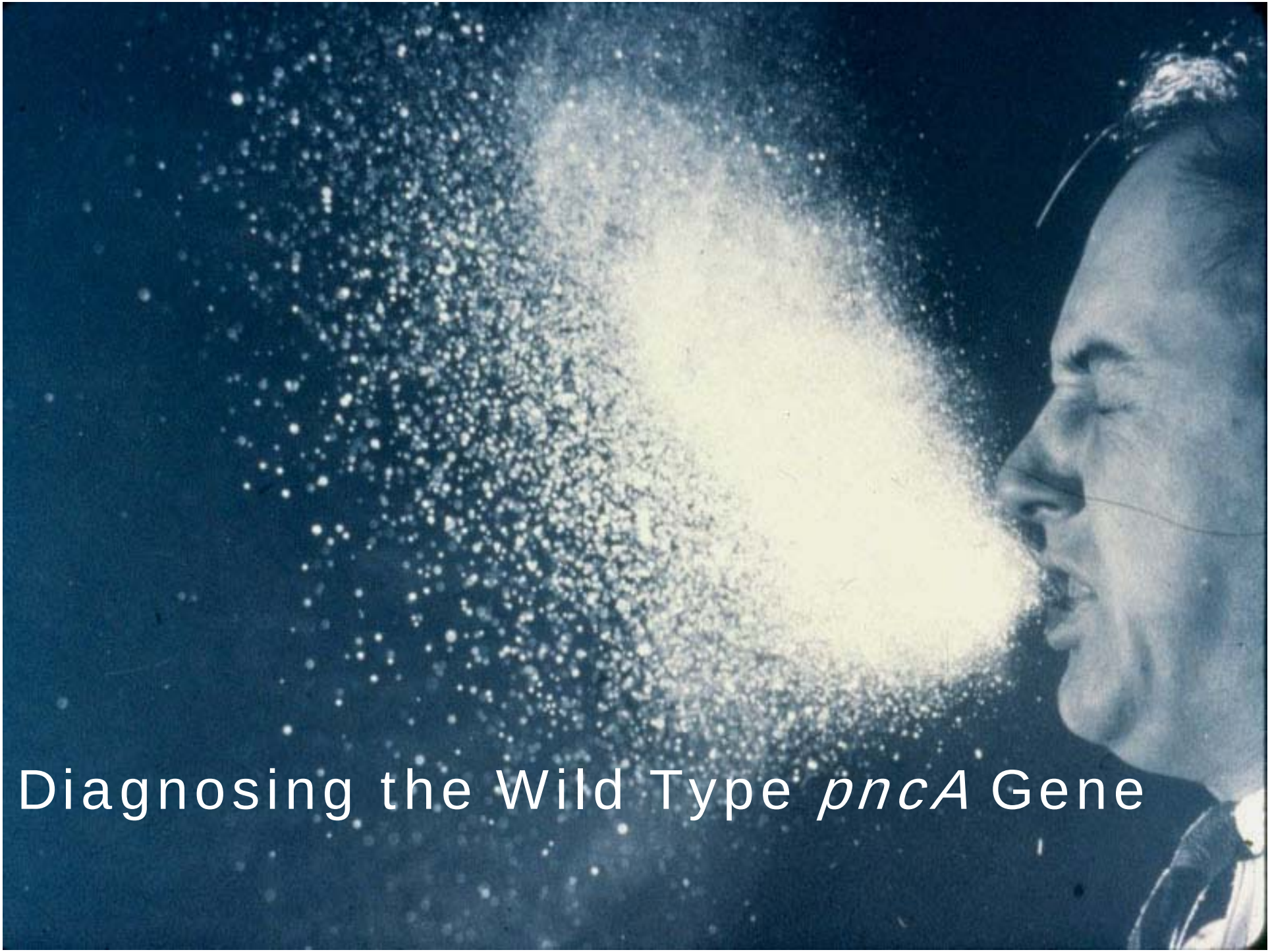

Diagnosing the Wild Type *pncA* Gene

Mycobacteriology testing in the clinical laboratory

It is not the black sheep of the lab - - It is the pig!

Susceptibility Testing of Mtb

Non-radioactive Bactec MIGIT 960 System

- First-line susceptibility testing takes 3-4 weeks
- Second-line against MDR takes 3-4 weeks

Comments:

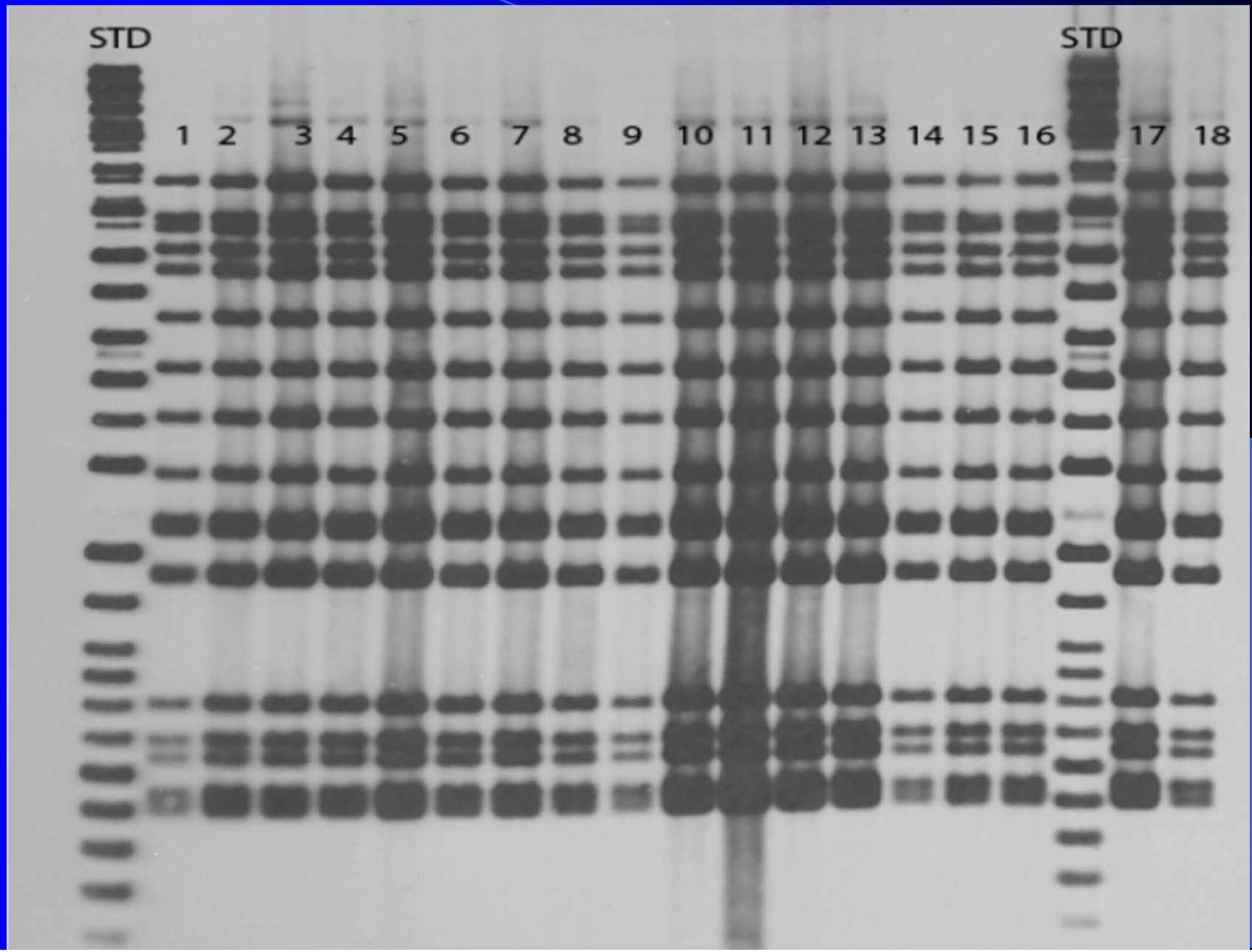
- Initial culture should be tested against both
- We should stop testing streptomycin
- We should replace it with a ciprofloxacin

Susceptibility Testing of PZA

Non-radioactive Bactec MIGIT 960 System

- Correlating MIC values to resistance varies
- High inoculums leads to false positive calls
- Low pH affects the viability of some *Mtb* isolates

Outbreak of the Multidrug Resistant “W” Tuberculosis Clone



Discrepant Results Between Pyrazinamide Susceptibility Testing by the Reference BACTEC 460TB Method and *pncA* DNA Sequencing in Patients Infected With Multidrug-Resistant W-Beijing *Mycobacterium tuberculosis* Strains*

Jillian Dormandy, BS; Akos Somoskovi, MD, PhD; Barry N. Kreiswirth, PhD; Jeffrey R. Driscoll, PhD; David Ashkin, MD; and Max Salfinger, MD

Chest 2007;131:497

- New York City DOH – Agar proportion and Bactec used to test 102 “W” strains tested. All have the same mutation in codon 47
- 44 Reported Susceptible, 58 Reported Resistant

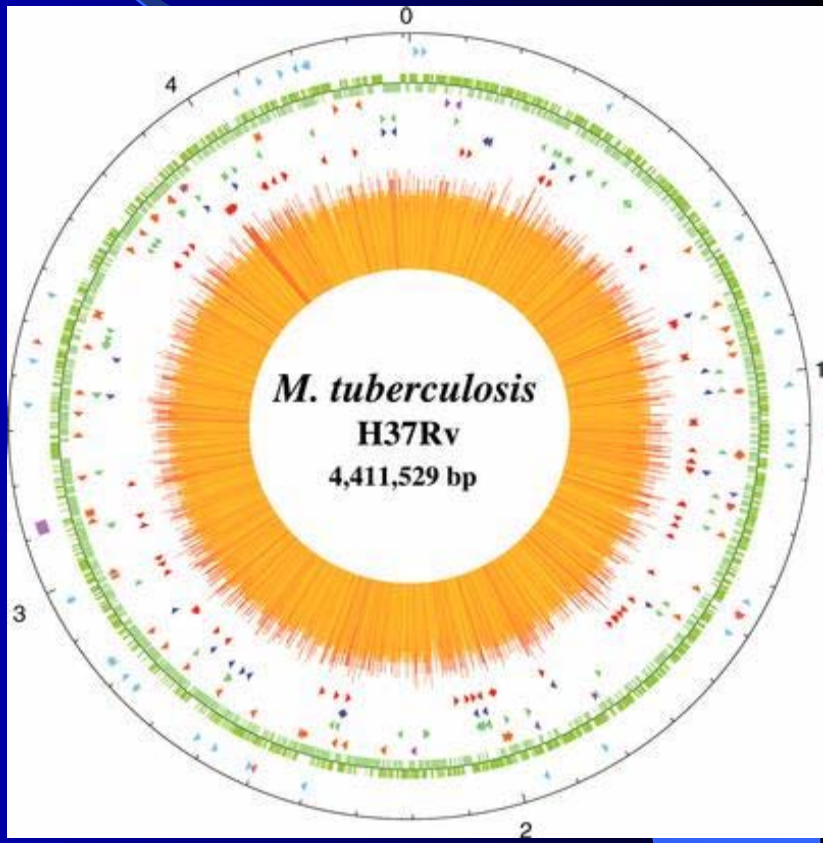
PHENOTYPIC SUSCEPTIBILITY TESTING

IT'S JUST TOO SLOW!

M. tuberculosis Genome – H37Rv

Genome size (bp): 4,411,532

- **3,959 predicted ORFs (90.8%)**
- **2,441 attributed functions**
- **912 conserved hypotheticals**
- **606 unkonwns**

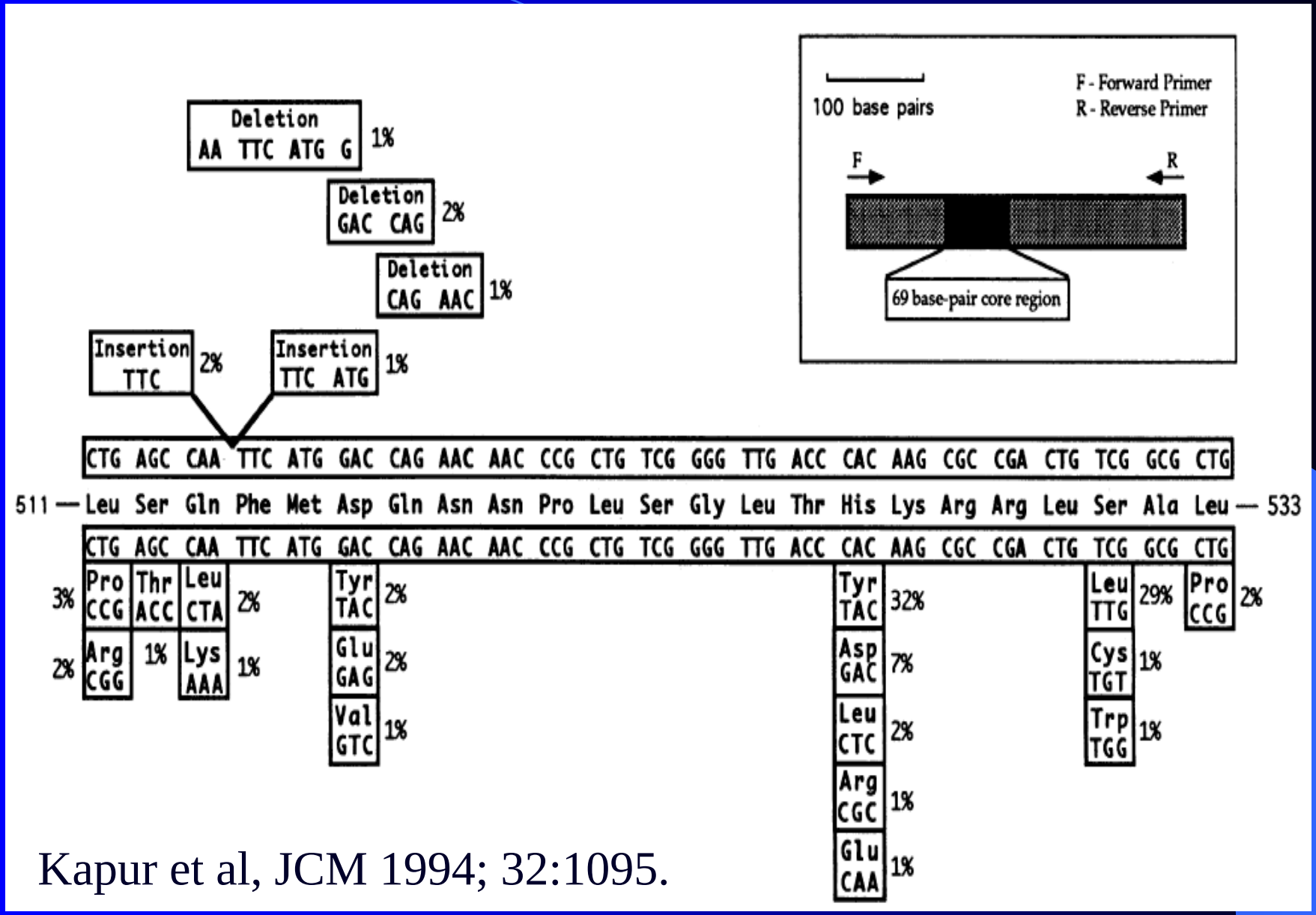


Comparative Sequence Analysis

- ***M. tuberculosis*** **genome is highly conserved**
- ***M. tuberculosis*** **is a monomorphic species**
- **Uniquely, ~2:1 number of Non-Synonymous: Synonymous base pair changes**
- **Synonymous mutations rare**
- **Non-synonymous changes in drug resistance targets correlate with clinical resistance**

NOTE: and wild type predicts susceptibility

Rifampin Resistance & *rpoB* Mutations



Kapur et al, JCM 1994; 32:1095.

Genotyping Drug Resistance

- Hain reverse hybridization line probe assay (Hain Lifescience, Nehren, Germany)
  - Multiplex PCR and reverse hybridization
  - Identifies major mutations in *rpoB, katG* and *inhA* – Detects MDR
  - Demonstrated with specimens and culture
- Cepheid (Sunnyvale, CA)
  - PCR and molecular beacon detection
  - Detection of *rpoB* – surrogate for MDR
  - Closed system with primary specimens
- Abbott's Ibis, PLEX ID (Abbott Park, IL)
  - Multiplex PCR and mass spectrometry
  - Detection platform able to detect XDR
  - Not evaluated with primary specimens

pncA Genotyping Dilemma

- There are over 100 mutations that span *pncA*
- Do not know which ones correlate with resistance
- Cannot develop a genotypic test for resistance

- No mutations in wild type *pncA*
- Only one major synonymous mutation – codon 65

DETERMINING THE WILD TYPE *pncA* GENE
WILL PREDICT PZA SUSCEPTIBILITY

Analyzing the *pncA* Gene

- **Sequencing PCR amplicon direct approach**
- **Direct sequencing is not yet routinely performed in clinical laboratories**
- **Surrogate methods for DNA sequencing**

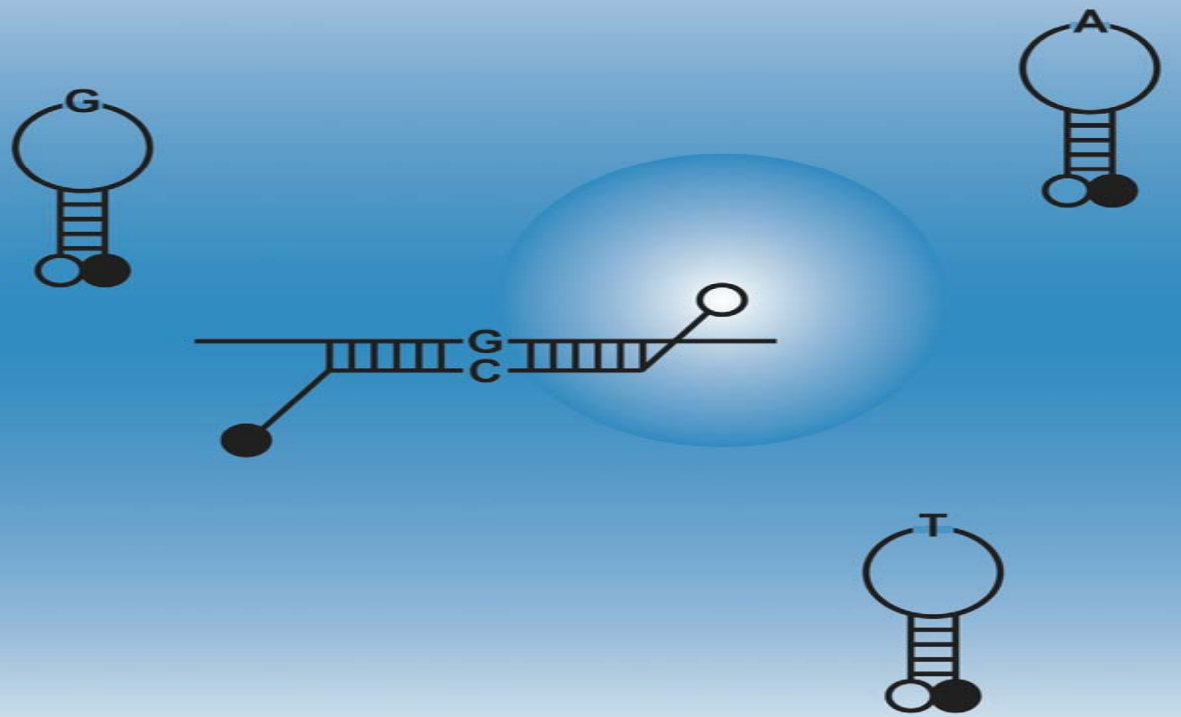
Analyzing the *pncA* Gene

THREE TECHNOLOGIES

- **Molecular Beacons**
- **High Resolution Melt Curve Analysis**
- **Lights-On / Lights-Off**

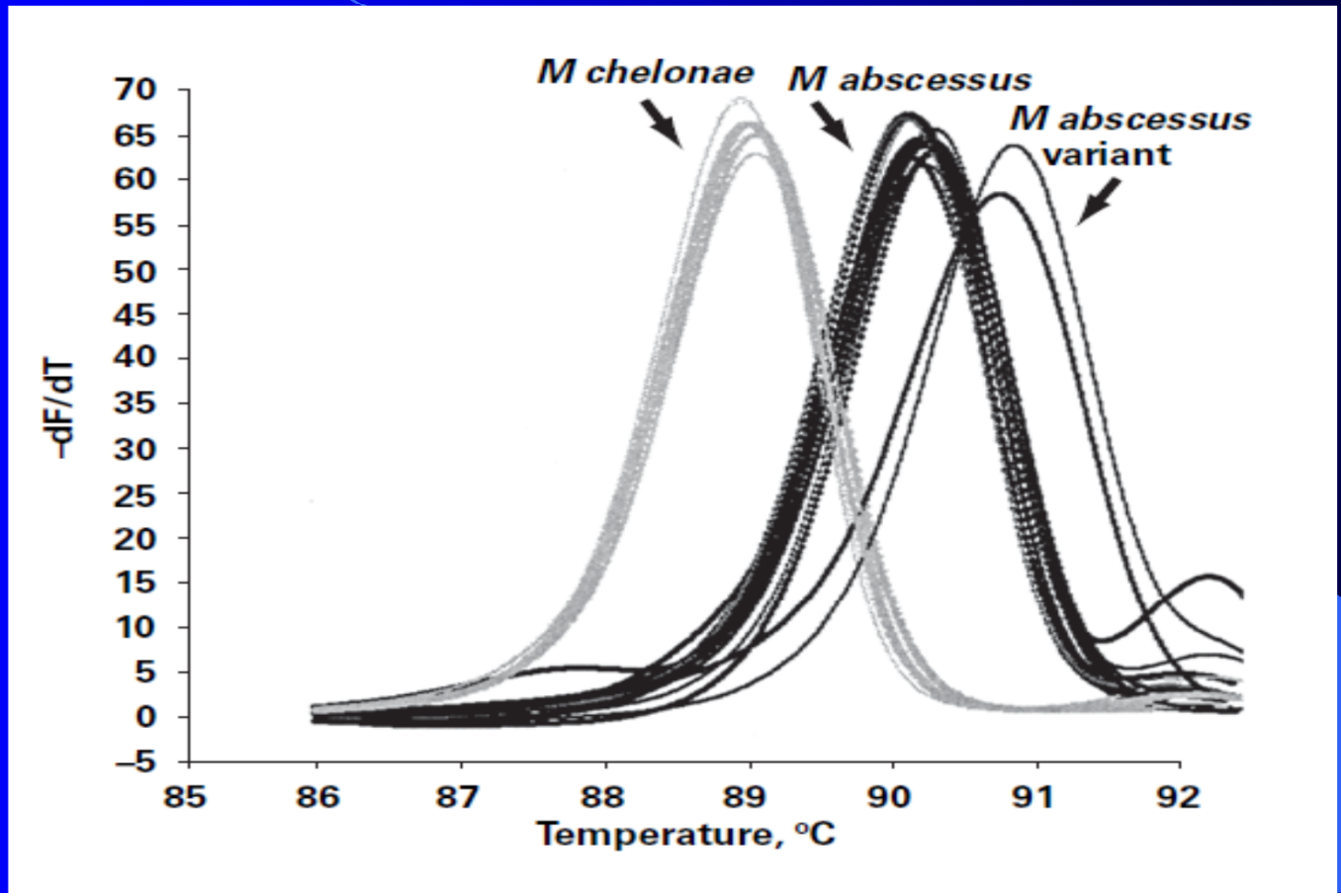
EACH TECHNOLOGY REQUIRES PROBE AND AMPLICON DESIGN THAT HAVE UNIQUE MELTING TEMPERATURES

molecular beacons

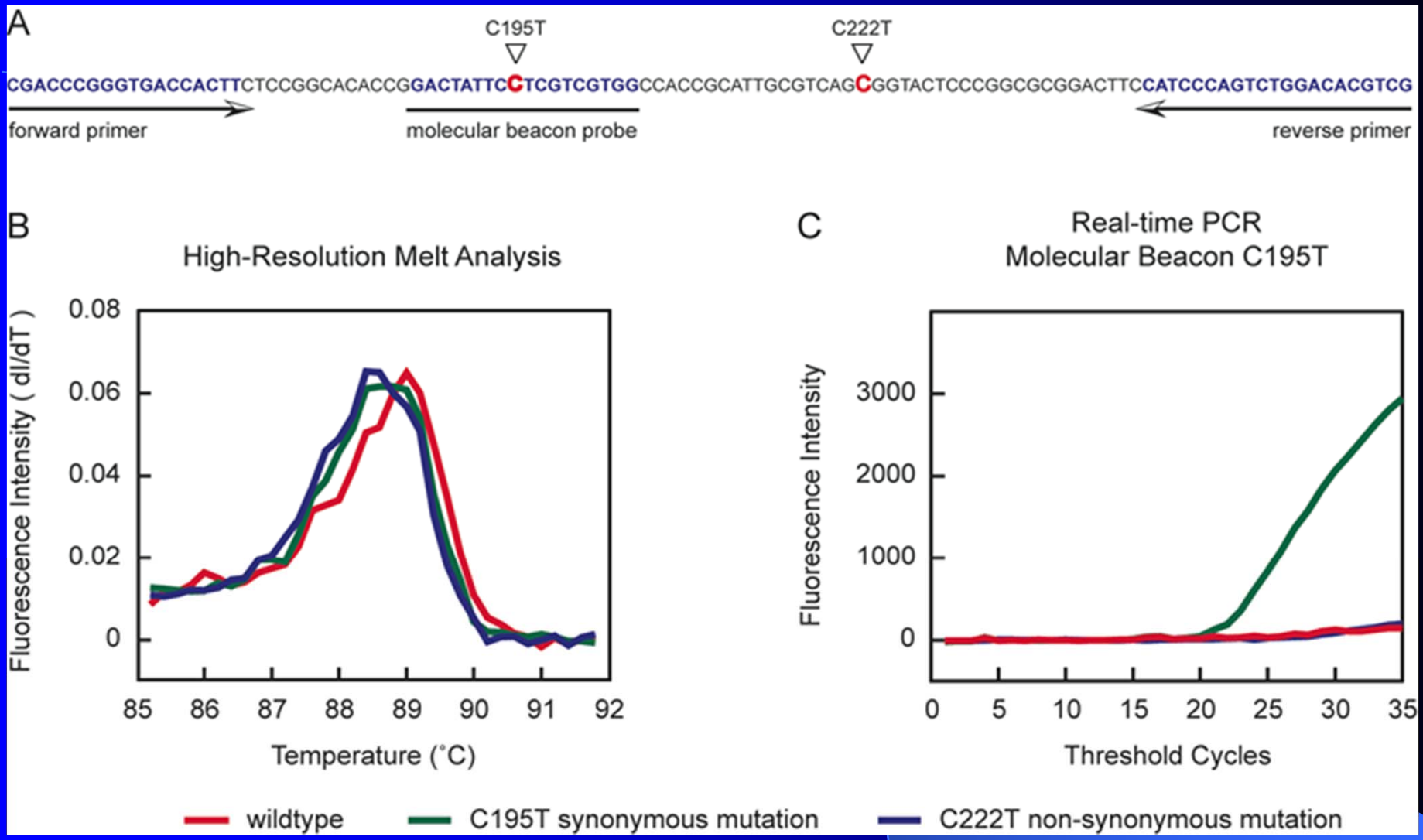


Rapid Species Identification Within the *Mycobacterium chelonae–abscessus* Group by High-Resolution Melting Analysis of *hsp65* PCR Products

Ian D. Odell,[1] Joann L. Cloud, MS, MT(ASCP),[2] Michael Seipp,[2] and Carl T. Wittwer, MD, PhD[1,2]



DETECTING *pncA* WILD TYPE USING HIGH RESOLUTION MELT CURVES PROFILES



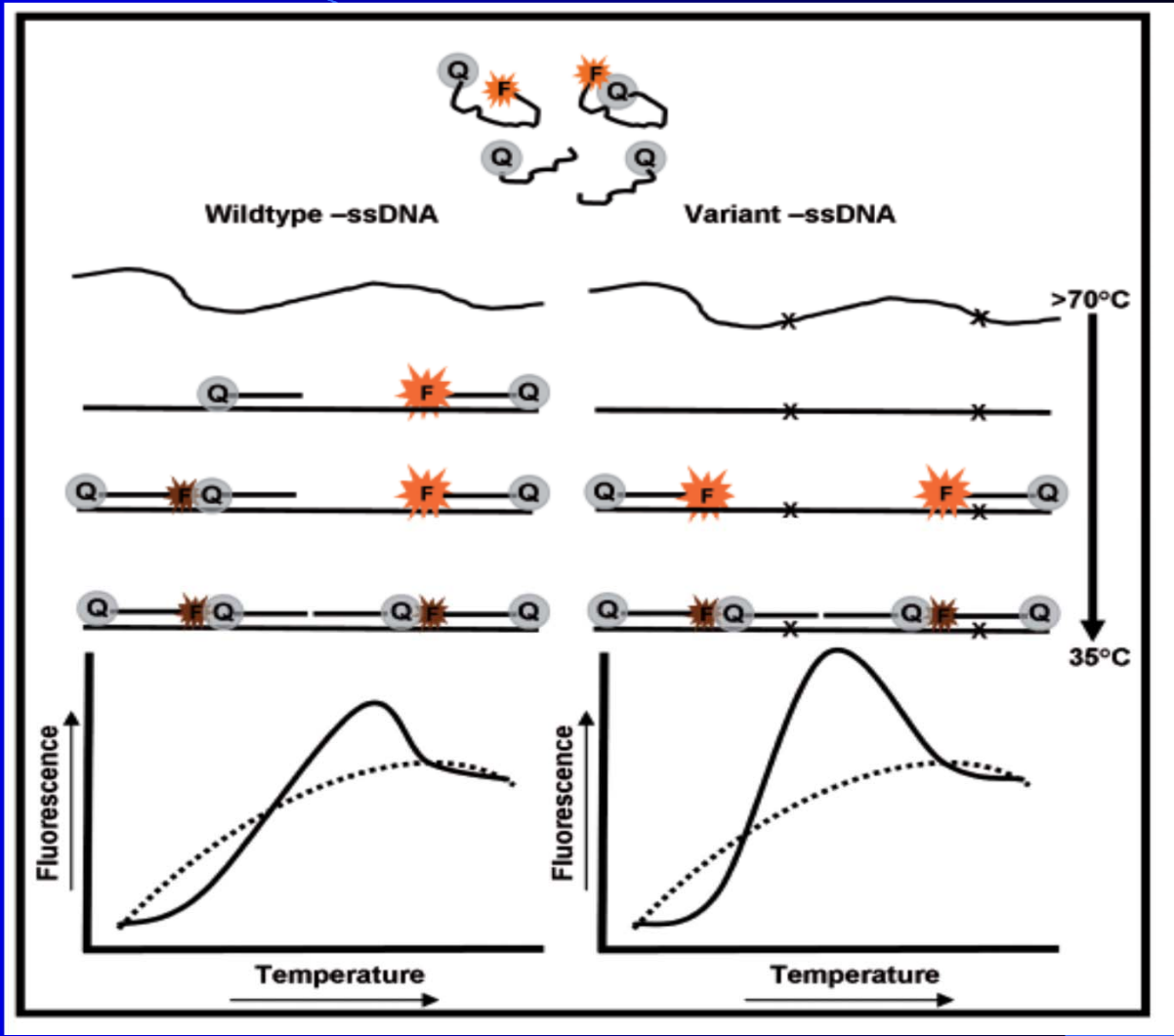
Nucleic Acids Research, 2012, 1–10
doi:10.1093/nar/gks731

Fluorescent signatures for variable DNA sequences

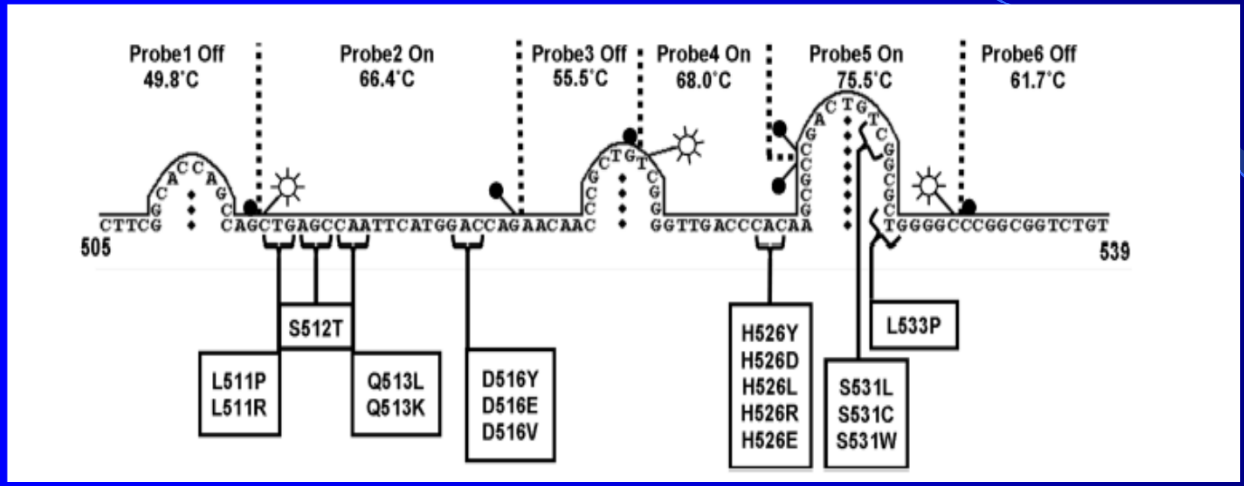
John E. Rice, Arthur H. Reis Jr, Lisa M. Rice, Rachel K. Carver-Brown and Lawrence J. Wangh*

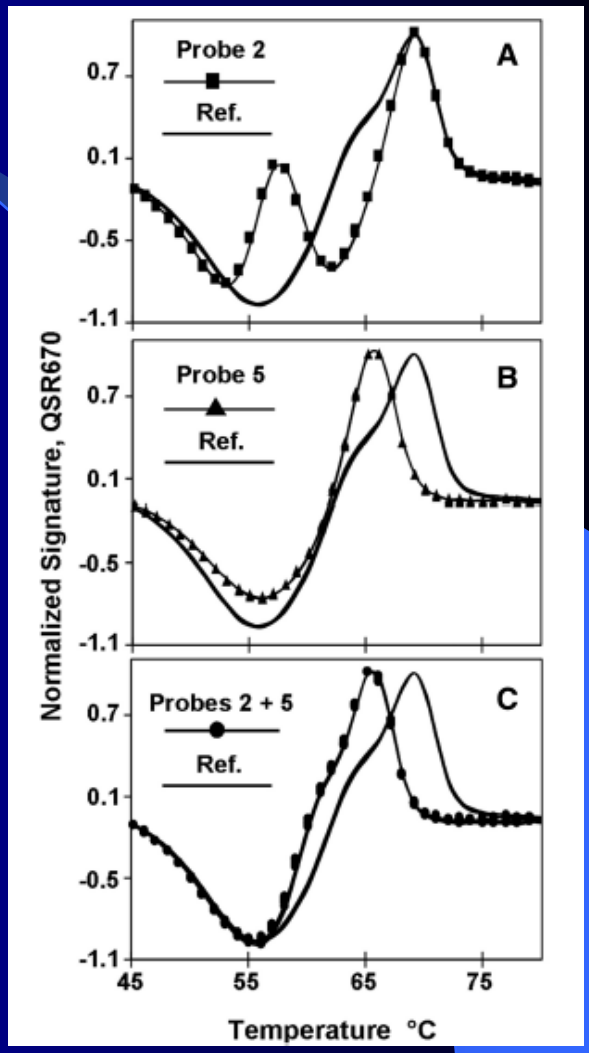
Department of Biology, Brandeis University, Waltham, MA 02454, USA

LIGHTS-ON / LIGHTS-OFF PROBES



LIGHTS-ON / LIGHTS-OFF DETECTION OF MUTATIONS IN THE RRDR REGION OF *rpoB*





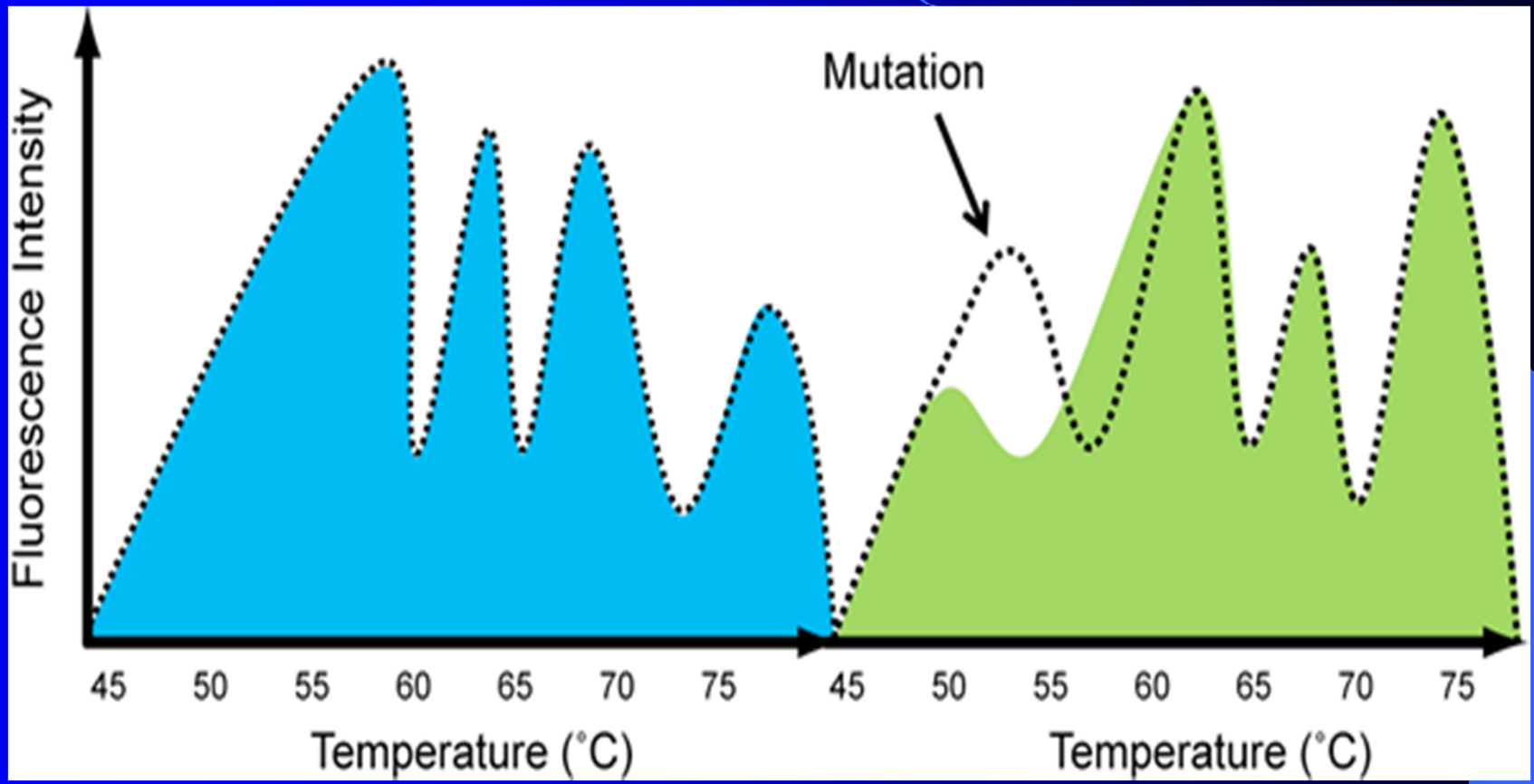
FLUORESCENT SIGNATURES

A: 516 A>G mutation

B: 533 T>C mutation

C: 516 A>G and 533 T>C mutations

PREDICTED LIGHTS-ON / LIGHTS-OFF FLUORESCENT SIGNATURES COMPARING WILD TYPE *pncA* TO A MUTANT



Current Strategy

- Develop a robust curated *pncA Mtb* strain repository with Dr. James Posey at the CDC
- Develop and test HRM and Lights-On / Lights-Off
- Both assays <2 hrs on standard RT-PCR machines
- HRM done in microtiter plates
- Lights-On / Lights-Off done in a single tube