

Development of qPCR Capabilities for Enterococci Analysis

NJ Water Monitoring Council
January 31, 2007

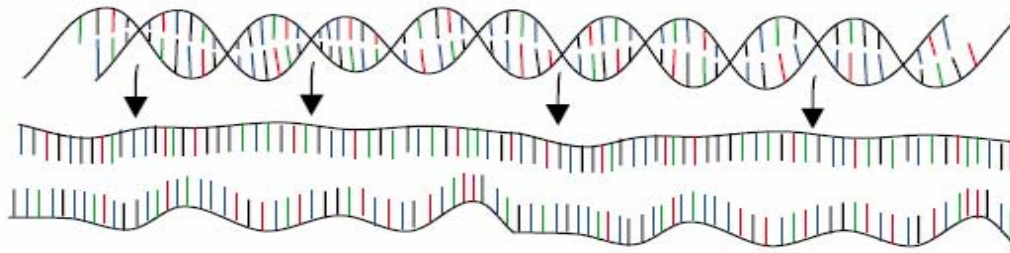
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723-906-6999

Overview

- How PCR works
- Conventional PCR vs qPCR
- Our thermal cycler
 - Six steps to run qPCR
- EPA Programs qPCR could support
- Enterococci qPCR Studies
- NJ Beach Study 2007

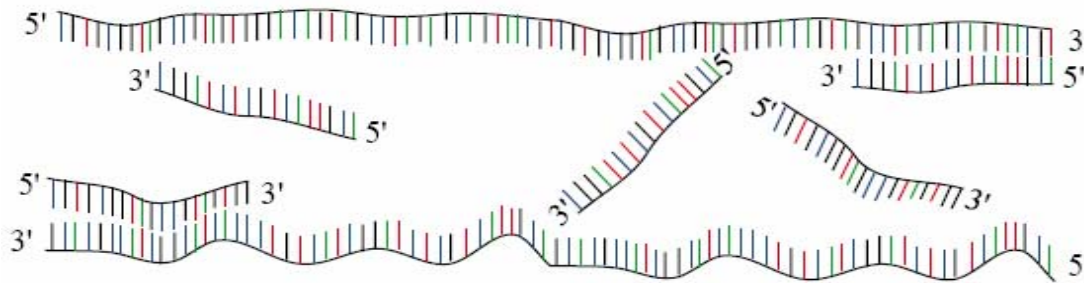
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



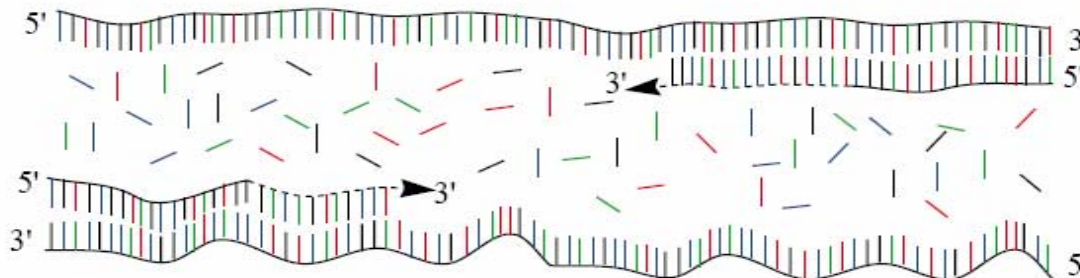
Step 1 : denaturation

1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C



Step 3 : extension

2 minutes 72 °C

(Andy Vierstraete 1999)

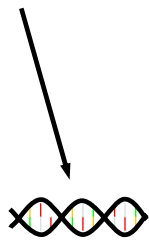


PCR 101



Target DNA

From 1 original copy



1st cycle



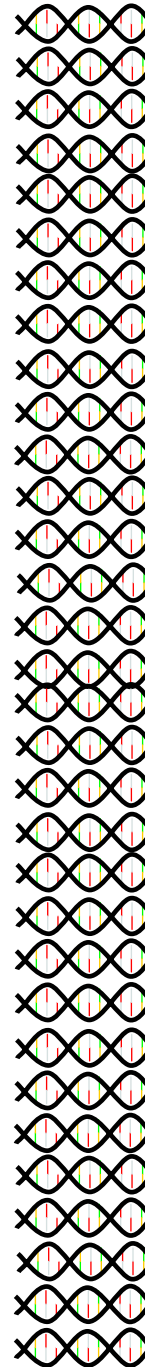
2nd cycle



3rd cycle




4th cycle



2^n copies per cycle

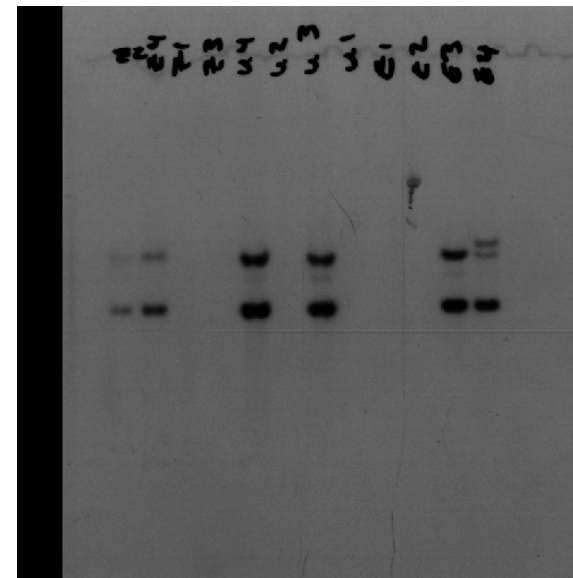
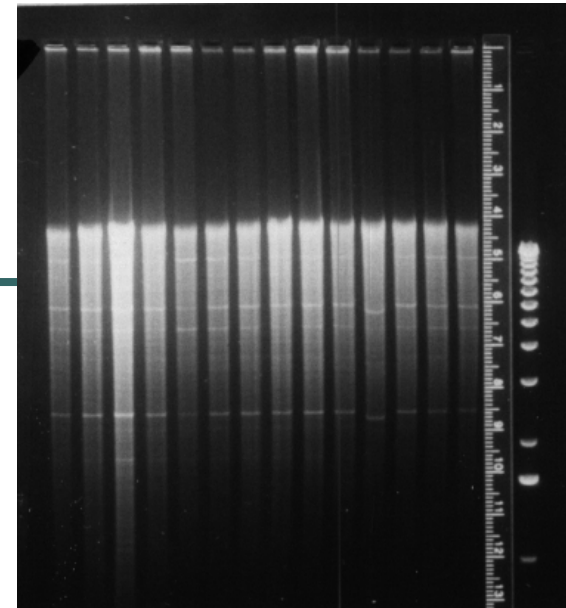
To Millions or Billions of Copies

20 PCR Cycles
 **30 to 40 minutes**

$2^{35} = 34$ Billion Copies

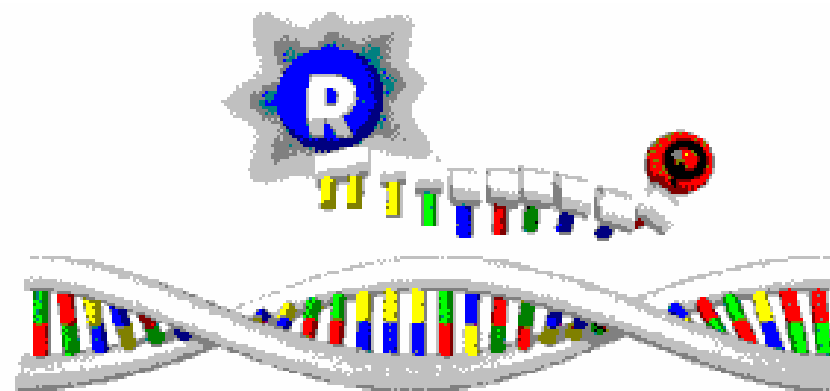
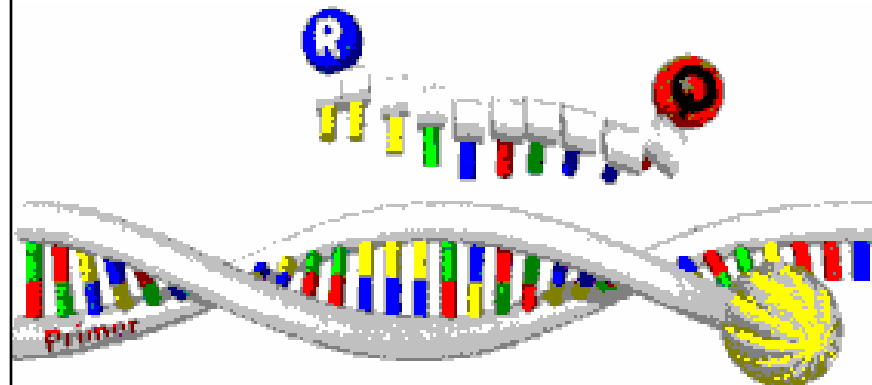
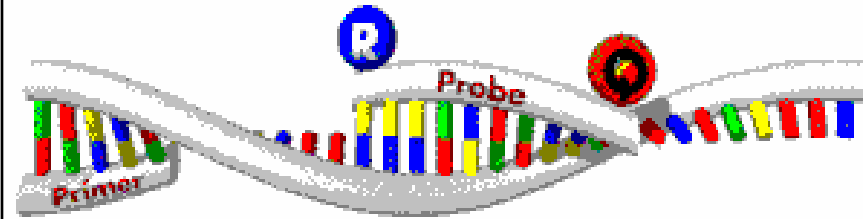
Pitfalls of Conventional PCR

- **Longer time to results**
- **Post-amplification processing**
 - Gels
 - DNA blot
- **Interpretation subjective**
 - Bands on a gel
 - Considerable expertise required

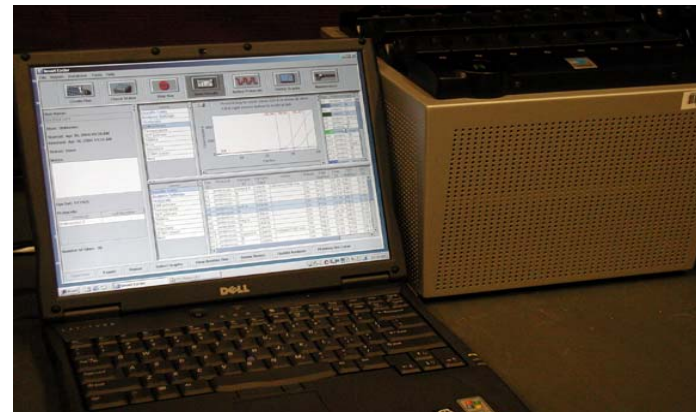


qPCR Primer/Probes

- Specific
- Proprietary
- Examples
 - TaqMan[®]
 - Scorpion[®]
 - Amplifluor[®]

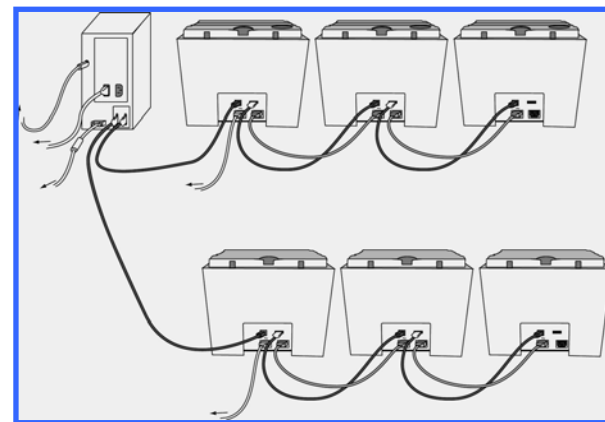


Applied Biosystems Prism™ VS Cepheid SmartCycler™

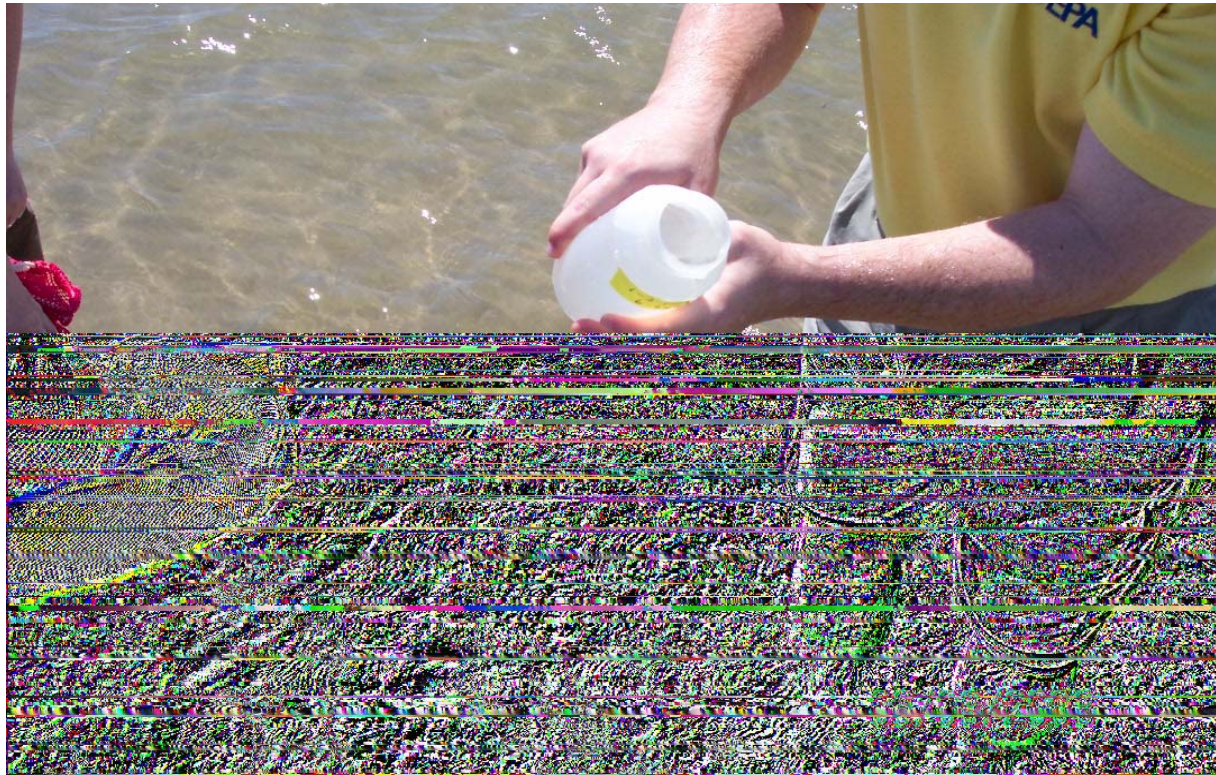


SmartCycler™ Modularity

- **16 Independent Modules**
- **Daisy Chain**
 - up to 6 units
 - 96 tests
- **Versatility**
 - run different tests
 - vary start times
 - multiplexing
 - portable



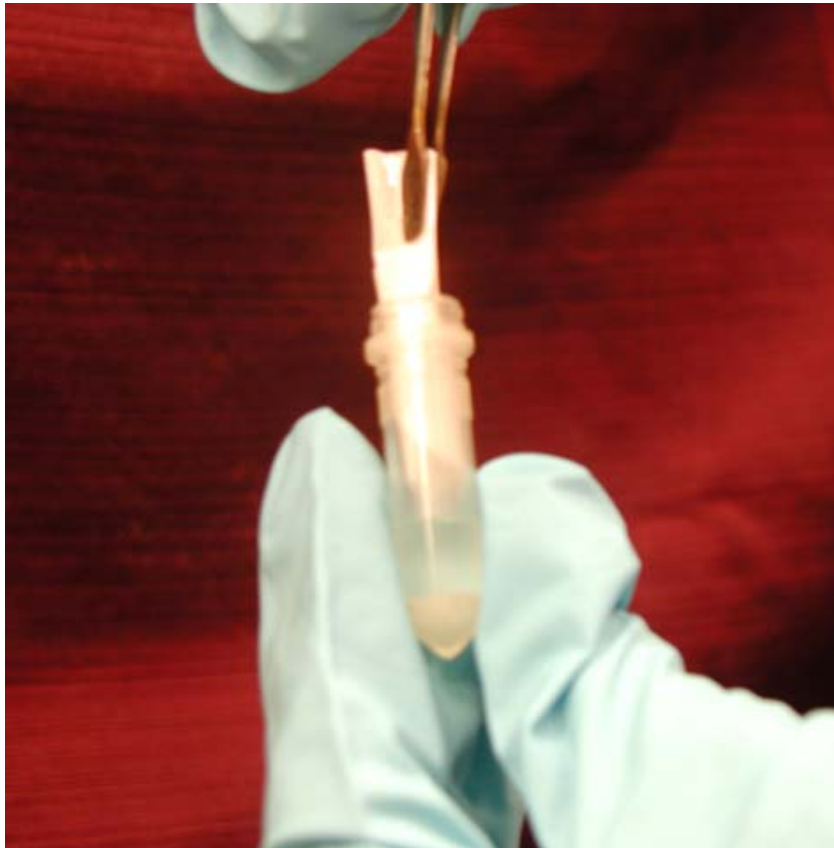
Step 1. Collect Water Sample



Step 2. Filter Water Sample



Step 3. Extract DNA



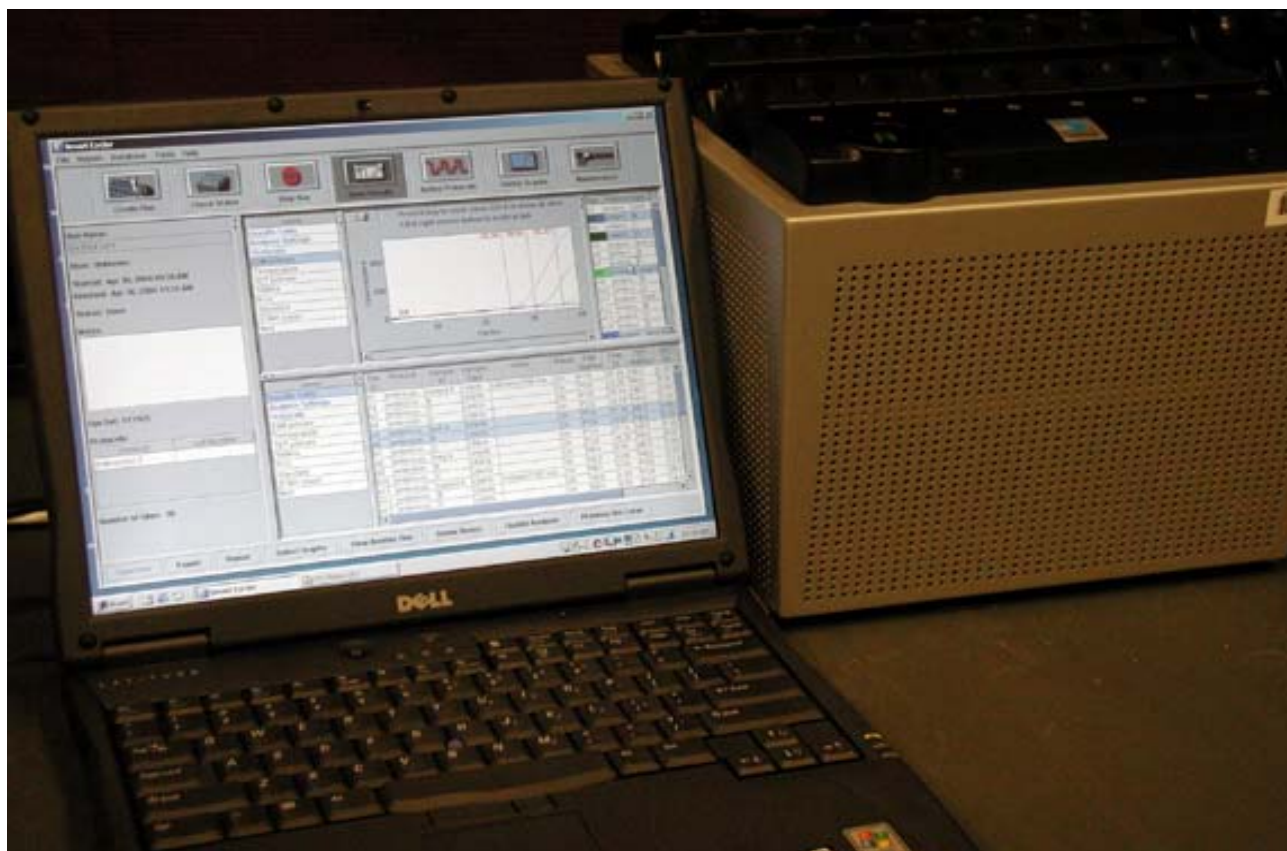
- **SmartMix™ Bead**

- Taq enzyme
- Buffer
- dNTPs
- MgCl₂

Step 4. Transfer to reaction tube

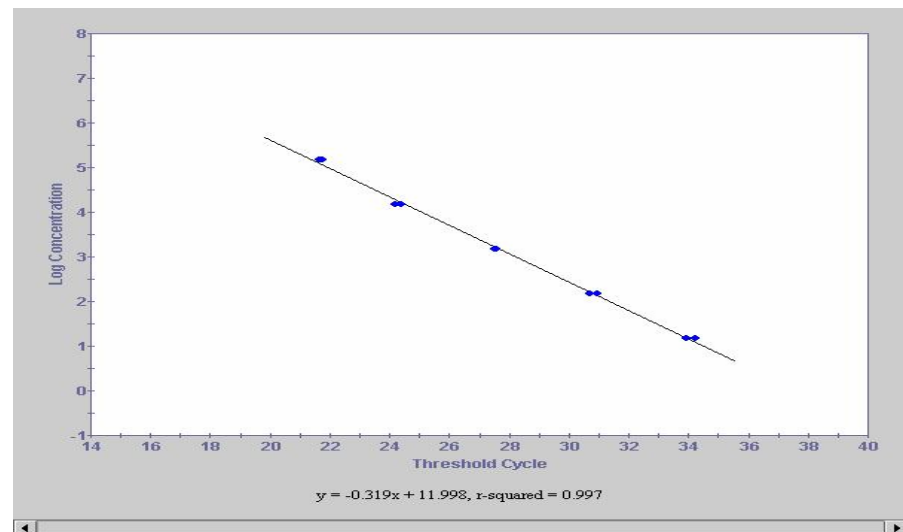
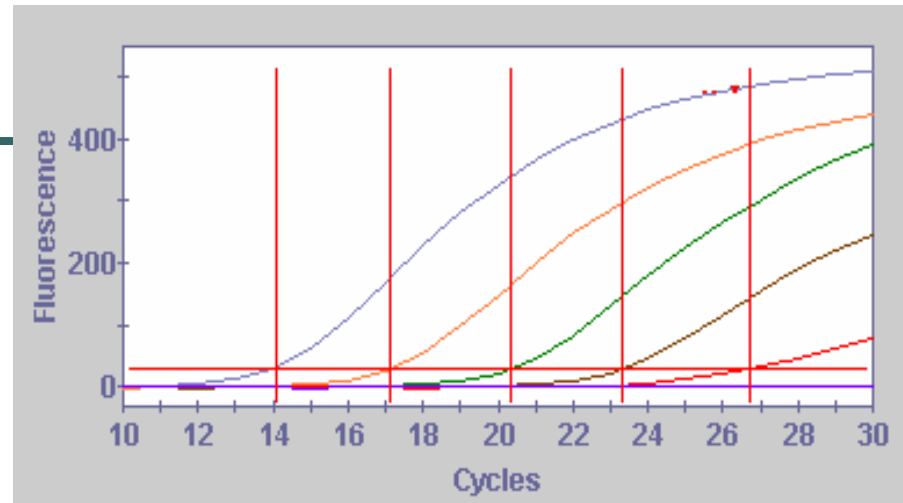


Step 5. Run Reaction in SmartCycler™



Step 6. Results

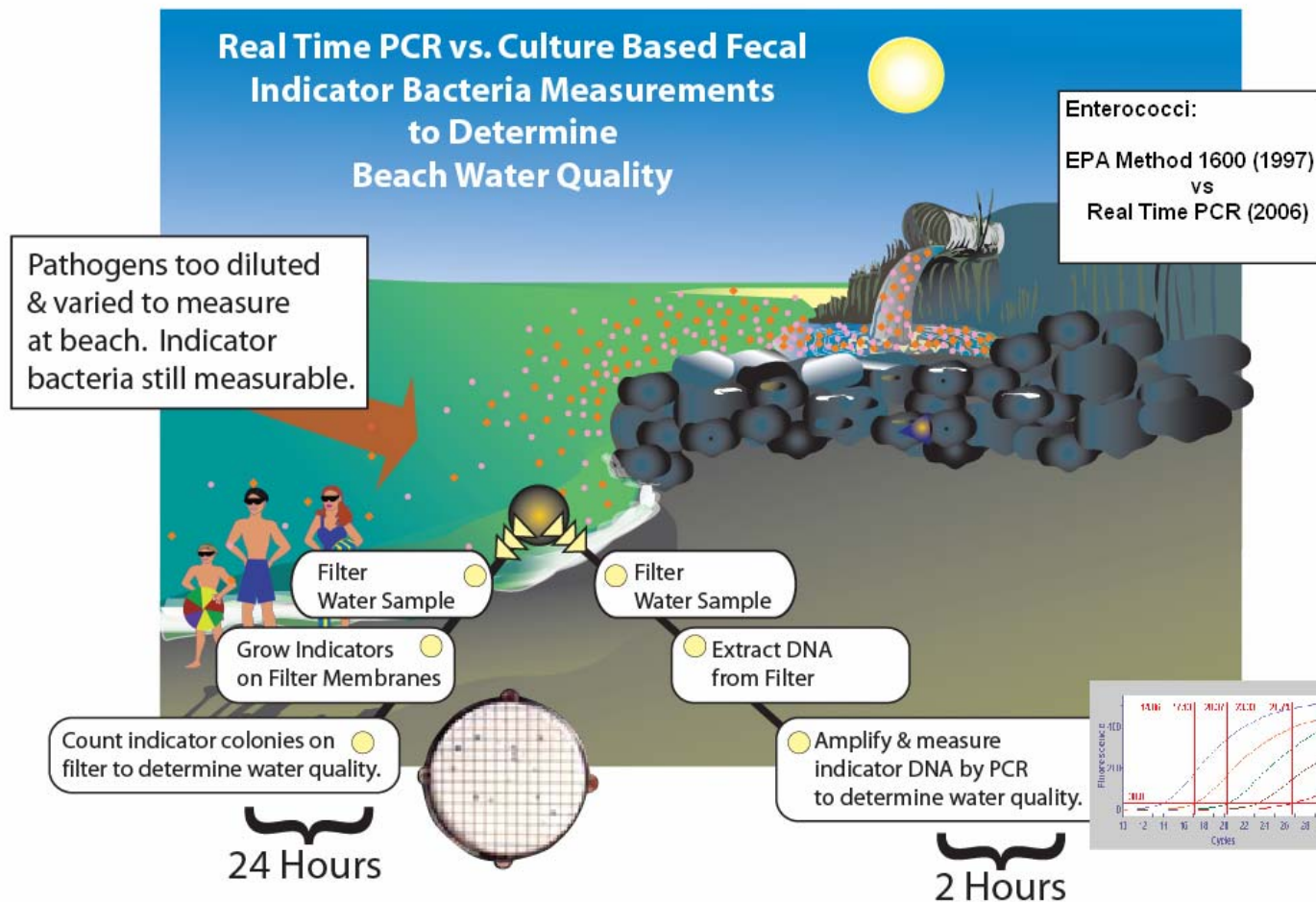
- **Real time**
 - Individual tests
 - Superimpose for comparisons
- **Quantitative Results**
 - Standard curve developed
 - cells/ml or cfu/ml



Programs qPCR Can Support

- Clean Water Act
 - National Pollutant Discharge Elimination System (NPDES)
 - Total Maximum Daily Loads (TMDL)
- Safe Drinking Water Act
- Homeland Security Act
- Beaches Environmental Assessment and Coastal Health (BEACH) Act

Enterococci Probe Developed

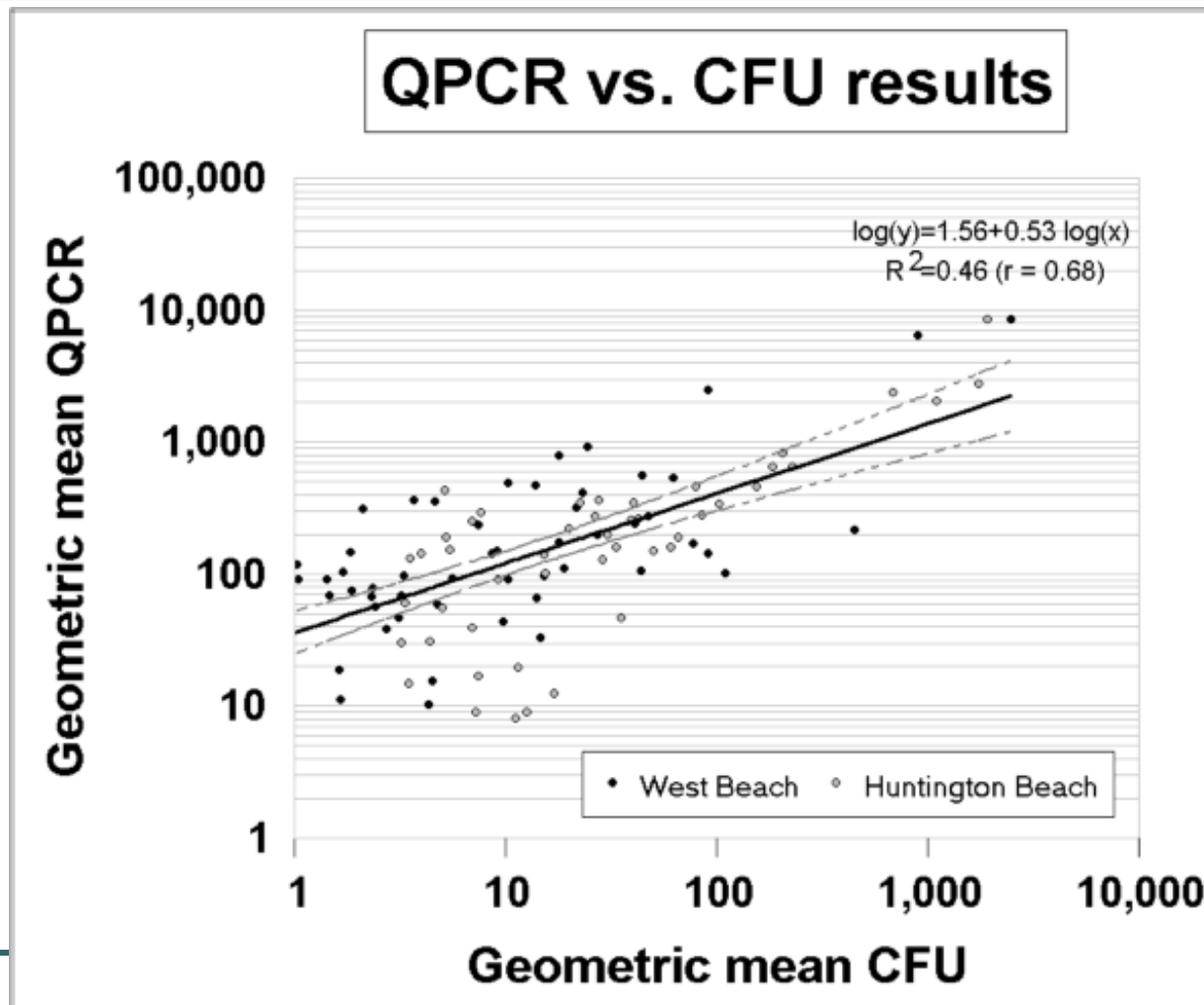


Enterococci qPCR Studies

- 2003 EPA Two Freshwater Beaches
- 2003 EPA Epidemiological Study
Great Lakes
- 2005 California Rapid Methods
Comparison Study

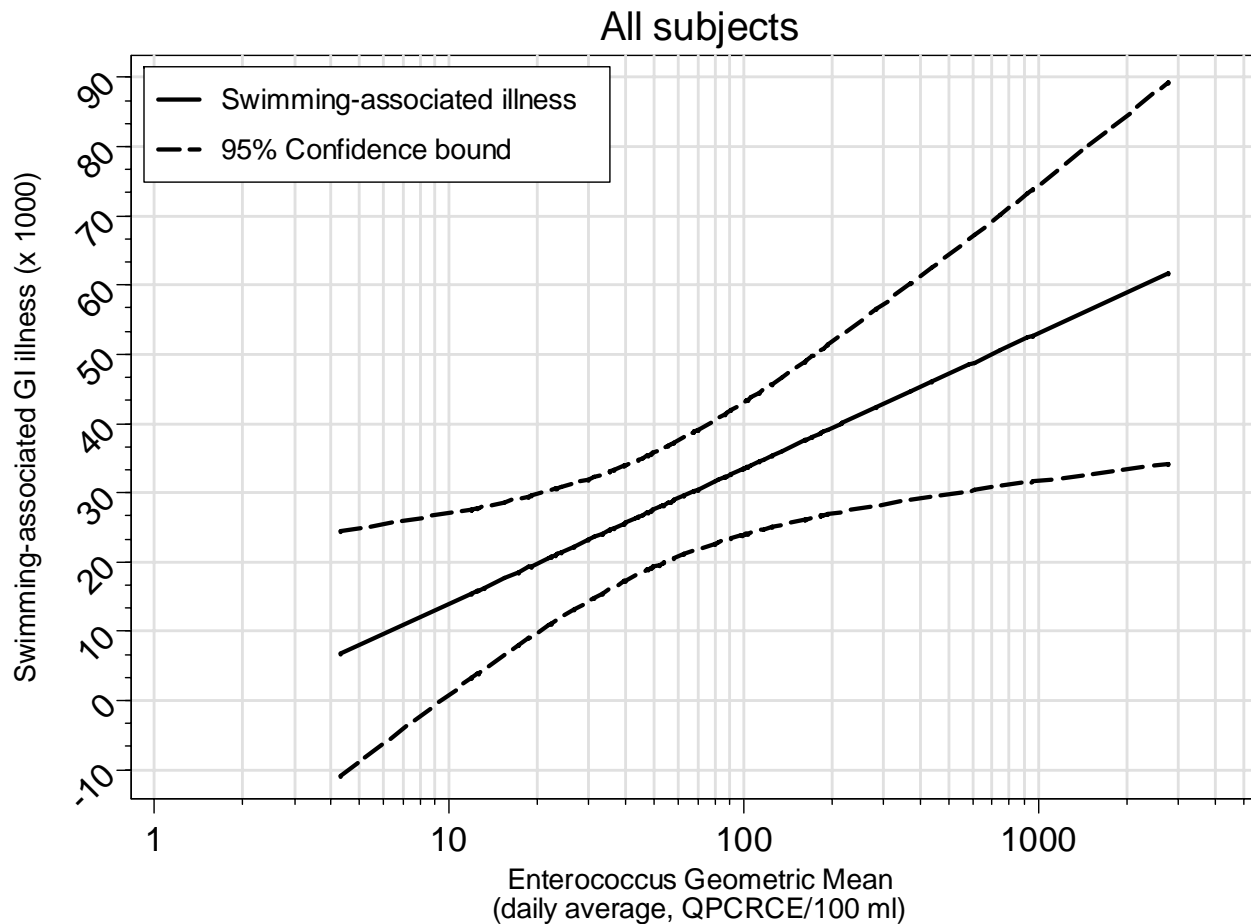
Results

Two Freshwater Beaches



Results

Epidemiological Study Great Lakes



Adjusted estimates from linear model. Censored values missing

Results

CA Rapid Method Comparison Study

- **Agreement between EPA-ORD qPCR method results and reference laboratory culture method results with respect to being above or below the 104 Enterococci/100 ml marine beach water posting criterion**

| Agreement among all samples | Agreement among samples below posting criterion by culture | Agreement among samples above posting criterion by culture |
|-----------------------------|--|--|
| 84% | 39% | 100% |

2007 NJ Beach Sampling

- Weekly sampling
 - June - August
 - 44 NJ stations
 - 26 Long Island stations



Referenced Studies

- Comparison of Enterococcus measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis
 - http://water.rutgers.edu/Source_Tracking/Enterococcus/ComparisonofEnterococcusmeasurementsinfreshwaterattwobeachesbyqPCRandEPA1600.pdf
- Rapidly Measured Indicators of Recreational Water Quality Are Predictive of Swimming-Associated Gastrointestinal Illness
 - <http://http://www.ehponline.org/realfiles/members/2005/8273/8273.pdf>
- *Evaluation of Rapid Microbiological Methods for Measuring Recreational Water Quality*, May 2006, Southern California Coastal Water Research Project
 - ftp://ftp.sccwrp.org/pub/download/PDFs/485_rapid_methods_II.pdf

Links

- The EMPACT Beaches Project - Results from a Study on Microbiological Monitoring in Recreational Waters
EPA 600/R-04/023, August 2005
 - <http://www.epa.gov/nerlcwww/empact.pdf>
- Microbial Source Tracking Guidance Document
EPA 600/R-05/064, June 2005
 - <http://www.epa.gov/ord/NRMRL/pubs/600r05064/600r05064.pdf>
- Rutgers Cooperative Research & Extension Water Resources Program
 - http://water.rutgers.edu/Source_Tracking/MST.htm

Thank you for your attention

- Any questions?