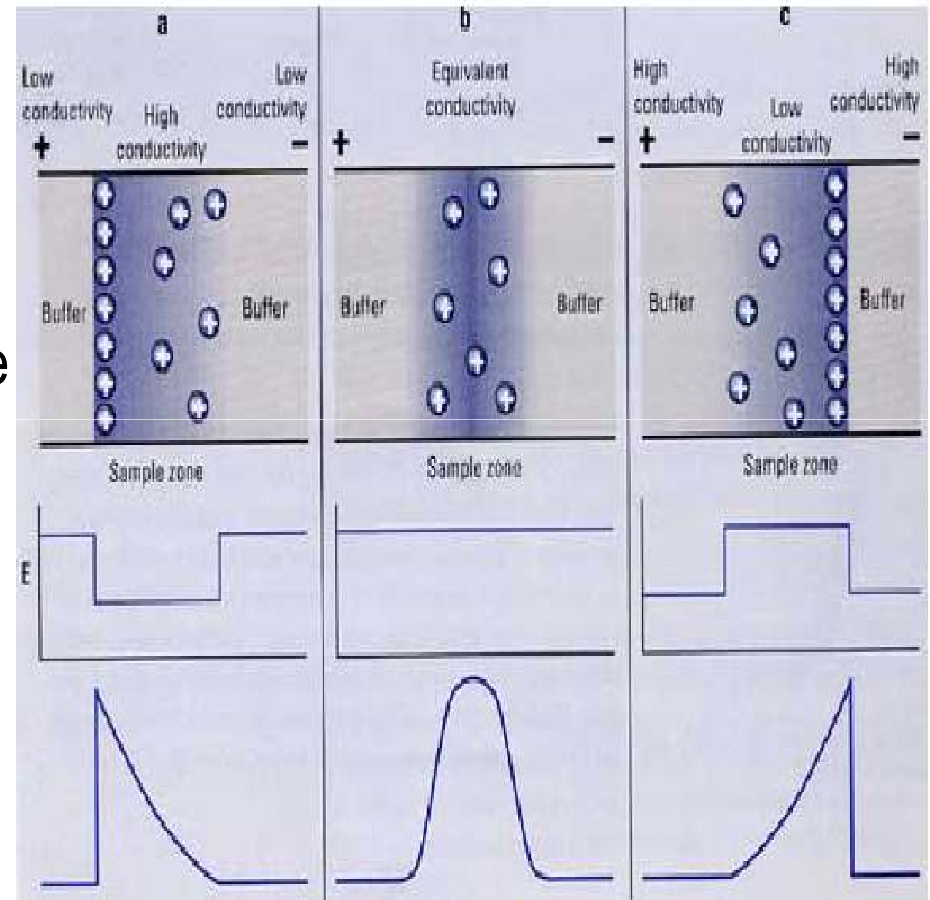


PD Grossman, et al (1992)

Strengths and Limitations of CGE

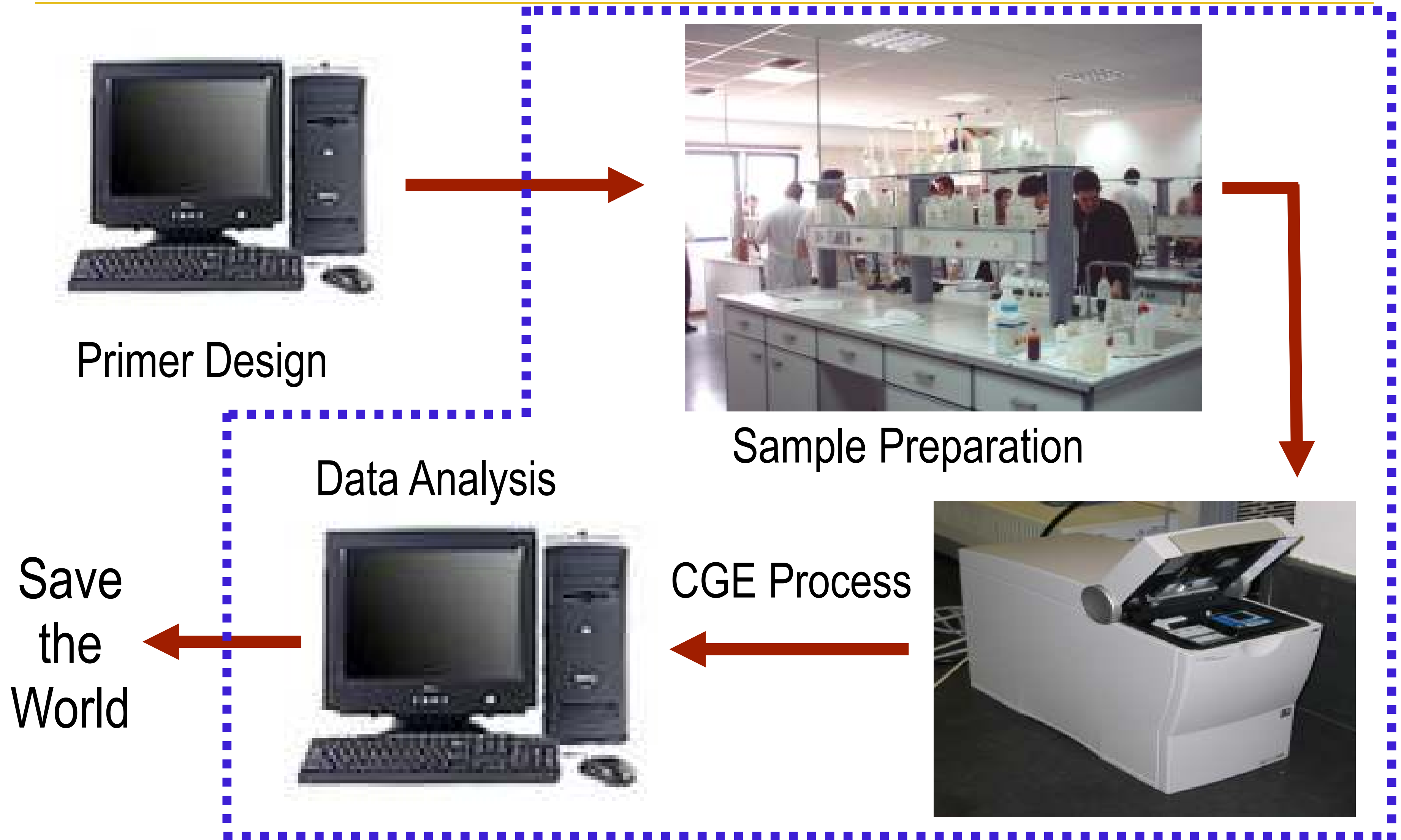
Limitations

- Error due to Electrodispersion
  - Difference in sample zone and buffer conductivity
- Single gel that separates a wide range of different length DNA strands has not been developed
- Only molecules that can carry a charge can be separated
- Solute-wall interactions

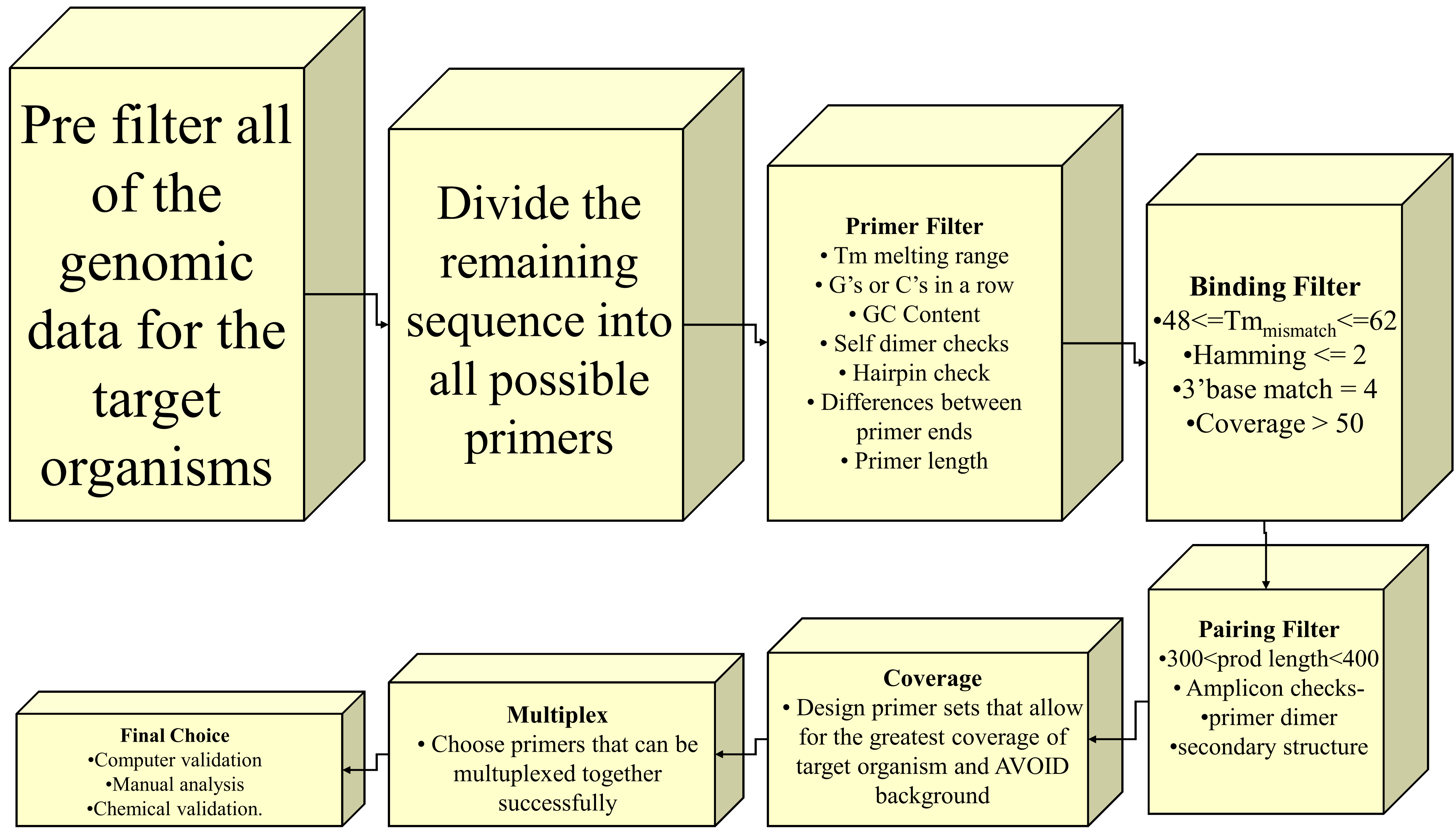


PD Grossman, et al (1992)

Sample to Detection Process

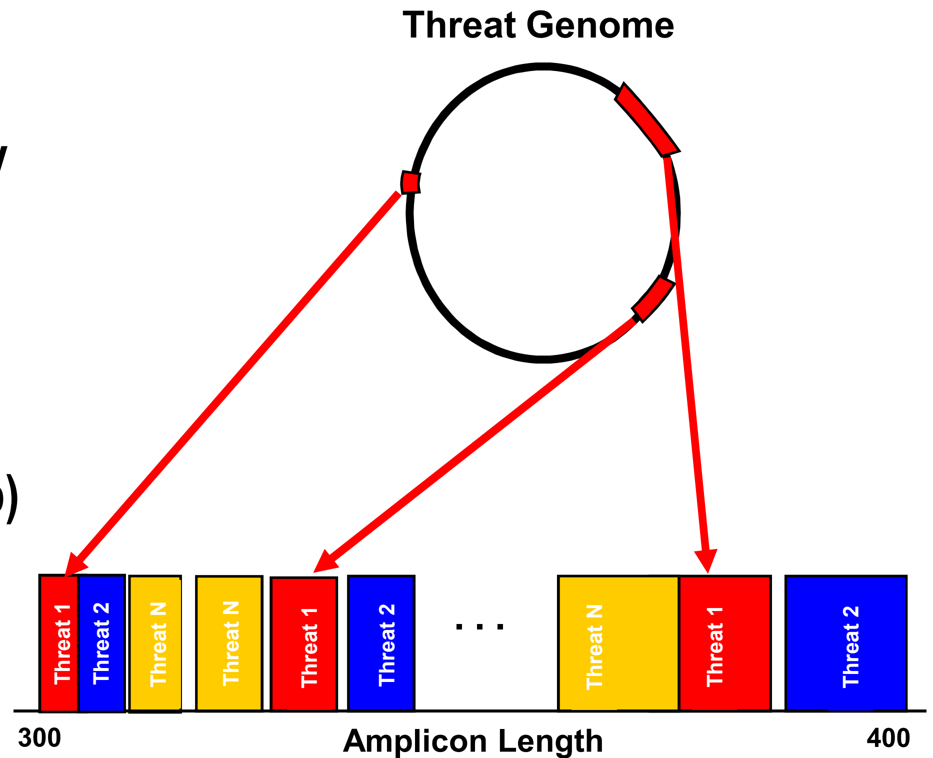


Clever Primer Design – The Foundation for Detection



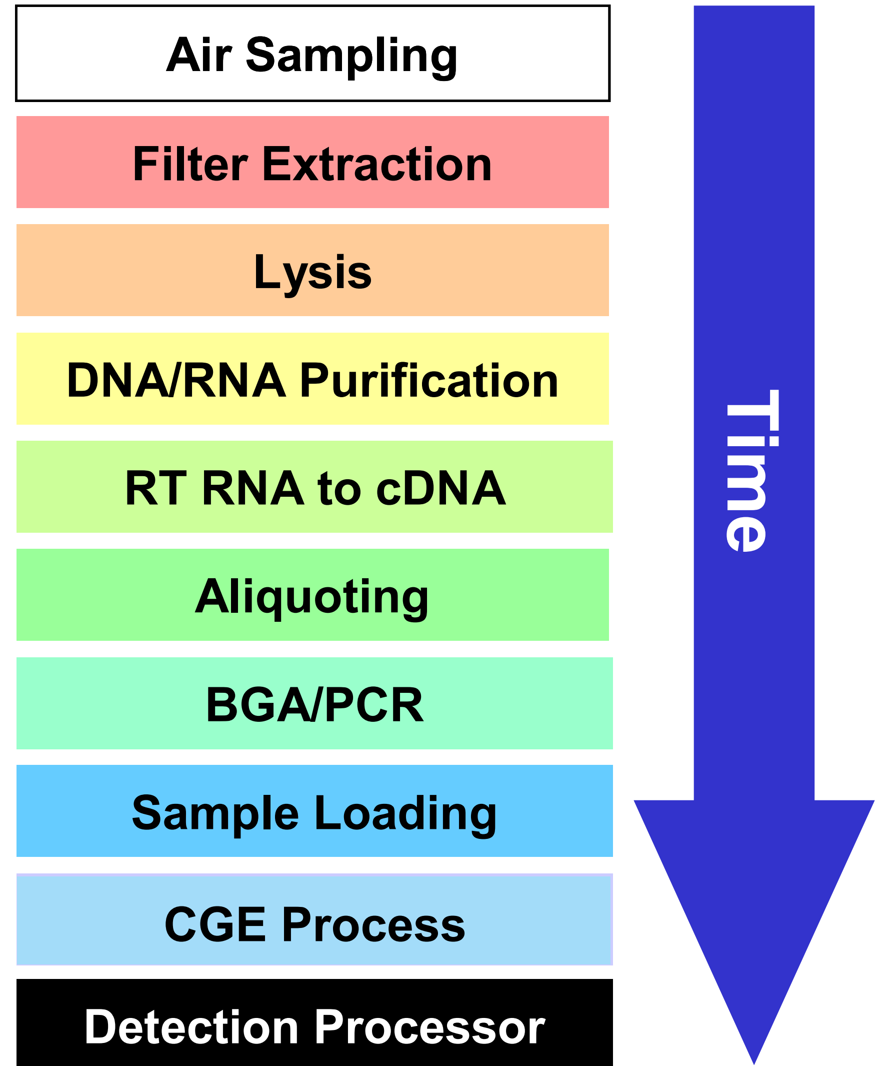
Gives Specificity Needed for Detection

- PCR Primer Design
  - Primers designed to specifically amplify only intended target organisms
  - Entire set of primers designed for simultaneous multiplexing
  - All amplicons designed to be within the CGE detection "window" (~300 – 400 bp)
  - Multiple amplicons are designed to target each threat
  - Each primer pair is chosen to produce an amplicon length within the threat window



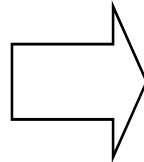
Sample Preparation for CGE

- Air collection done on filter
- Lysis done to expose DNA material within spores
- DNA purification done to prepare sample for PCR
- Capillary Gel Electrophoresis is not sensitive enough to work with raw sample – must be amplified through PCR
- Multiple CGE can be ran in parallel due to small input volume to improve detection results (repeats of data)

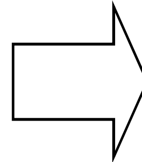


Capillary Gel Electrophoresis

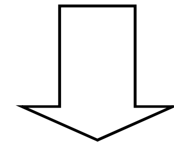
Primer design

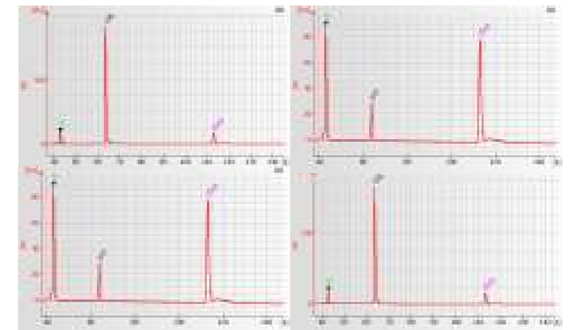


Multiplex PCR Amplification



Electrophoresis on Agilent Bioanalyzer



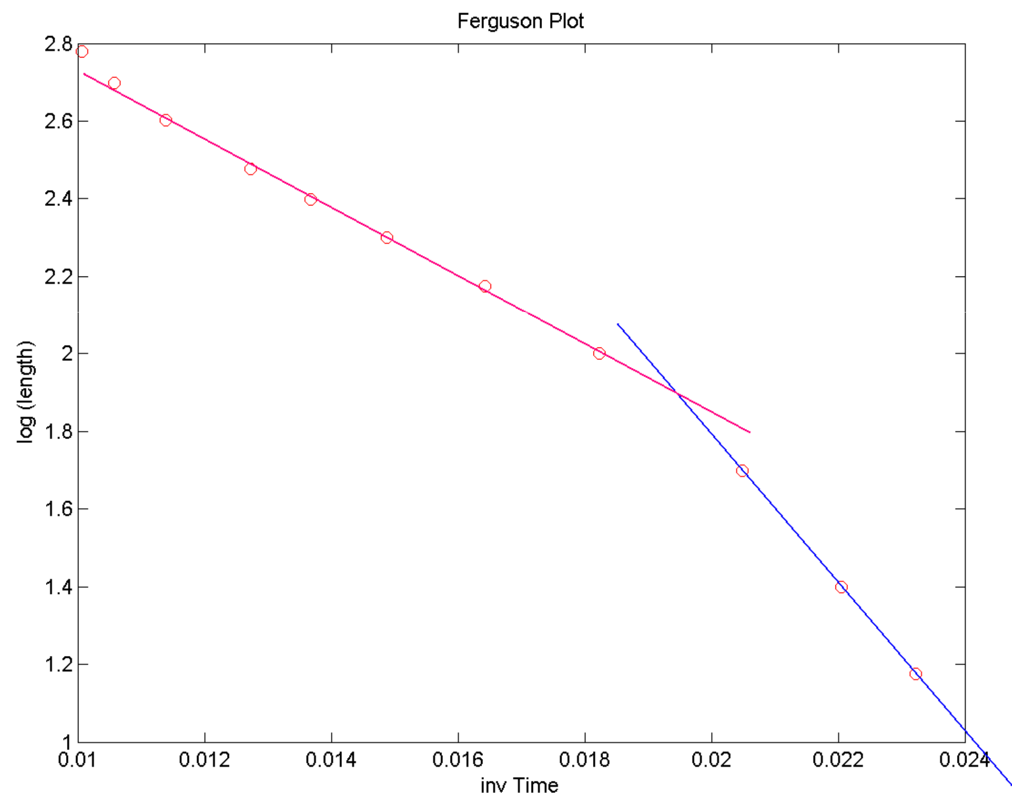


Fluorescent Peaks Represent Masses of PCR Amplicons

- DNA fragments are sent through a capillary filled with gel
- Movement driven by electrical current
- Output data are the lengths of of the masses of PCR amplicons

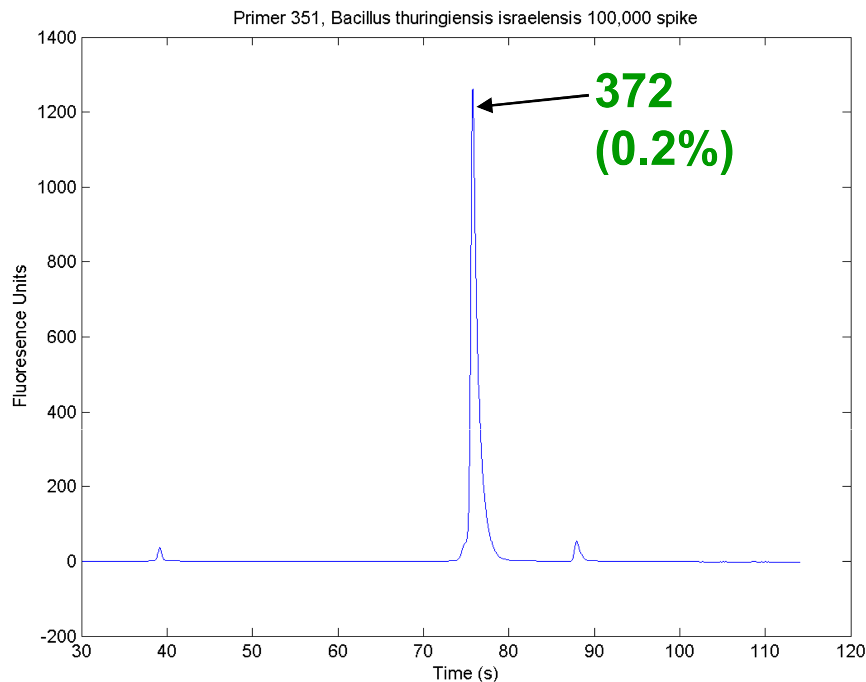
DNA Migration Theory – Ferguson Plot

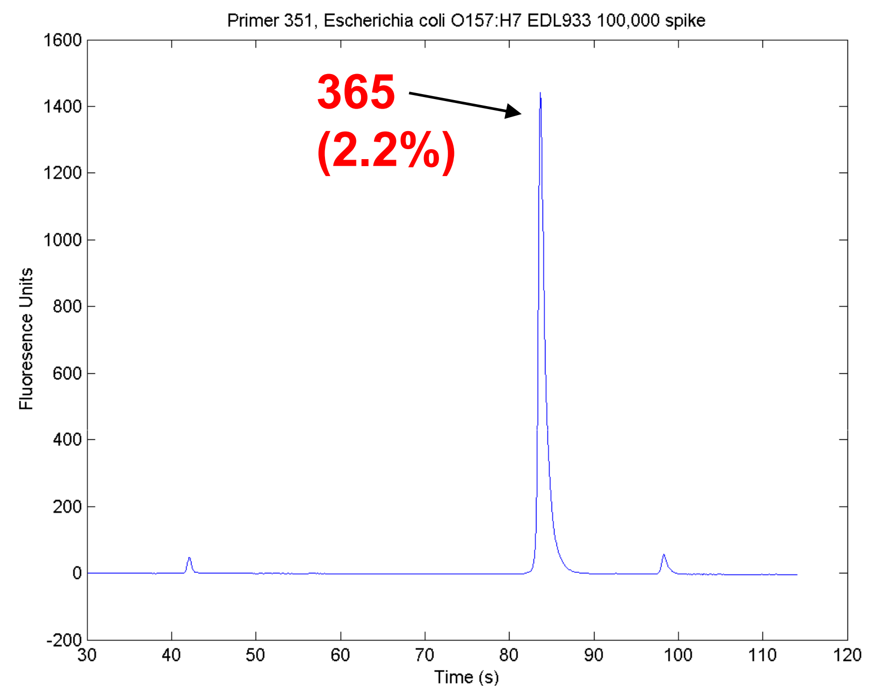
- Fragment migration follows two main theoretical models based on size
- Ogston model: fragments are small enough to fit through the pores in the gel
- Biased reptation model: fragments are too large to fit through the pores in the gel without a considerable amount of deformation and stretching of the molecules
- Leads to two distinct regions in the Ferguson plot
- Linear interpolation used to find migration times of unknown samples



How Data Compares to Theory

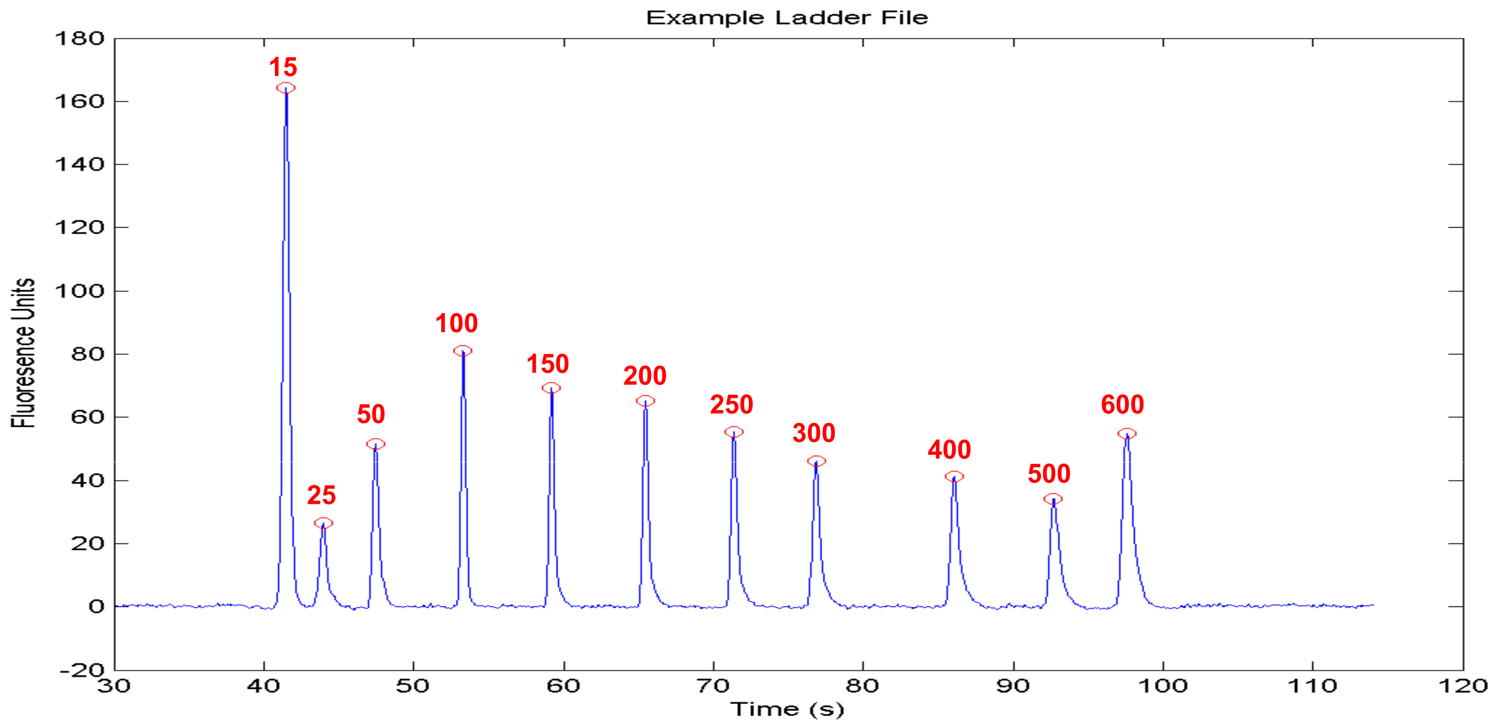
Broad Range Priming, Expected Size 373 for Both Target Organisms





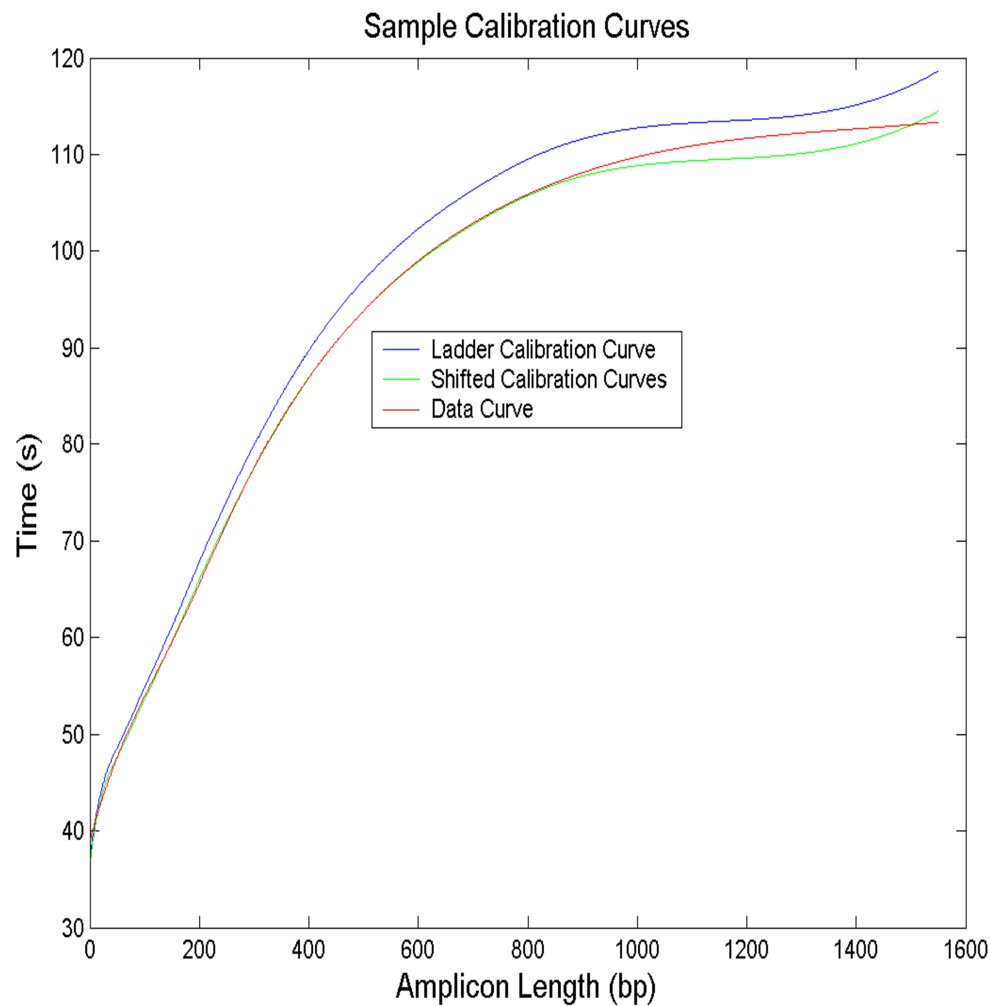
- Error rates vary from 0% to 5% with no apparent systematic error
  - For a particular amplicon, migration is constant, even if it doesn't agree with actual length (run to run variability less than 1%)
  - Though sequence analysis does not reveal trends, G, C, A, and T may migrate differently due to size
  - Bioinformatic data is not complete or accurate

Length Time Calibration

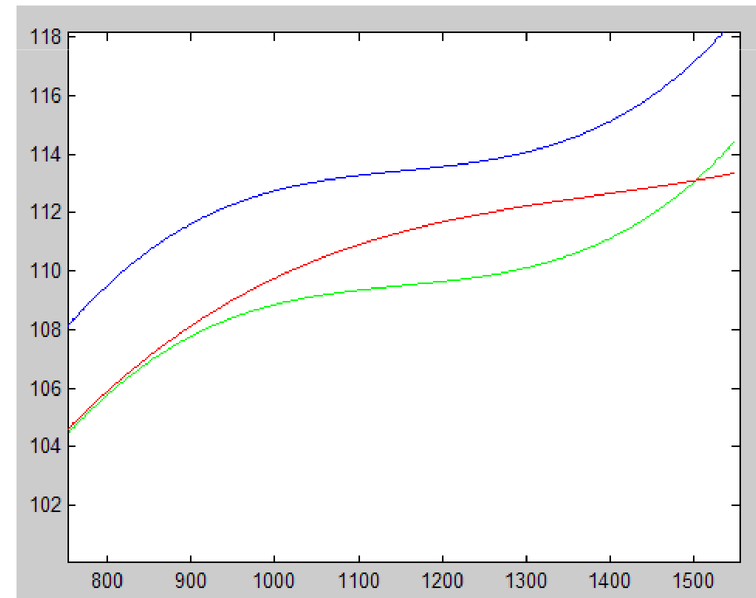


- Proper peak identification and time data gives ability to create an equation that relates migration time to the size of the DNA
- Heating decreases viscosity which decreases migration time
- Calibration peaks picked out of the data
- Calibration length-to-time line is shifted to go through the two calibration points of the data

Best Fit Model – Improvement over Commercial Software

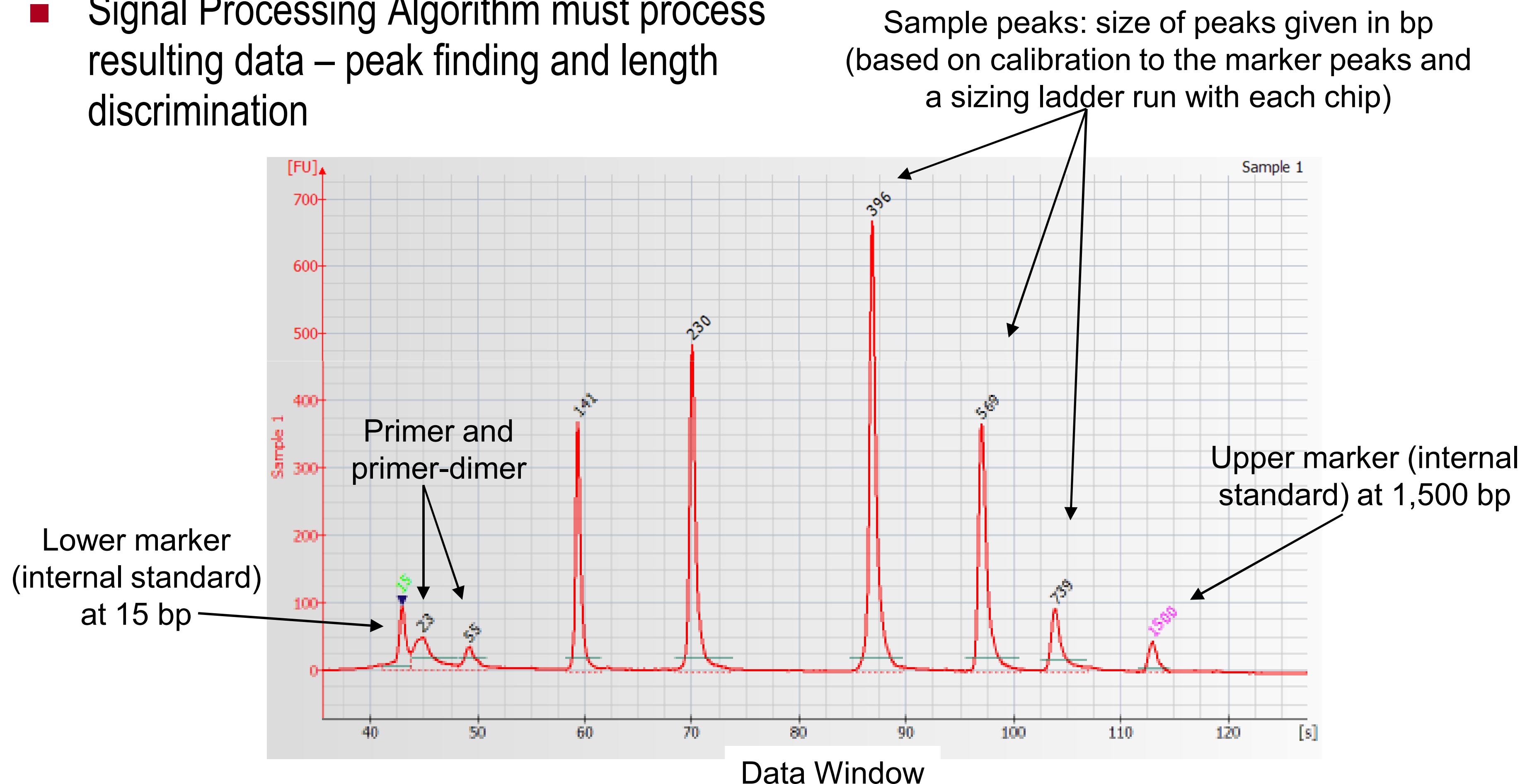


- Step 1
  - Spline fit the peaks found in the ladder file to create a length time calibibration curve – This file is done once for each set of runs (12 samples in one run)



Signal Processing – Agent Identification and Detection

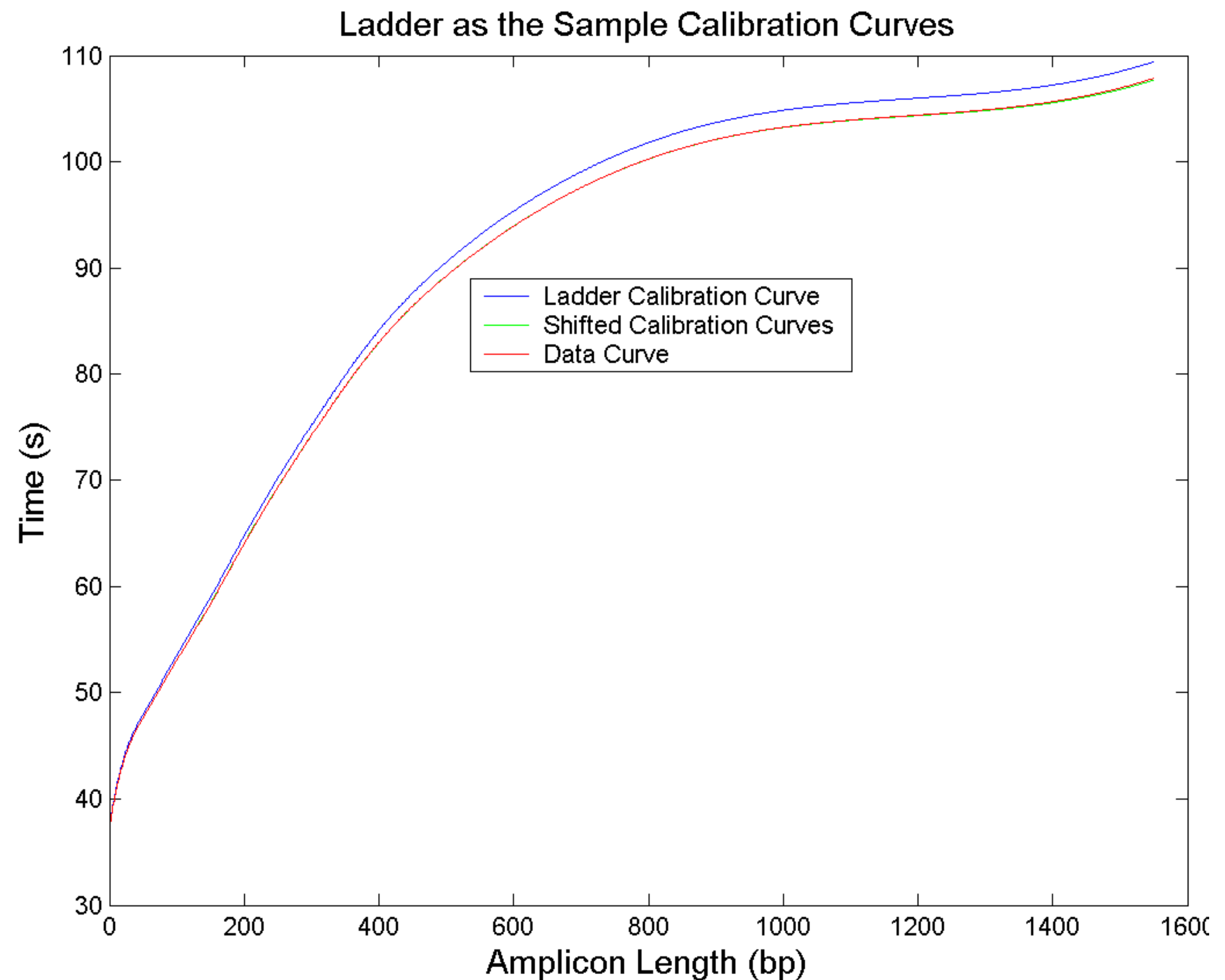
- Signal Processing Algorithm must process resulting data – peak finding and length discrimination



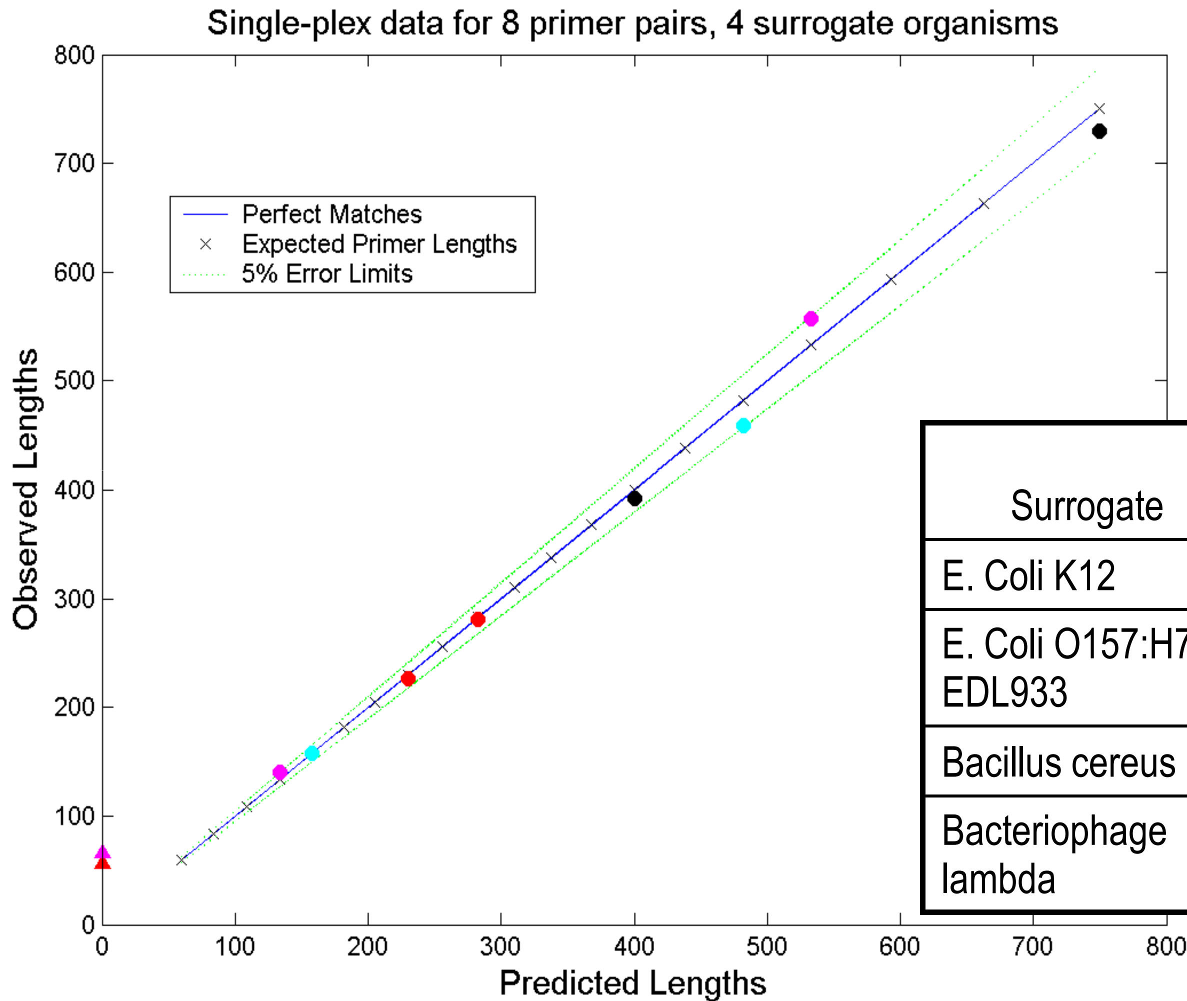
X-axis = Migration time of DNA (i.e. how big it is)
Y-axis = Fluorescent intensity of DNA (i.e. how much is there)

Best Fit Model – Improvement over Commercial Software

- Step 2
  - Find the two calibration peaks in the sample data
  - Use the migration time of those peaks to correct the length time calibration curve
- Corrects for variation seen between sample 1 and sample 12 of a single run
- Results are better than commercially available software



Data Examples



- 2 primer pairs tested alone for each surrogate organism
- 100 org copies

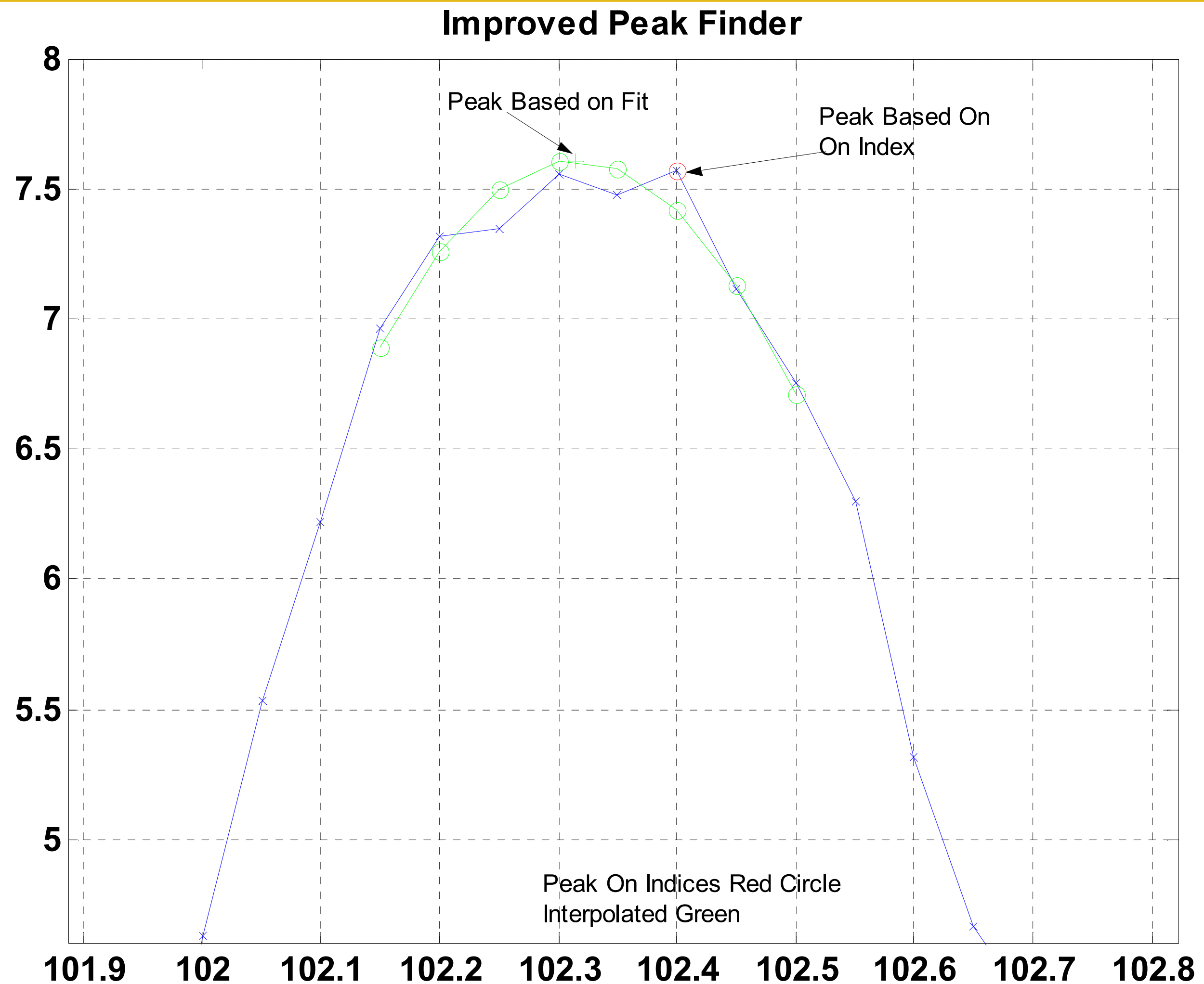
Surrogate	Primer Pairs	Expected Lengths	Color
E. Coli K12	104 & 114	400 & 750	●
E. Coli O157:H7 EDL933	103 & 113	230 & 283	●
Bacillus cereus	99 & 109	158 & 482	●
Bacteriophage lambda	101 & 111	134 & 533	●

●	- Expected Lengths
▲	- Unexpected Lengths

- All expected primer lengths found within 5% error

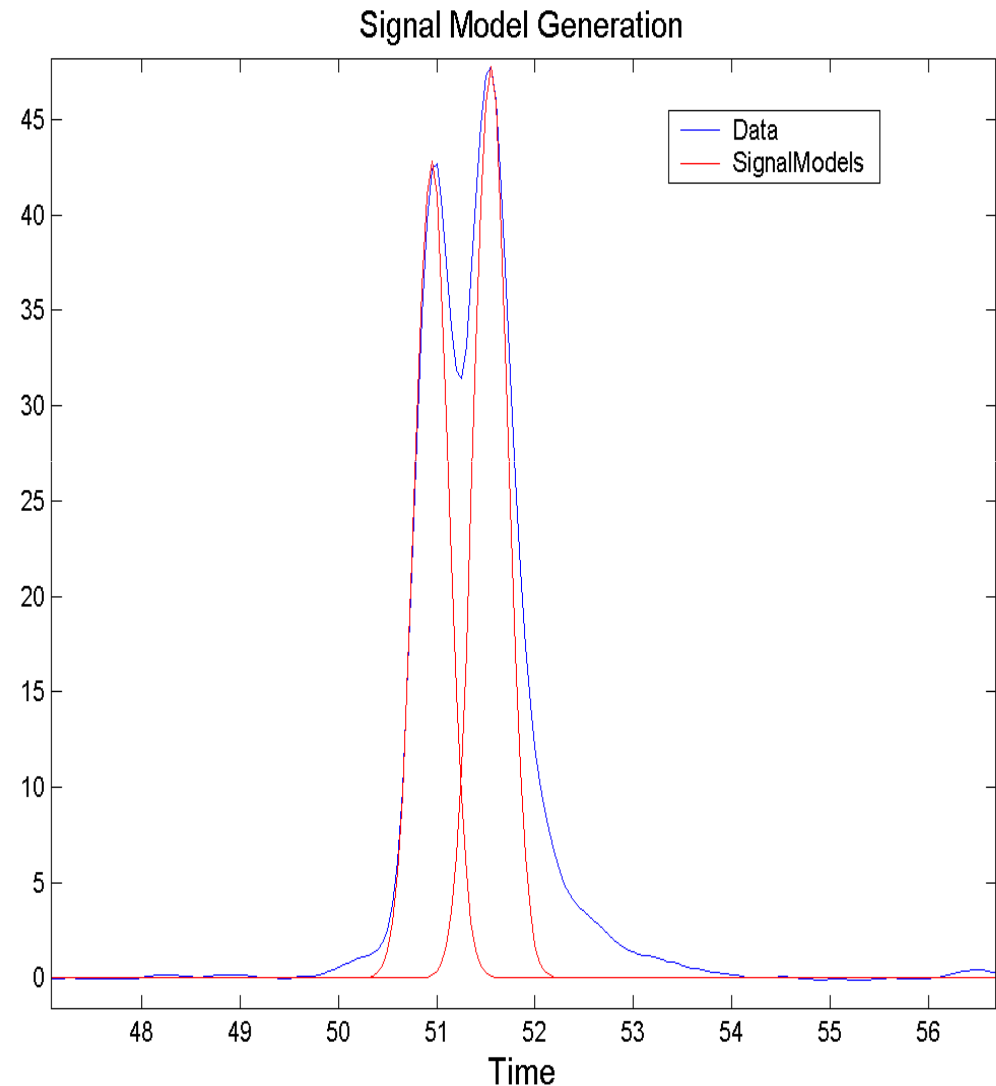
Peak Finding

- To locate peak interpolate with second order polynomial fit (parabola)
- Improves Peak Finding Especially Noisy or Sparse Peaks
- Peak Closest to Interpolated Peak is Chosen as Peak Index
- Also improves time to length calibration curve



Peak Resolution – Peaks too close together

- Peak Resolution:
  - $R_s = (t_1 – t_2) / (\text{Width Avg.})$
- Individual Peaks Resolved when $R_s > 1.5$
- High SNR Required for Detailed Resolution
- Each peak may be modeled as an Individual Waveform for Analysis (Gaussian Waveform commonly used)

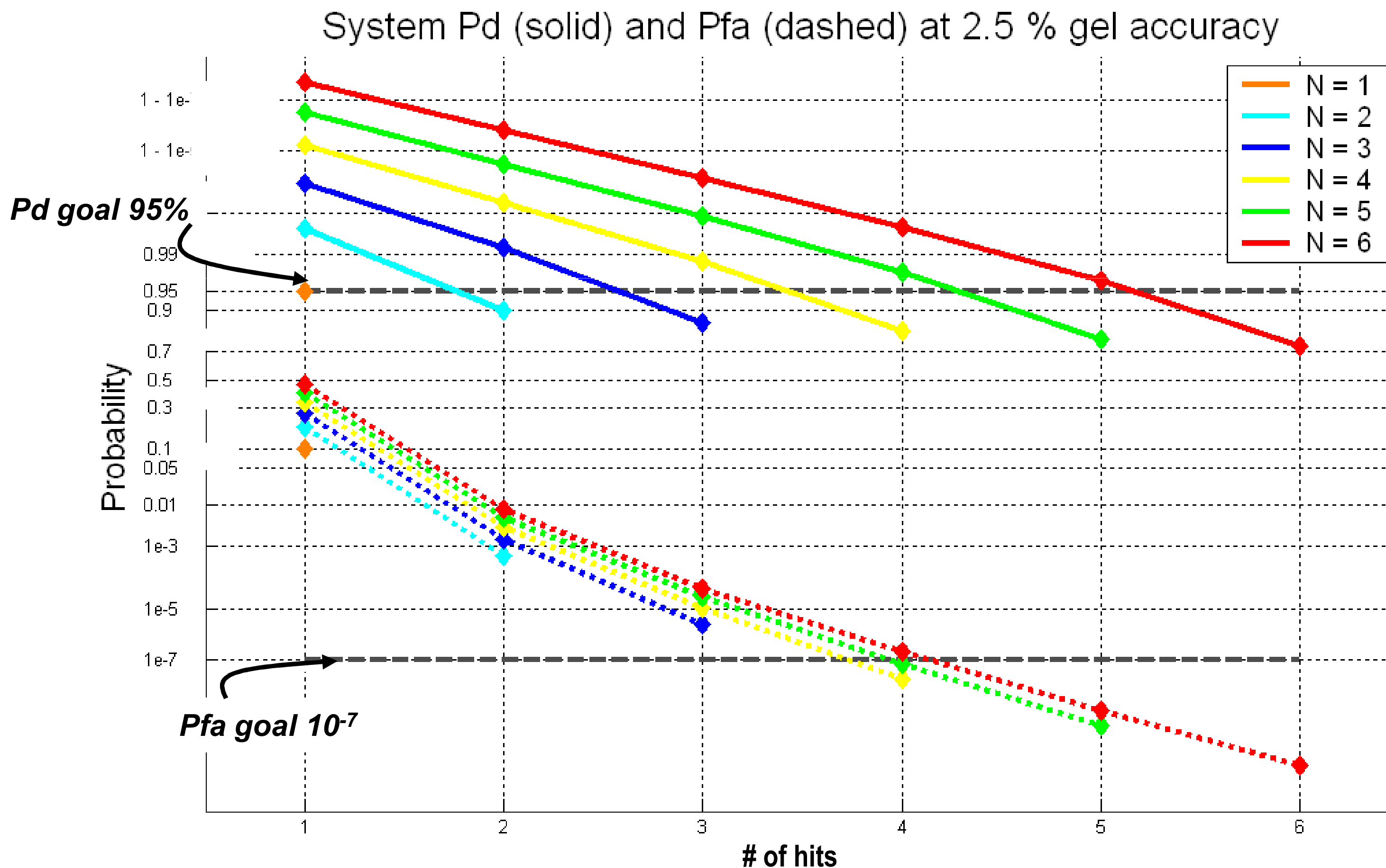


Detection Logic

- Detector takes in all in the information – how many peaks were present within the detection range, and how likely is that to happen without a threat being present
- Detection Logic gets more complex if organism discrimination is desired
- Likelihood of seeing gel peak in correct bin when target is present is P_d= 95 %
  - Missed detections from PCR failures, too-low peaks, etc.
  - Likely conservative
- Likelihood of seeing a peak in any given bin when intended target is *not* present is P_{fa} = 1 %
  - Caused by unintended cross-priming of background, system noise, air bubbles in gel, etc.
  - Can be improved through better primer design

Approximate Pd / Pfa Trade to Identify Organisms

- Probability of detection (Pd) = likelihood of correctly detecting one target
- Probability of false alarm (Pfa) = likelihood of falsely detecting at least one target



Strengths and Limitations of CGE for detection

Strengths

- Speed
  - Three hour sampling time
  - One hour processing time
- Sensitivity and Accuracy
  - Only 8μl of sample is needed for each test
  - 99% with >3ppb sampled from air with careful primer selection
- Stability
  - BP length invariant on one gel
  - Temperature invariant
  - Low space/energy footprint

Limitations

- Speed
  - Four hours between exposure and detection
- Accuracy
  - Background interference/noise
  - Peaks close together
- Maintenance
  - Gels are one-use

Future Possibilities

- Parallel capillaries to increase sample space
- Increase light path to increase sensitivity
  - Bends in capillary or rectangular capillaries
- Interfacing between CGE and mass spectrometry
- Interfacing with Micellar electrokinetic capillary chromatography
  - Extend biowarfare detection CGE to molecules that cannot carry a charge

Conclusions

- CGE alongside PCR amplification can be used for Bio-Agent detection
  - Accurate, Stable, Can be Automated
- Technology already exists to build and use a CGE bio-detection system
  - PCR, CGE, DSP chips, etc., only need is for integration
- Signal Processing is a powerful tool to account for and correct the chaotic factors inherent in biological testing
  - Flexible Technology that will only get stronger as computing/processing advances

Questions?

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