

# DNA Bar Coding and Pyrosequencing to Identify Rare HIV Drug Resistance Mutations

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# Background

- Treatment of HIV with antiretroviral agents selects for drug-resistant mutants
- Undetected rare resistance mutations facilitate viral escape
- Concern for increasing HIV drug resistance in the developing world

## Aim

- Develop methods capable of screening for drug resistance in multiple samples in parallel
  - apply pyrosequencing to HIV drug resistance testing
- Detect minor drug resistance mutations

# Previous methods for characterizing minor drug resistant mutations

- Microarrays

*Kozal et al. (1996) Nat Med 2, 753-759*

- Allele-specific RT-PCR

*Halvas et al. (2006) J Clin Microb 44, 2612-2614*

- Massively parallel polony sequencing

*Cai et al. (2007) Nat Methods 4, 123-125*

- Single genome sequencing

*Palmer et al. (2005) J Clin Microb 43, 406-413*

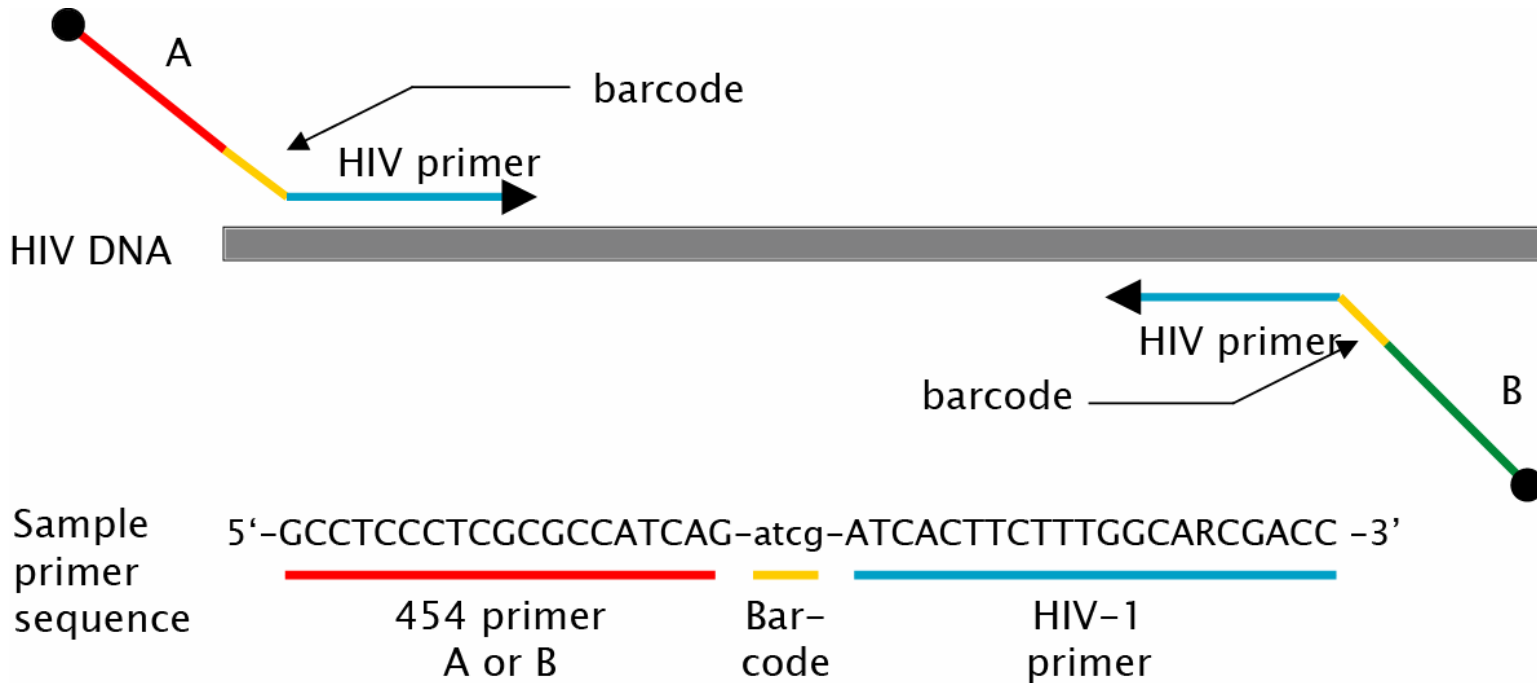
- Pyrosequencing

*O'Meara et al (2001) J Clin Microb 39, 464-473*

# Our approach

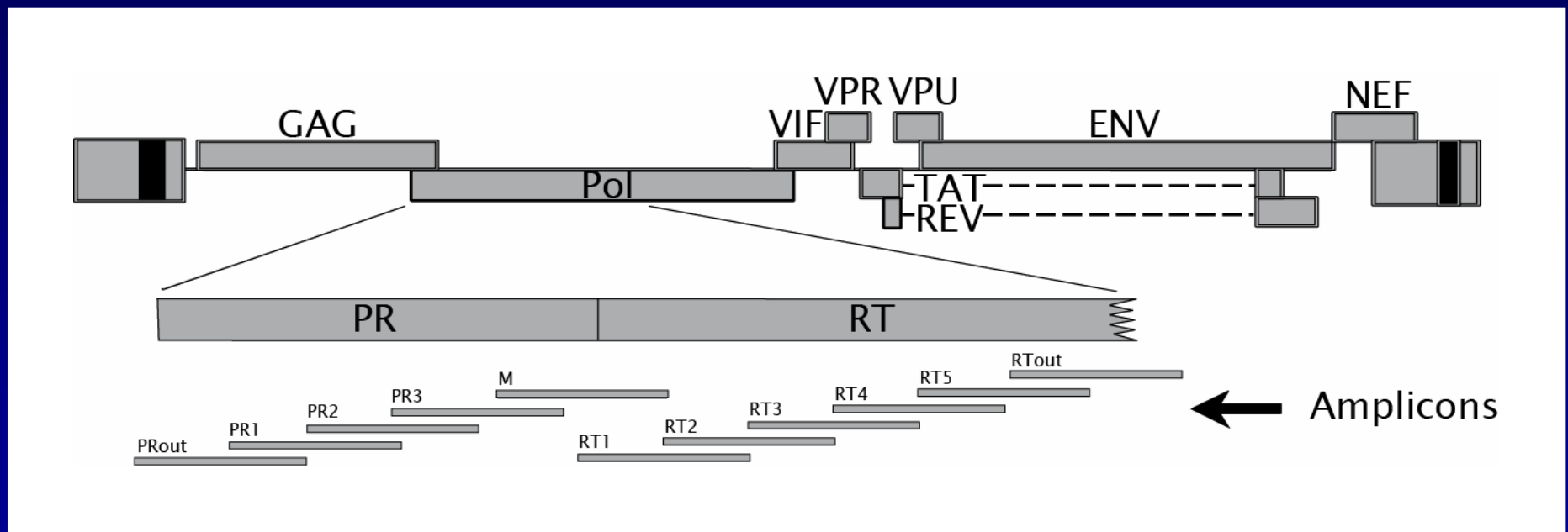
- Interrogate the PR and RT-coding regions
- Analyze multiple samples in a single sequencing run
- Extract HIV RNA
- RT-PCR
- 11 amplicons per sample
- Pyrosequencing (454 Life Sciences)
- DNA bar coding system to sequence multiple samples in parallel in a single pyrosequencing run
- query 62 positions of drug resistance (IAS-USA)

# DNA bar coding



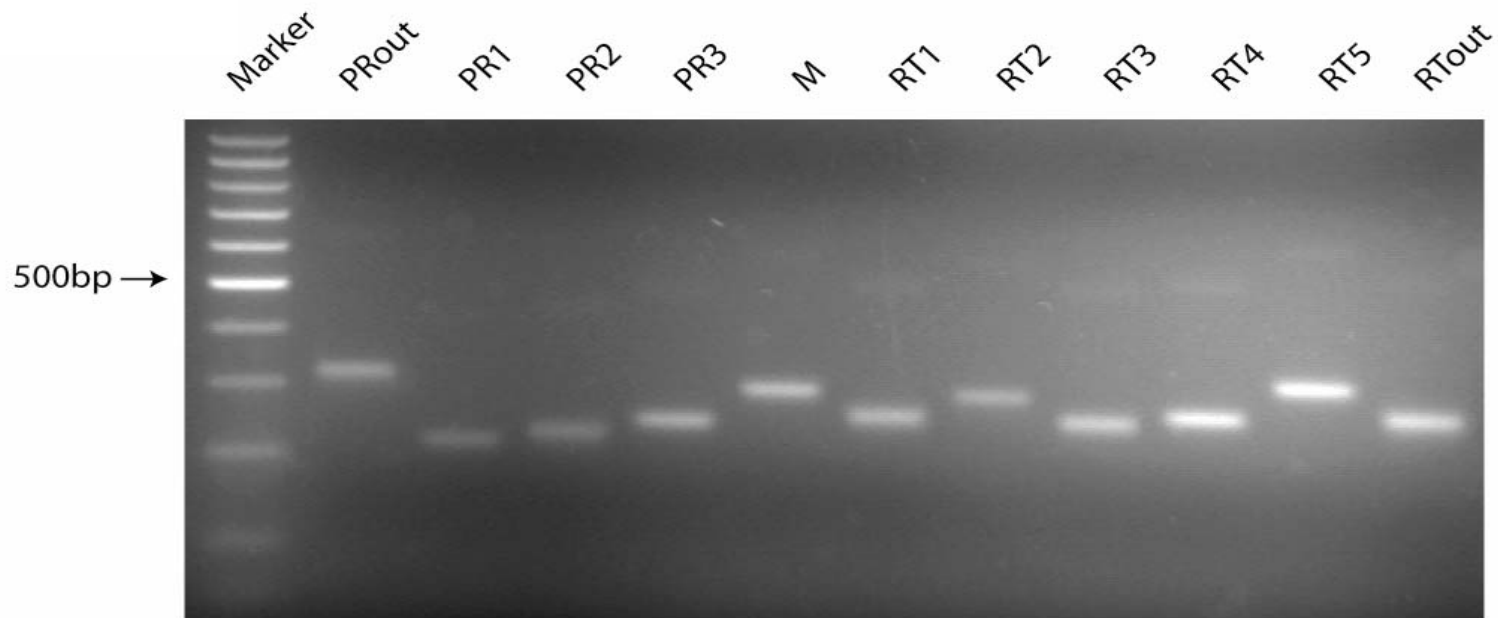
- Each PCR sample is tagged with a unique barcode
- Allows pooling of samples for a single pyrosequencing run

# 11 Amplicons to Interrogate PR and RT Drug Resistant Alleles



All 62 positions associated with drug resistance are covered in the 11 amplicons

# HIV amplification using pan-HIV primer set



All major circulating subtypes of HIV  
can be amplified using our pan-HIV primer set

# Viral populations analyzed

## HIV samples tested for drug resistance mutations.

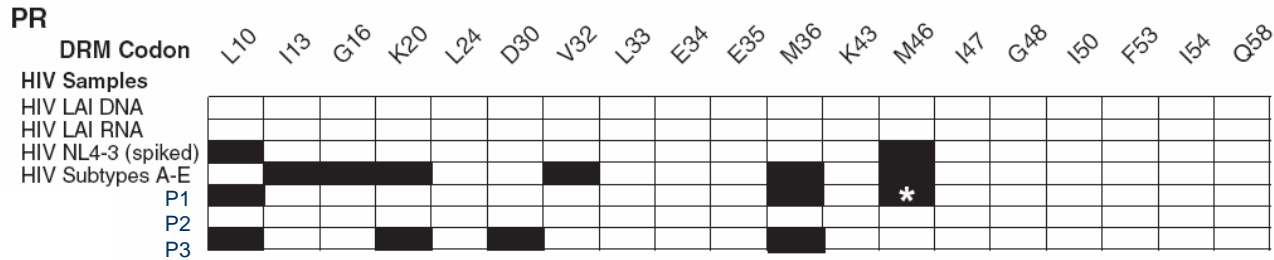
Sample	Amplification template	Description	Barcode	Number of sequence reads
1	Viral RNA	HIV LAI RNA from particles	d(ACTG)	17434
2	Plasmid DNA	HIV LAI cloned DNA	d(AGTC)	13856
3	Viral RNA	HIV NL4-3 mixed with drug resistant NL4-3	d(CTGA)	7398
4	Viral RNA	Mixture of HIV particles from subtypes A-D and 01_AE	d(CGTA)	28751
5	Viral RNA	HIV quasispecies from patient 1	d(GCTA)	19079
6	Viral RNA	HIV quasispecies from patient 3	d(TAGC)	10730
7	Viral RNA	HIV quasispecies from patient 2	d(TGAC)	20845

# Pyrosequencing results

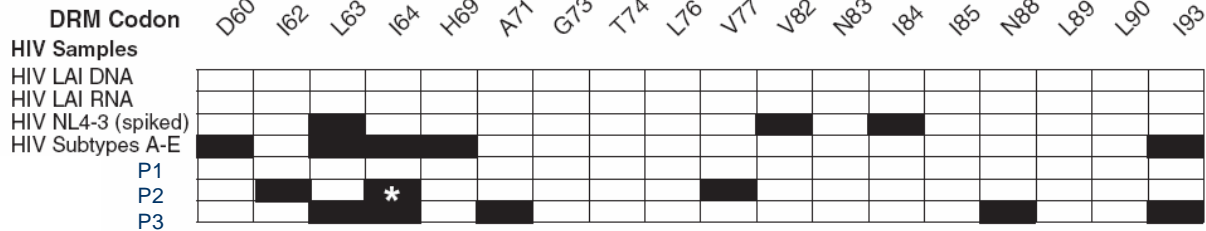
- total sequence reads = 135,528
- Average length ~100 bp
- 118,093 sequences available for analysis
- good representation of all barcodes

# Summary of drug resistance alleles detected

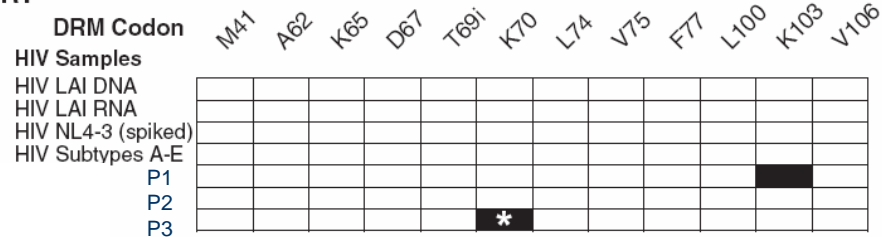
PR



PR (cont.)

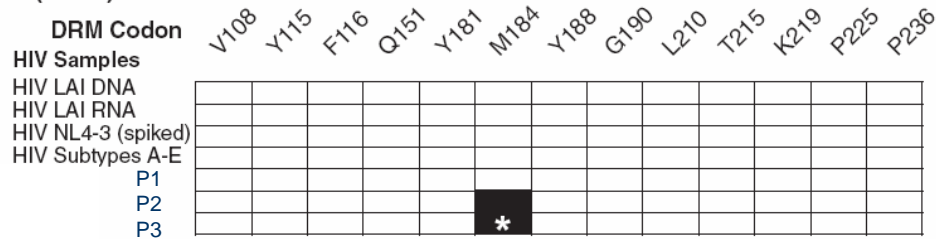


RT



Pyrosequencing can detect additional drug resistance alleles not called by the standard genotyping method

RT (cont.)



RT

## NL4-3 mixed with drug resistant NL4-3 (5%)

- The measured frequencies of the drug resistance alleles
  - L10: 4.6%
  - M46: 1.3%
  - L63: 4.8%
  - V82: 2.9%
  - I84: 3.1%
- Sensitivity depends on the number of sequences in each amplicon

## Summary

- Pyrosequencing can now deliver 400,000 reads of 200-300 bp
- DNA bar coding allows many samples to be analyzed in a single run
  - distinct samples can be processed in parallel
  - simultaneously screening all 62 positions implicated in HIV drug resistance
  - pan-HIV primers allow detection and analysis of several HIV subtypes
- sensitivity depends on the number of sequences in each amplicon

# Summary

- Minor variants can readily be detected as a few percent of the viral population
- Possible applications
  - analysis of the clinical importance of minor drug resistant alleles in HIV quasispecies
  - cheap genotyping in resource-limited settings
  - drug resistance genotyping of diverse pathogens
  - analysis of genetic changes in microbes under diverse selective pressures

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