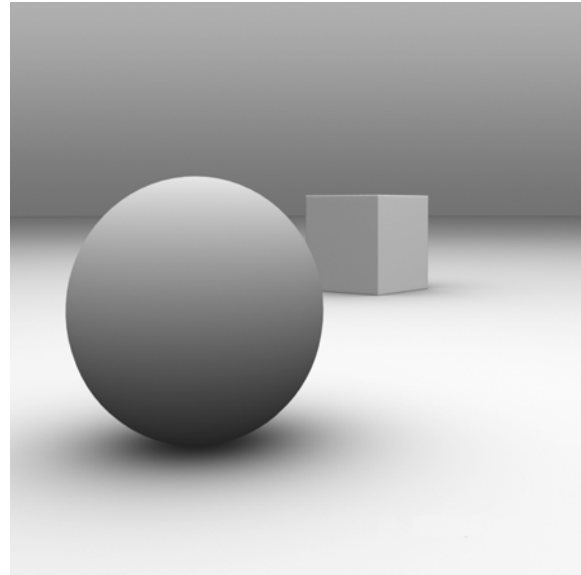


**454** LIFE  
SCIENCES

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***Taking High  
Throughput  
Sequencing To  
A New Level***



*Measuring Life One Genome at a Time*

# 454 Life Sciences

## Proven Scalable Technology



**Genome Sequencer 20**

- ✓ >20 million bases
- ✓ 100 bp reads
- ✓ 200,000+ clonal reads
- ✓ Single 5-hour run

- ❑ Complete system from library preparation through to *de novo* assembly
- ❑ Sold 20 instruments in 2005
- ❑ Peer reviewed publications (Nature, Science, etc)
- ❑ Roche Applied Sciences Worldwide distribution partner
- ❑ Sequences can now be submitted to NCBI in the SFF format

International weekly journal of science  
**nature**

15 September 2005

Volume 437 Number 7057 pp376-380

**Genome sequencing in microfabricated high-density picolitre reactors**

**454 Sequencing  
Technology Publication**

**454 Sequencing  
Woolly Mammoth**

**Scienceexpress**

**Report**

**Metagenomics to Paleogenomics: Large-Scale Sequencing of Mammoth DNA**

Hendrik N. Poinar,<sup>1a,b\*</sup> Carsten Schwarz,<sup>1a</sup> Ji Qi,<sup>2</sup> Beth Shapiro,<sup>3</sup> Ross D. E. MacPhee,<sup>4</sup> Bernard Buigues,<sup>5</sup> Alexei Tikhonov,<sup>6</sup> Daniel H. Huson,<sup>7</sup> Lynn P. Tomsho,<sup>2</sup> Alexander Auch,<sup>7</sup> Markus Rampp,<sup>8</sup> Webb Miller,<sup>2</sup> Stephan C. Schuster<sup>2\*</sup>

**Scienceexpress**

**Research Article**

**A Diarylquinoline Drug Active on the ATP Synthase of *Mycobacterium tuberculosis***

Koen Andries,<sup>1\*</sup> Peter Verhasselt,<sup>1</sup> Jerome Guillemont,<sup>2</sup> Hinrich W. H. Göhlmann,<sup>1</sup> Jean-Marc Neefs,<sup>1</sup> Hans Winkler,<sup>1</sup> Jef Van Gestel,<sup>1</sup> Philip Timmerman,<sup>1</sup> Min Zhu,<sup>3</sup> Ennis Lee,<sup>4</sup> Peter Williams,<sup>4</sup> Didier de Chaffoy,<sup>1</sup> Emma Huitric,<sup>5</sup> Sven Hoffner,<sup>5</sup> Emmanuelle Cambau,<sup>6</sup> Chantal Truffot-Pernot,<sup>6</sup> Nacer Lounis,<sup>6</sup> Vincent Jarlier<sup>6</sup>

**454 Sequencing  
Antibiotic Resistance**

**454 Sequencing  
Microbial Ecology**

Research article

**Using pyrosequencing to shed light on deep mine microbial ecology under extreme hydrogeologic conditions**

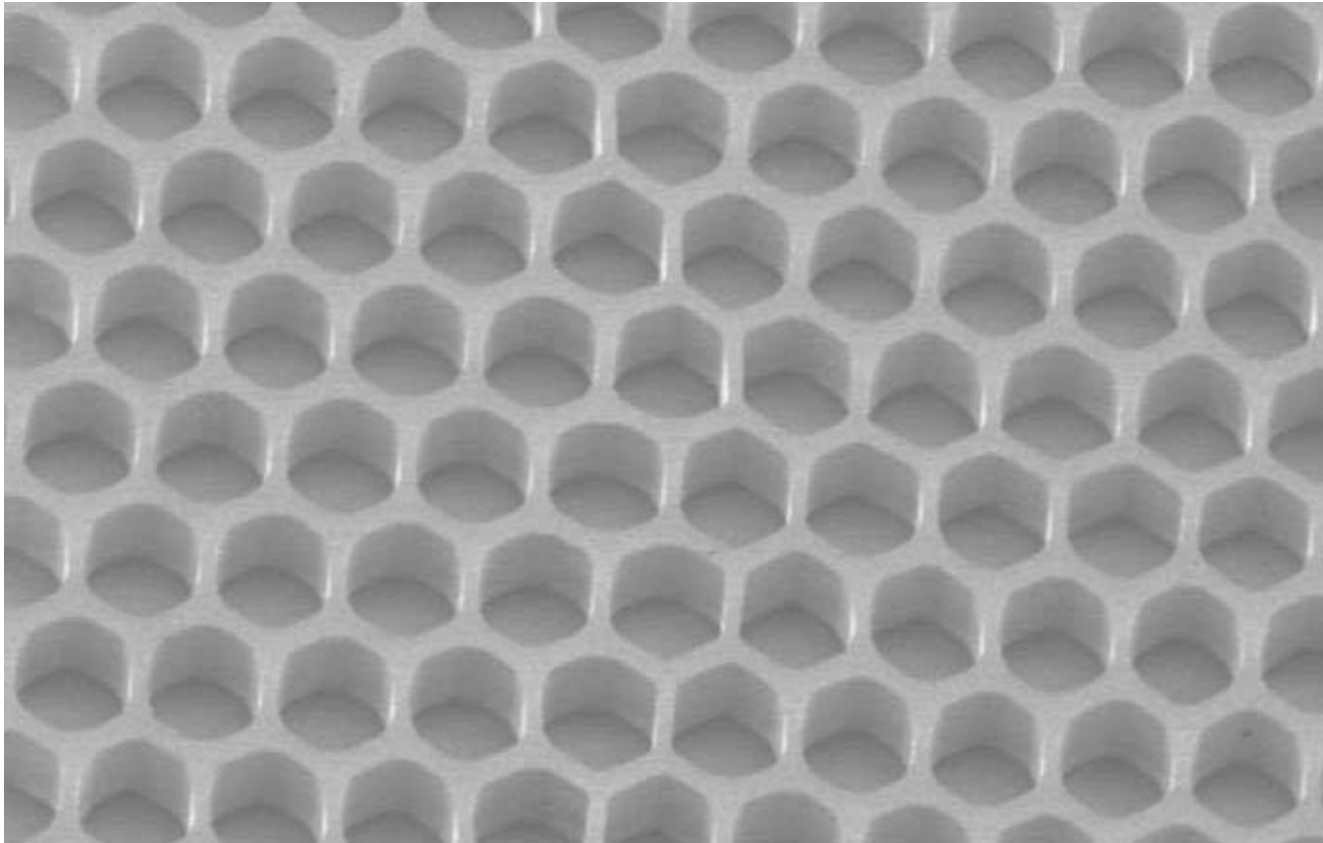
**Robert A Edwards** ✉, **Beltran Rodriguez-Brito** ✉, **Linda Wegley** ✉, **Matthew Haynes** ✉, **Mya Breitbart** ✉, **Dean M Peterson** ✉, **Martin O Saar** ✉, **Scott Alexander** ✉, **E. Calvin Alexander Jr** ✉ and **Forest Rohwer** ✉

*BMC Genomics* 2006, **7**:57 doi:10.1186/1471-2164-7-57

**Published** 20 March 2006

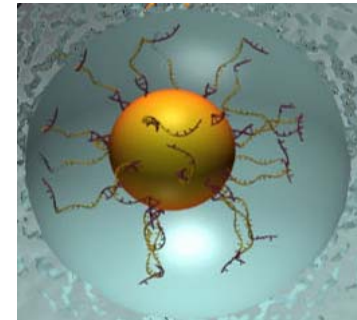
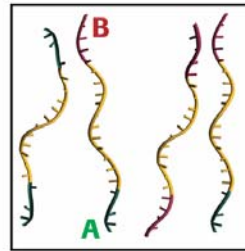
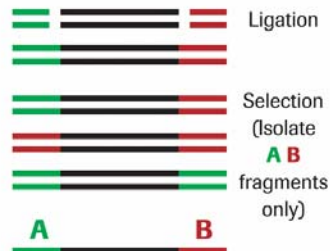
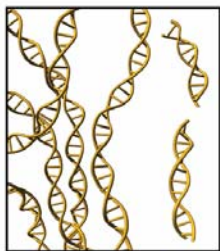


# PicoTiterPlates™



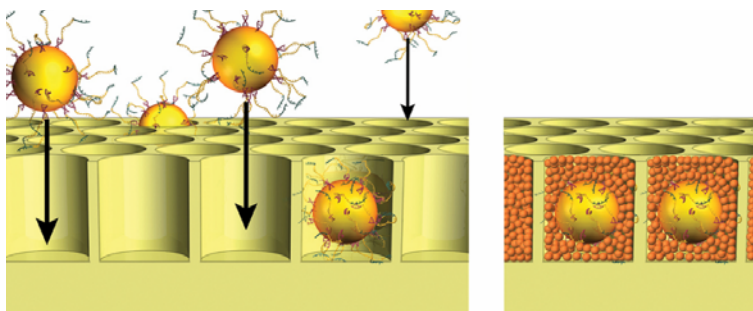
- ❑ Multiple optical fibers are fused to form an optical array
- ❑ Selective removal of core material generates reaction wells
- ❑ Reactions occurring in the ‘test tubes’ are monitored optically, through the remaining fiber

# Overview of The 454 Sequencing System

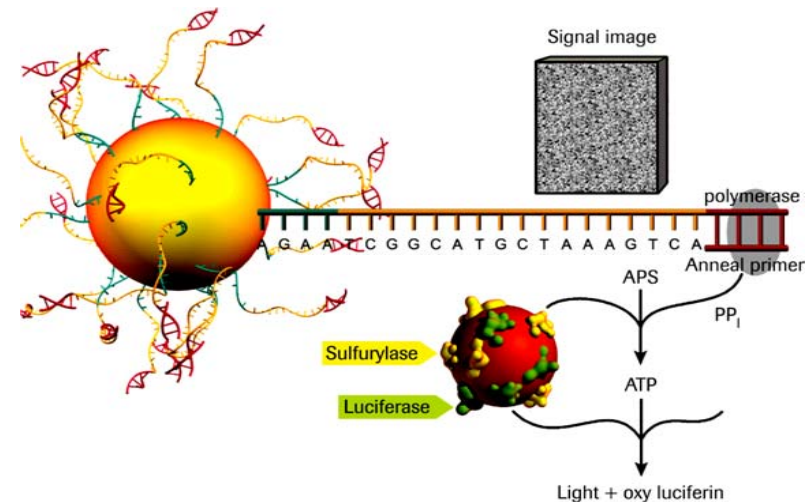


1) Prepare Adapter Ligated ssDNA Library

2) EmPCR: Clonal Amplification on beads

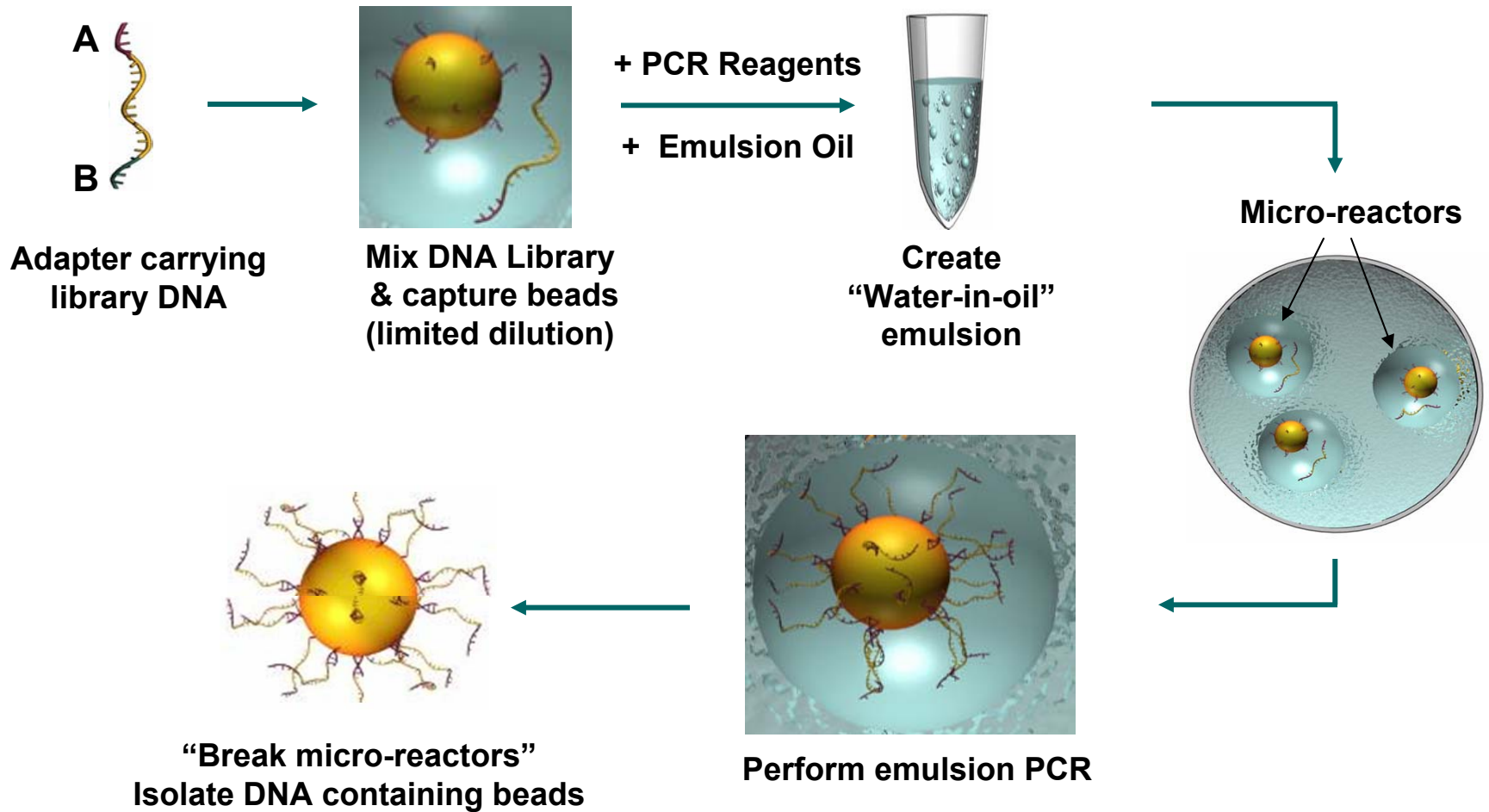


3) Load beads and enzymes in PicoTiter Plate™



4) Sequencing-by-synthesis on the GS 20 Sequencer

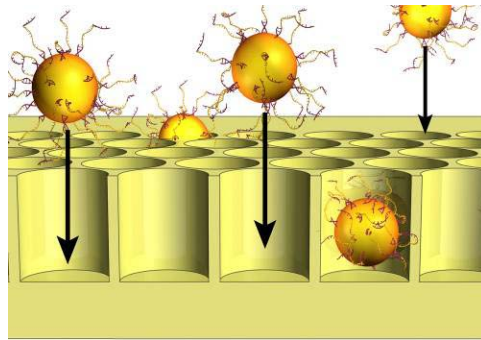
# Emulsion Based Clonal Amplification



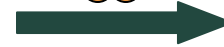
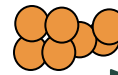
- Generation of millions of clonally amplified sequencing templates on each bead
- No cloning and colony picking

# Depositing DNA Beads into the PicoTiter™ Plate

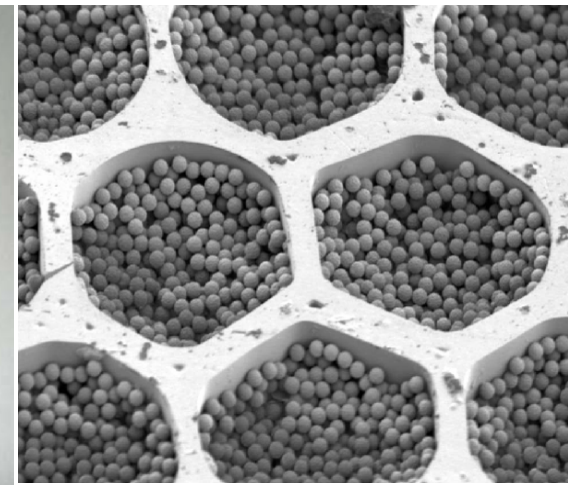
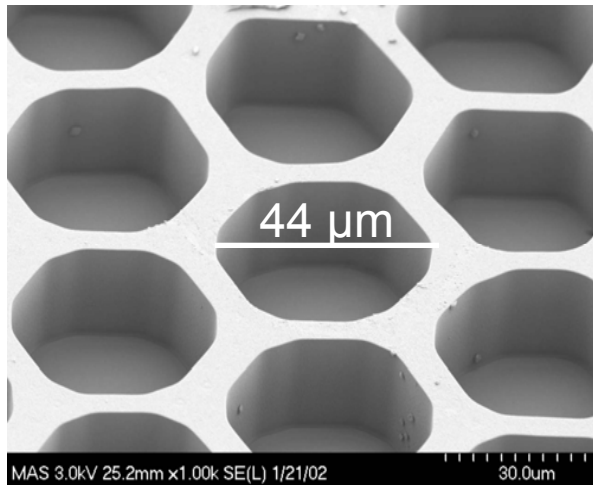
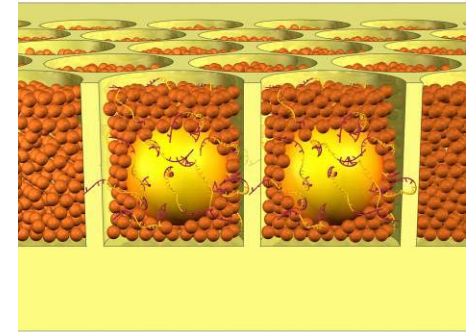
Load beads into PicoTiter™ Plate



Load Enzyme Beads



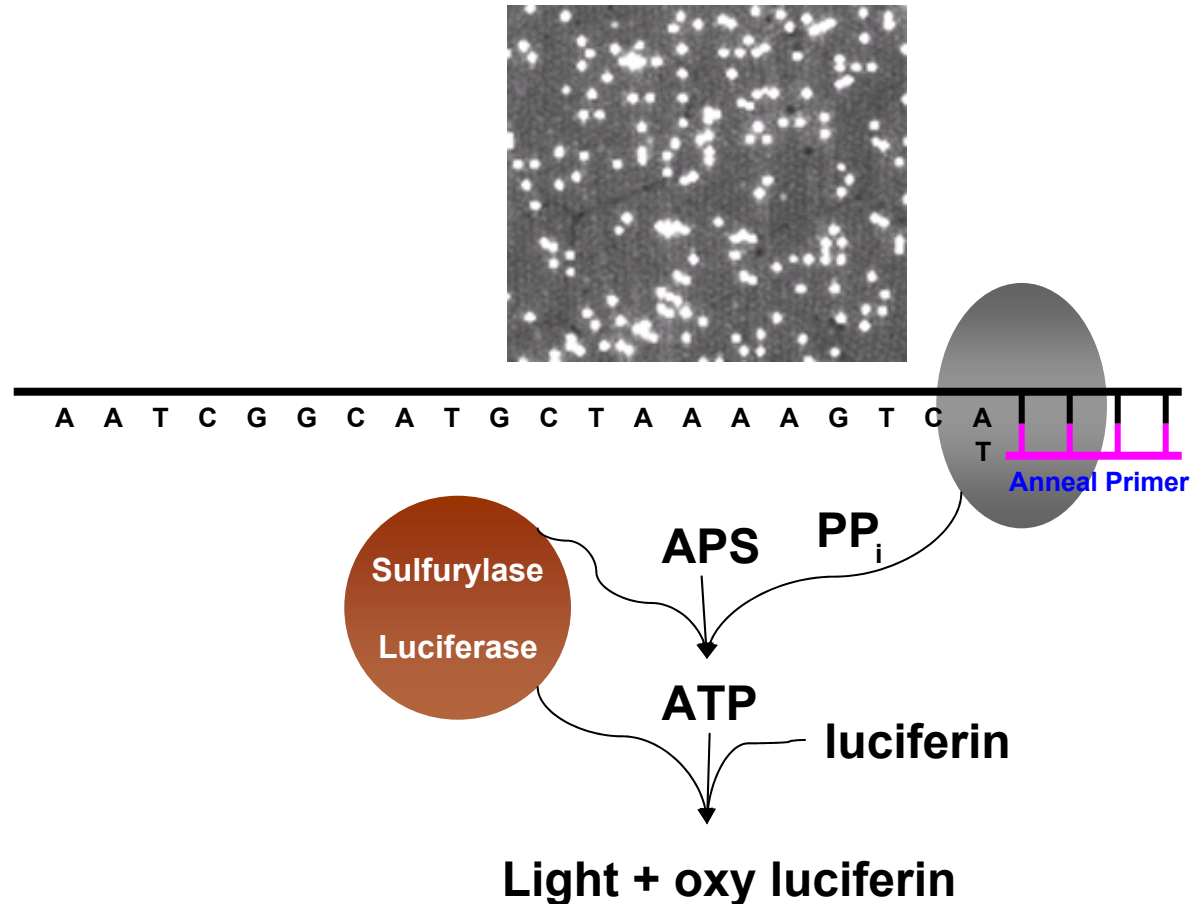
Centrifuge Step



# Sequencing-By-Synthesis

- ❑ Simultaneous sequencing in hundreds of thousands of picoliter-size wells
- ❑ Pyrophosphate signal generation

DNA Capture Bead  
Containing Millions of  
Copies of a Single  
Clonal Fragment





# Microbial Genome Sequencing with the 454 Life Sciences GS20

# Results from *de novo* assembler

	<i>M. genitalium</i>	<i>S. pneumoniae</i>	<i>E.coli</i>	<i>B.licheniformis</i>
<b>Genome Size:</b>	<b>580,069</b>	<b>2,014,239</b>	<b>4,639,675</b>	<b>4,222,645</b>
<b>Number of Runs:</b>	<b>0.5</b>	<b>2</b>	<b>3</b>	<b>3</b>
<b>Assembly Contigs:</b>	<b>19</b>	<b>228</b>	<b>140</b>	<b>105</b>
<b>Assembly Cover:</b>	<b>96.66%</b>	<b>92.46%</b>	<b>97.46%</b>	<b>98.62%</b>
<b>Overall Accuracy:</b>	<b>99.993%</b>	<b>99.991%</b>	<b>99.998%</b>	<b>99.993%</b>
<b>Avg. Contig Size:</b>	<b>29.5 kb</b>	<b>8.8 kb</b>	<b>32.4 kb</b>	<b>39.7 kb</b>
<b>N50 Contig Size:</b>	<b>41.0 kb</b>	<b>14.0 kb</b>	<b>67.2 kb</b>	<b>74.3 kb</b>
<b>Largest Contig:</b>	<b>130 kb</b>	<b>66 kb</b>	<b>164 kb</b>	<b>262 kb</b>

- Contig breaks are repeat sequences

# 454 Sequencing: Genetic Study of a 9MB “Evolved” Genome

## ❑ Project

- Given 9MB genome that was experimentally evolved for 1000 generations
- Sequence to 19x, map against original strain sequence and identify mutations

## ❑ Results

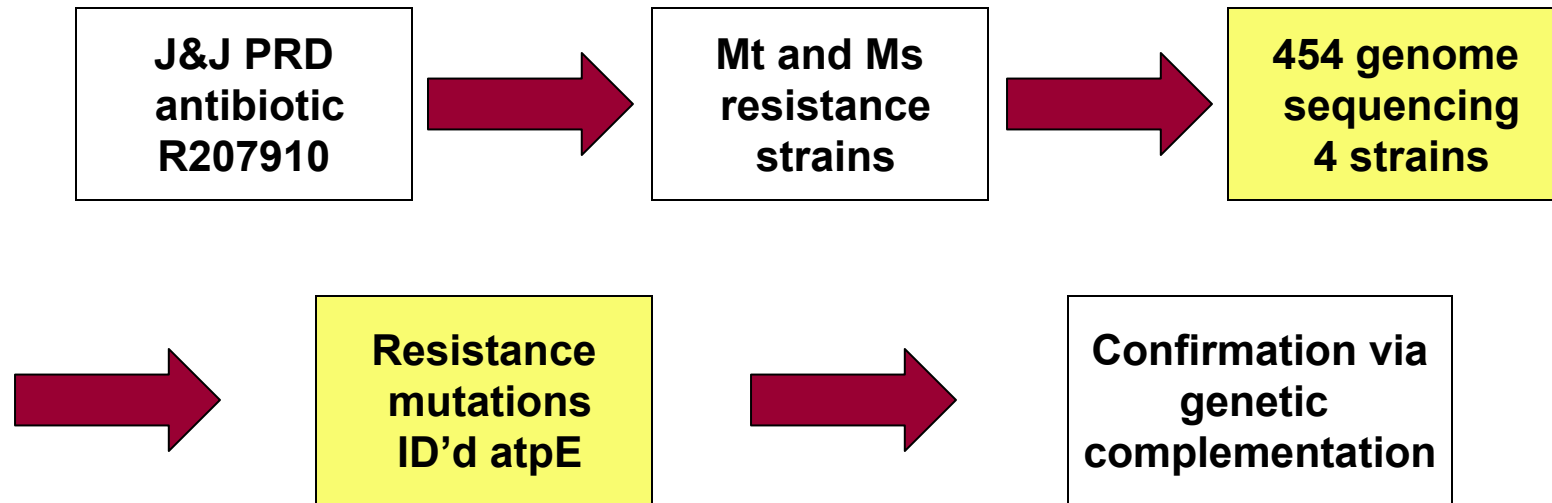
- 15 mutations identified by 454 sequencing
  - All confirmed by PCR and Sanger sequencing
- No additional mutations found by confirmatory sequencing
  - 45% of evolved strain sequenced by Sanger sequencing
- Manuscript has been submitted

# 454 Sequencing and Drug Development

## *M. tuberculosis* antibiotics

- ❑ Johnson & Johnson PRD developed novel antimycobacterial drug
  - R207910, a diarylquinoline, with potent and selective antimycobacterial activity in vitro
  - R207910 does not inhibit DNA gyrase – the target of quinolones
  - Resistance mutants were selected to study mechanism of action
- ❑ Mechanism of action through whole genome sequencing
  - 4 genomes sequence to near completion
    - 1 resistant *M. t.* (4 Mb), 2 resistant *M. smegmatis* (6 Mb) strains, parent *M. s*
  - Comparison of genomes from resistant and parental strain
  - Validation of results through complementation experiments

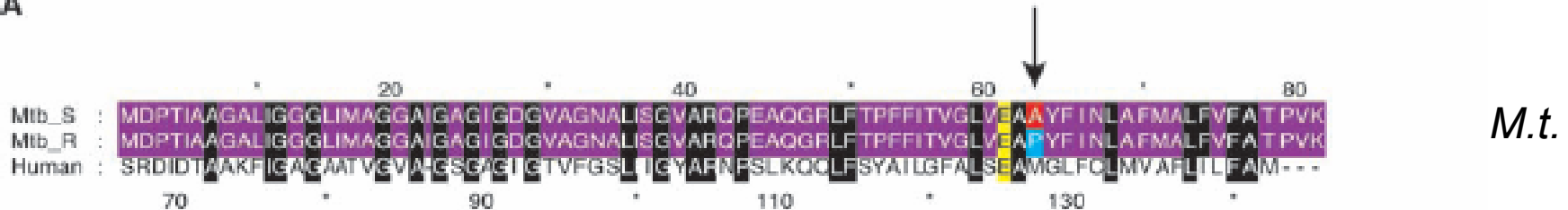
# 454 Sequencing and Drug Development *M. tuberculosis* & *M. smegmatis*



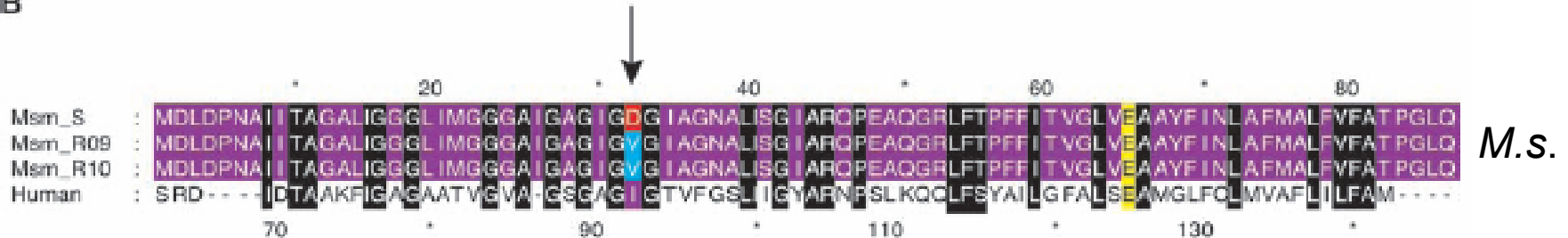
- ❖ GS20 generates >20Mbp/run
- ❖ Mycobacterial genome ~4Mbp to 6Mbp
- ❖ 3 to 4 fold coverage of Mycobacterial genome in 5 hour run
- ❖ Sequencing four bacterial genomes to near completion is less than a two week project with the 454 GS20

# 454 Sequencing reveal R201970 resistance mutations in *M. smegmatis* and *M. tuberculosis*

A



B



- Resistance mutations in the membrane spanning region of atpE.
- No mutations found in DNA gyrase genes as typical for resistance to quinolone drugs

# 454 Sequencing in Drug Development

- ❑ The genomes of the resistant *M. tuberculosis* strain BK12 and the two resistant *M. smegmatis* strains R09 and R10, as well as the parental *M. smegmatis*, were sequenced to near completion.
- ❑ R207910-resistance-conferring point mutations were identified by comparative analysis of the genome sequences of susceptible and resistant strains of *M. tuberculosis* and *M. smegmatis*
- ❑ The only gene commonly affected in all three independent mutants encodes for **atpE**, a part of the F0 subunit of ATP synthase.
- ❑ This finding indicates that R207910 inhibits the proton pump of *M. tuberculosis* ATP synthase.
- ❑ These findings confirmed by genetic complementation experiments

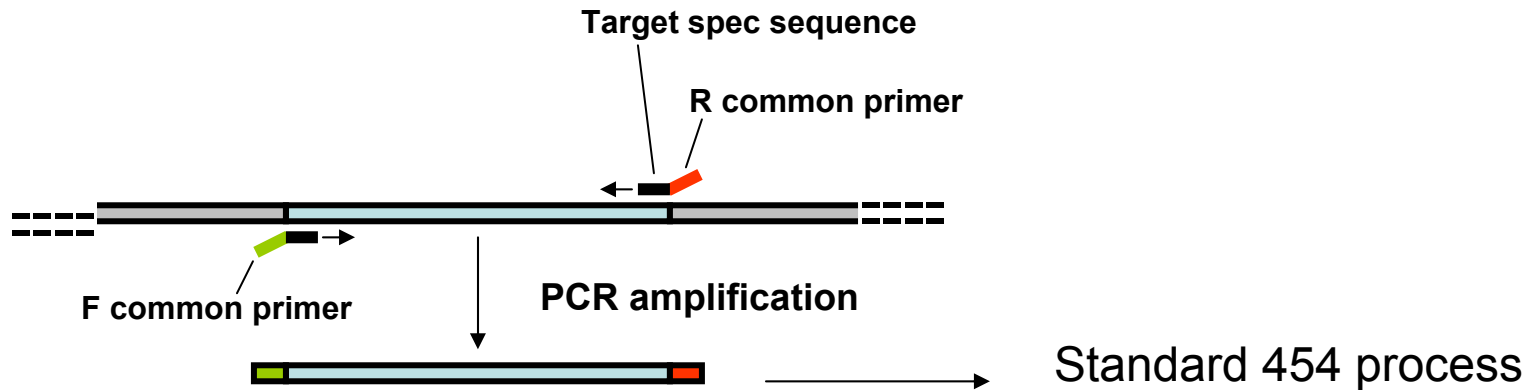
**Science**express

Research Article  
2005 Jan 14;307(5707):223-

**A Diarylquinoline Drug Active on the ATP Synthase of *Mycobacterium tuberculosis***

Koen Andries,<sup>1\*</sup> Peter Verhasselt,<sup>1</sup> Jerome Guillemont,<sup>2</sup> Hinrich W. H. Göhlmann,<sup>1</sup> Jean-Marc Neefs,<sup>1</sup> Hans

# Ultra Deep Sequencing of PCR Amplicons

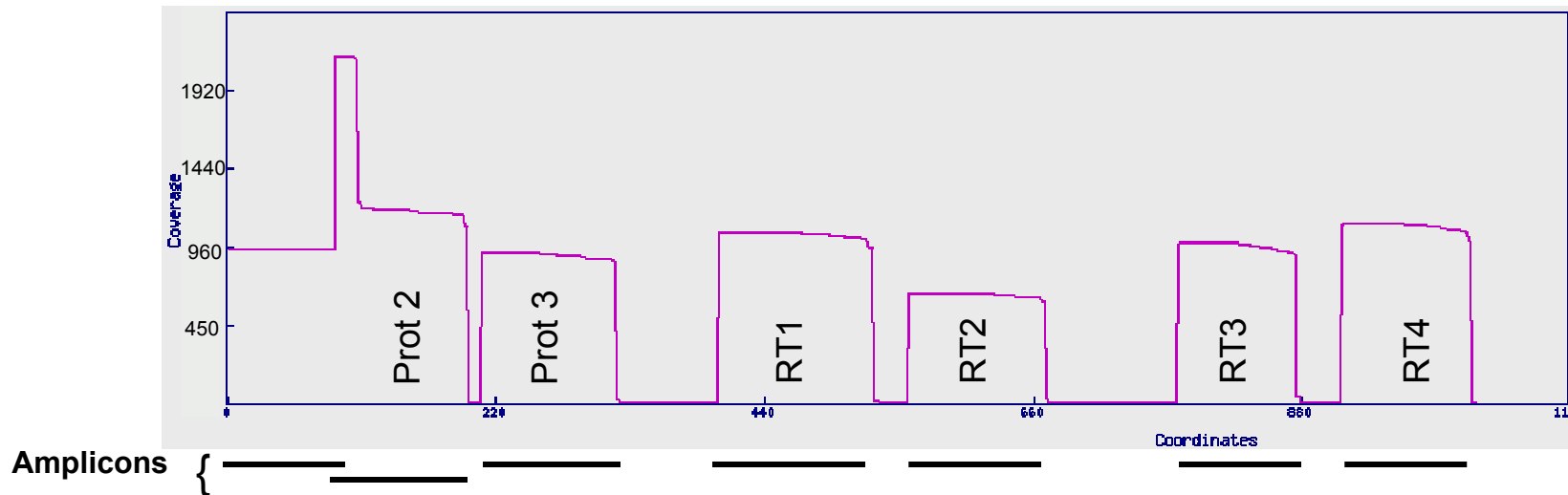


- ❑ Exploits the “instant cloning” in the emulsion PCR process and the 1000s of reads generated in a single run
- ❑ No *a priori* knowledge of sequence variants required
  - Detection of substitutions, insertions and deletions **in complex mixtures**
- ❑ An unlimited number of amplicons can be processed in the same emulsion PCR and sequencing reaction
- ❑ Applications include:
  - Detection of somatic mutations in tumor biopsies (mixed with normal cells)
  - Low abundance viral variant detection
  - Population based studies of sequence variants
  - Bacterial strain identification

# 454 Sequencing Mutation Detection

## Ultra Deep Sequencing of HIV

- Coverage plot for 7 simultaneously sequenced amplicons from regions of HIV protease and RT genes



- Multiplexing only limited by accuracy of quantification and desired overall coverage depth

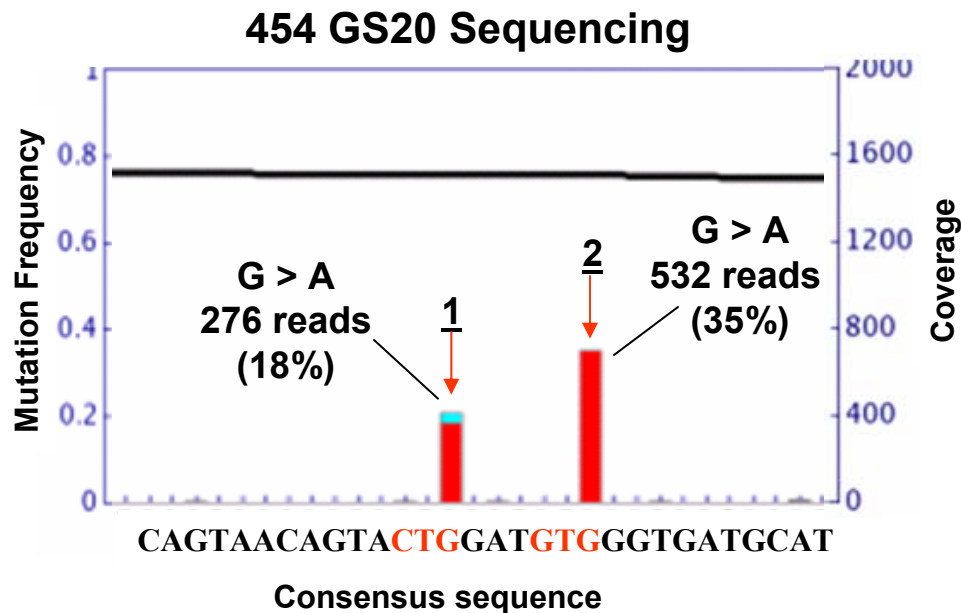
In collaboration with Dr. M. Kozal, Yale VA Hospital

# Ultra Deep Sequencing of HIV

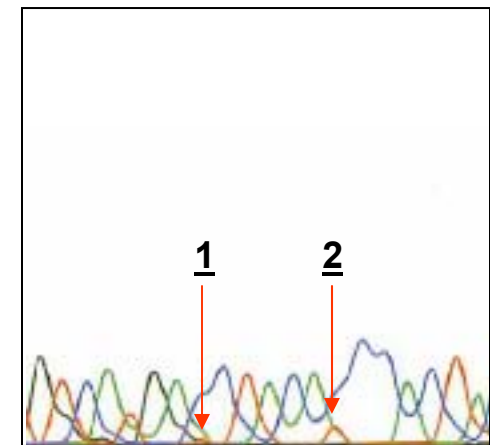
## Sensitive and Accurate Mutation Detection

- Direct amplification and sequencing of HIV RT gene from clinical sample

Sequencing of 156 bp amplicon covering codons 84 to 124 of RT gene



### Corresponding Sanger Trace



GTCATGACCTACACCCACTA

Consensus sequence (complement)

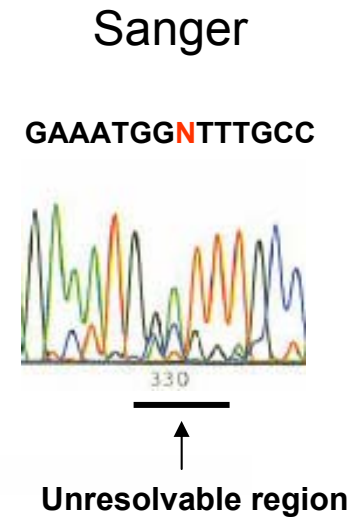
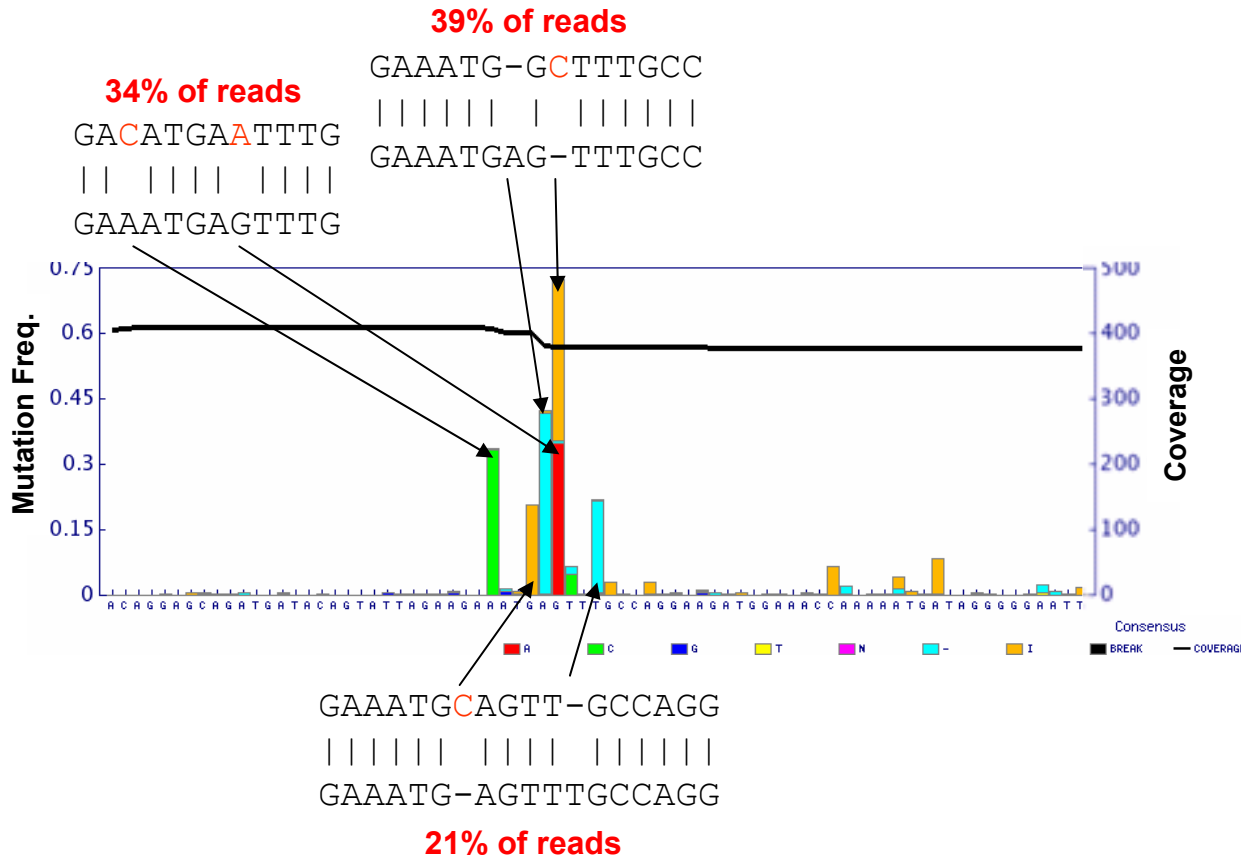
- Digitalized mutation detection provides superior sensitivity

In collaboration with Dr. M. Kozal, Yale VA Hospital

# Ultra Deep Sequencing of HIV Subspecies Identification

- Digital sequencing of each template allows identification and quantification of distinct subspecies, including haplotypes

Sequencing of 207 bp amplicon from protease gene

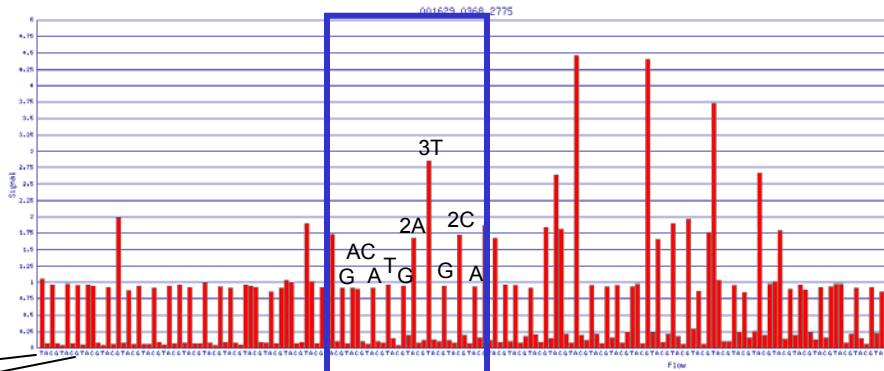


In collaboration with Dr. M. Kozal, Yale VA Hospital

# Ultra Deep Sequencing of HIV Individual Sequencing Traces

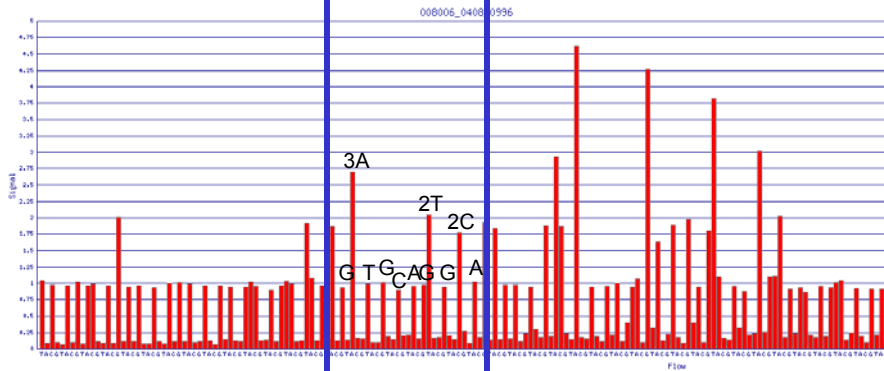
Nucl flow order:  
TACG

TACGTACG....



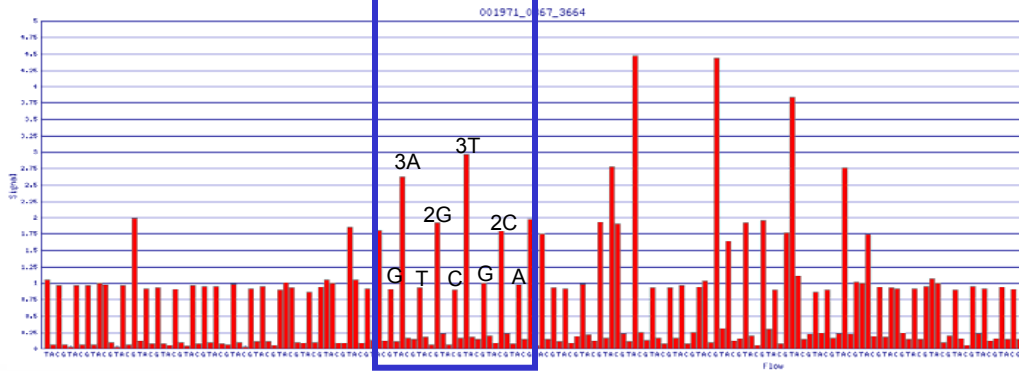
GACATGAATTGCCA

Ref GAAATGAGTTTGCCA



GAAATGCAGTT-GCCA

Ref GAAATG-AGTTTGCCA



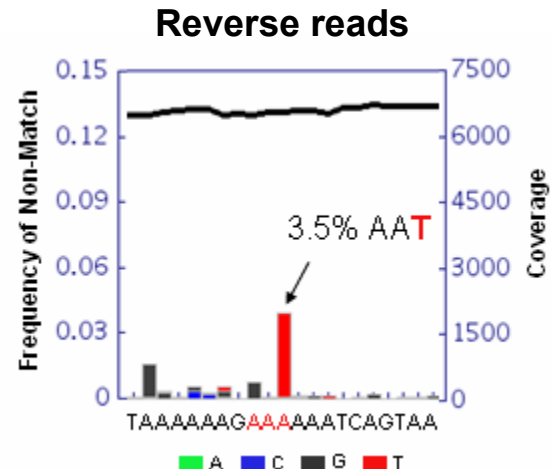
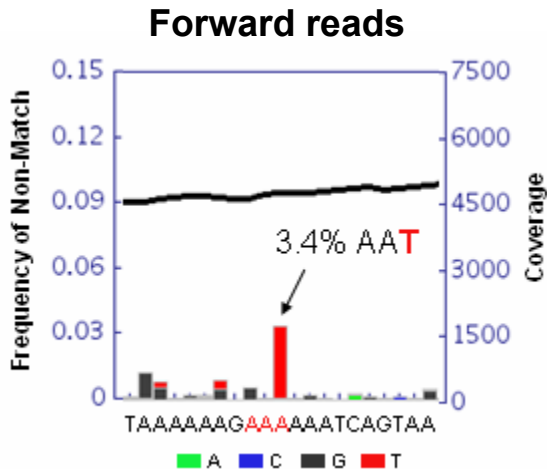
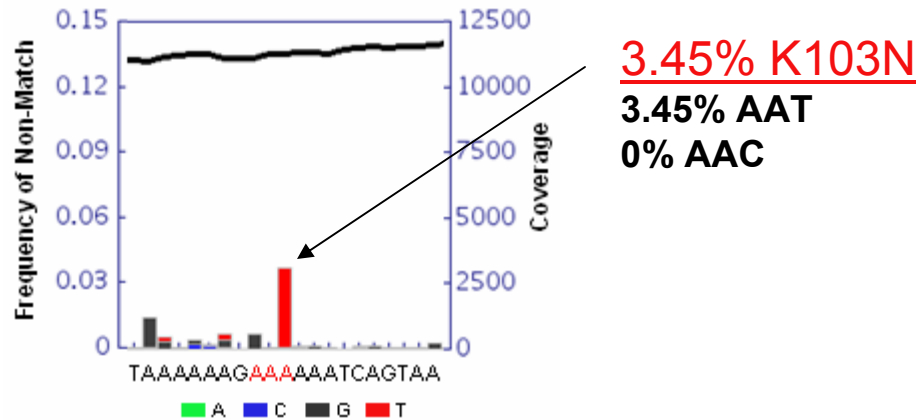
GAAATG-GCTTTGCCA

Ref GAAATGAG-TTTGCCA

# HAART-Experienced Patient Sample

- ▣ **K103N mutation detected in post-antiretroviral treatment sample**
  - Mutation undetectable by Sanger sequencing

Stavudine  
Lamivudine  
Efavirenz



# 454 Sequencing Overview

- ❑ 100X Increase in Throughput Over Sanger Sequencing
  - Sequencing over 20 Mbp per 5 hour run
  - Consensus accuracy of 99.99+% at 10-15X over-sampling
- ❑ One Person can Sequence a Genome from Start to Finish
  - Single sample preparation for any size genome
  - Sample preparation to sequencing requires only basic laboratory skills
  - Fast – days from isolate to assembled sequence
- ❑ Proven technology
  - Instruments sold to major genome centers, et al
  - Roche Applied Science distributes instruments and reagents
  - 454 Sequencing Center provides sequencing services
  - Award Winning
    - *Wall Street Journal*
    - *Popular Science*

