Monitoring for Drug Resistance by Genotyping

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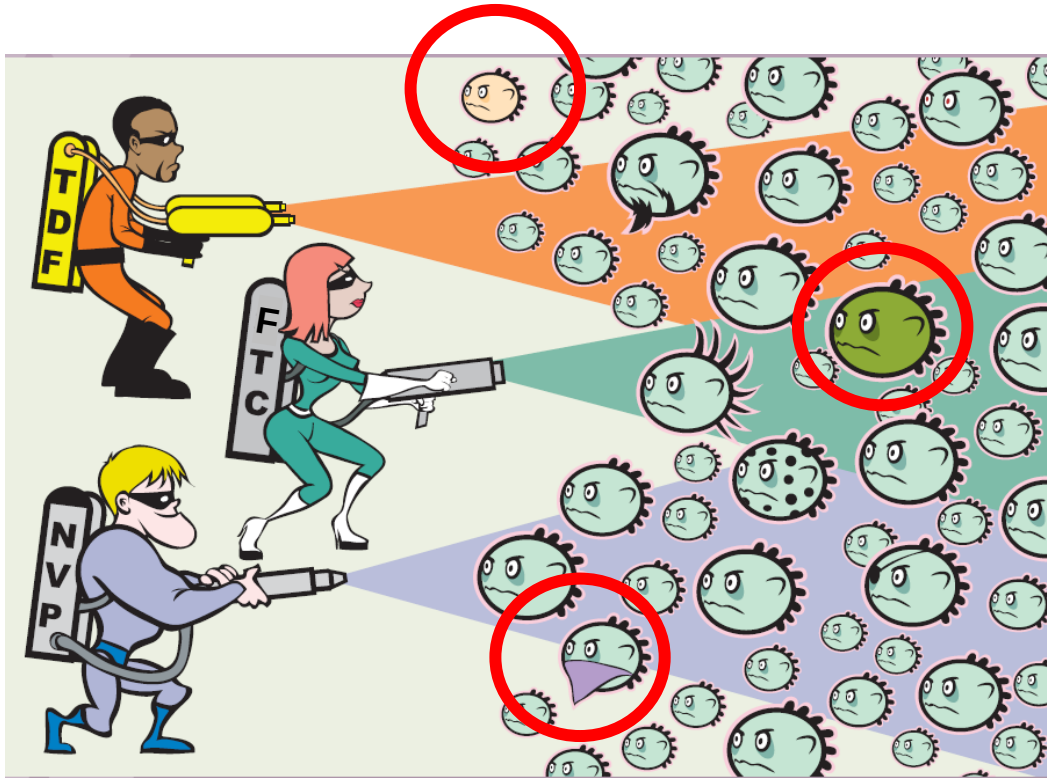
Outline

- What is Drug Resistance?
- Genotyping Algorithm
- Standard vs Sensitive Resistance Testing
- Sequencing Protocols
  - ViroSeq
  - Allele-specific PCR
  - Single Genome Sequencing
- Interpreting the Data

What is drug resistance?

- High error rate of HIV causes misincorporations, resulting in changes in genome
- Some changes enable HIV to replicate in presence of antiviral compounds, thus reducing drug effectiveness



www.thebody.com

MTN Study Drugs

- MTN-001 – **tenofovir**
- MTN-002 – tenofovir
- MTN-003 – tenofovir, TDF, TDF/**FTC**
- MTN-004 – SPL7013 (**VivaGel**™)
- MTN-005 – non-medicated intravaginal ring
- MTN-015 – “seroconverter”
  - HPTN-035 – **BufferGel, PRO2000/5 Gel**

Mutations of Interest

Tenofovir

- **K65R** (3%)
- K70E (0.24%)
- L74V (rare)
- Q151M (rare)
- T69SS (rare)
- A62V and S68G
  - Compensatory
  - Replication capacity

FTC

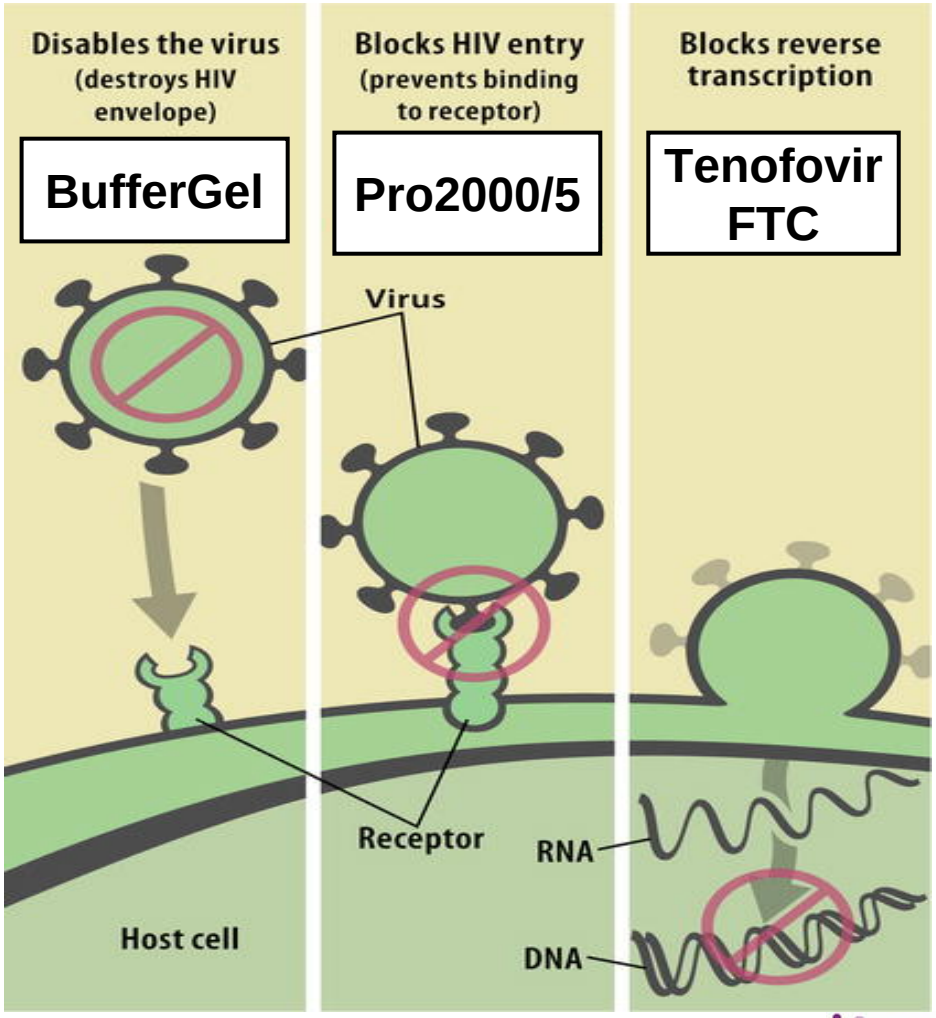
- **M184V**

Virus with K65R causes resistance to FTC

M184V makes the virus MORE SUSCEPTIBLE to Tenofovir

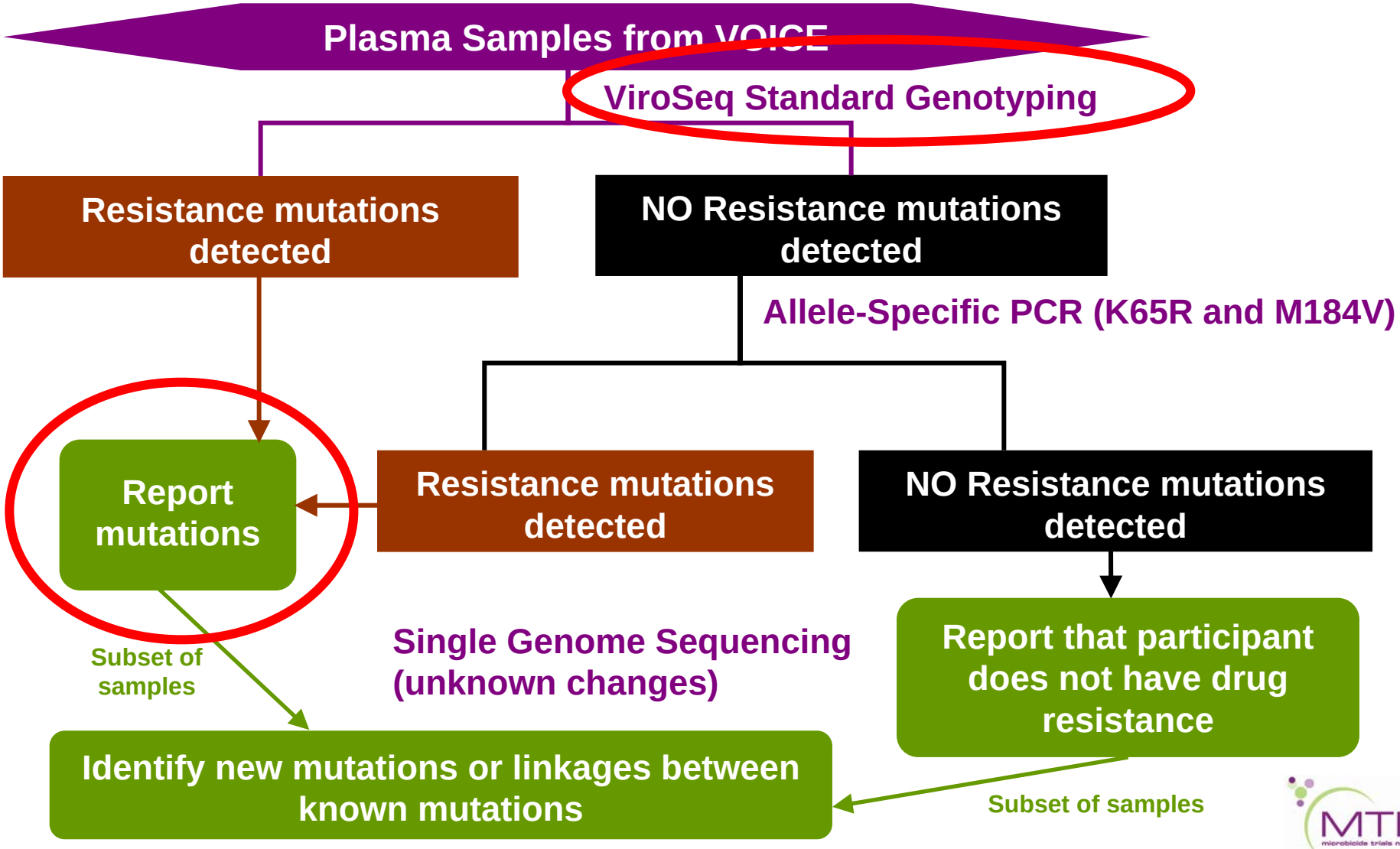
Microbicide Resistance Unlikely

- **BufferGel**
  - Carbopol974P
  - Maintains acidic pH of vagina
  - Virus inactivated at pH 4 – 5.8
- **Pro2000/5**
  - Inhibits virus entry into cells
  - Non-specific mechanism



From Weber PLOS Med 2005

Genotyping Algorithm



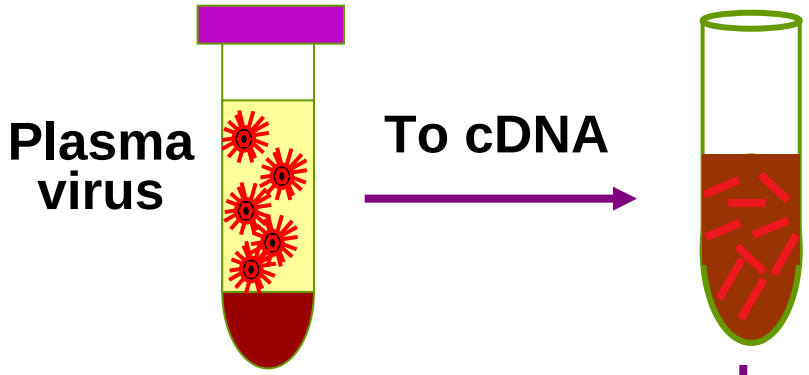
Why Sensitive Testing?

- Standard sequencing can miss mutations that are present at <25% of the population
- Minority variants can be associated with treatment failure (Johnson PLOS Med 2008)

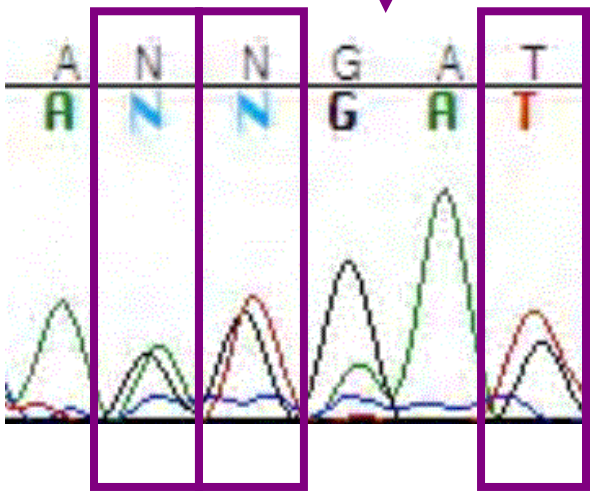
Sample ID	Baseline Minority Mutations	Bulk Genotype Mutations at Failure
11	M184V	Unk
25	M184V	M184V
31	K103N	K103N, M184V
41	K103N, M184V	K103N, M184V
44	K103N	K103N
63	K103N	Unk
67	Y181C	WT

All patients were reported as having wild type infection by standard sequencing

Standard Sequencing (ViroSeq)



PCR Bulk cDNA



STANDARD SEQUENCE (Population)

Detects the "majority" or "population" variant

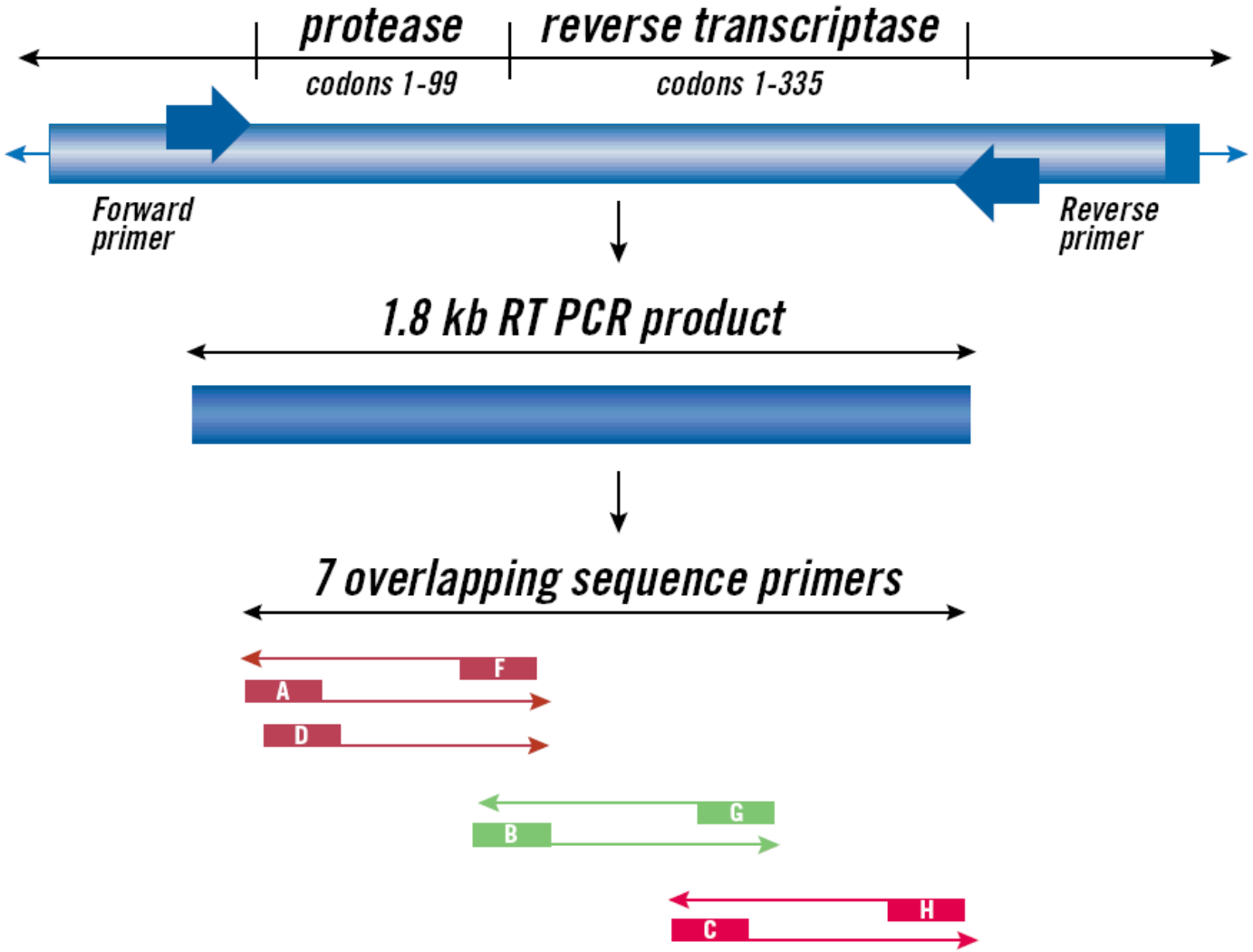
Misses bases present at <25%

N = G and A

N = G and T

Reported T
Actually T and G

Protocol: ViroSeq Coverage



ViroSeq™ HIV-1 Antiretroviral Drug Resistance Report

Patient ID	-----	Testing Laboratory	
Patient Name Last	-----		
Patient Name First MI	----- -	Lab Director	
Accession Number	-----	Department ID	
Patient Gender	Not Available	Mailstop	
Patient Birthdate & Age		Street Address1	
Report Generated By	admin	Street Address2	
Report Date & Time	13 Oct 2006, 03:07:47 PM, PDT	City	
Ordering Physician	-----	State/Province	
Institution	-----	Postal Code	
Date Drawn		Country	
Assay Operator	-----	Telephone/Fax	
Field1	-----	E-mail	
Field2	-----	Web Site	

Drug Class	Drug		Evidence of Resistance
NRTI	EPIVIR®	(lamivudine, 3TC)	Resistance**
	EMTRIVA®	(emtricitabine, FTC)	Resistance**
	RETROVIR®	(zidovudine, AZT)	Resistance**
	VIDEX®	(didanosine, ddI)	Resistance***
	ZERIT®	(stavudine, d4T)	Resistance**
	ZIAGEN®	(abacavir, ABC)	Resistance**
	VIREAD®	(tenofovir, TDF)	Resistance**
NNRTI	RESCRIPTOR®	(delavirdine, DLV)	None
	SUSTIVA®	(efavirenz, EFV)	None
	VIRAMUNE®	(nevirapine, NVP)	None
PI +	AGENERASE®	(amprenavir, APV)	Resistance*
	LEXIVA®	(fosamprenavir, FOS)	Resistance*
	CRIXIVAN®	(indinavir, IDV)	Resistance***
	FORTOVASE® / INVIRASE®	(saquinavir, SQV)	Resistance*
	KALETRA®	(lopinavir + ritonavir, LPV)	Resistance***
	NORVIR®	(ritonavir, RTV)	Resistance***
	VIRACEPT®	(nelfinavir, NFV)	Resistance***
	REYATAZ®	(atazanavir, ATV)	Resistance***
	APTIVUS®	(tipranavir, TPV)	Resistance***

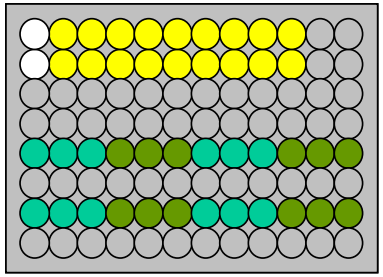
Drug Class	Drug Resistance Mutations Identified
NRTI	M41L, A62V, T69A, T69Ins, V118I, M184V, T215Y

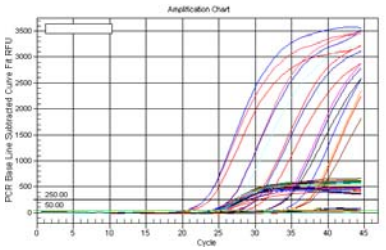
Allele-Specific PCR (ASPCR)

Plasma virus

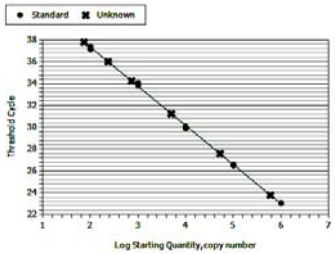
To cDNA

1st round PCR

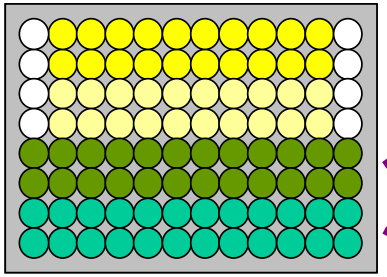




Amplify target region
Quantify with CYBR green



Dilute DNA to 10^7 copies/reaction

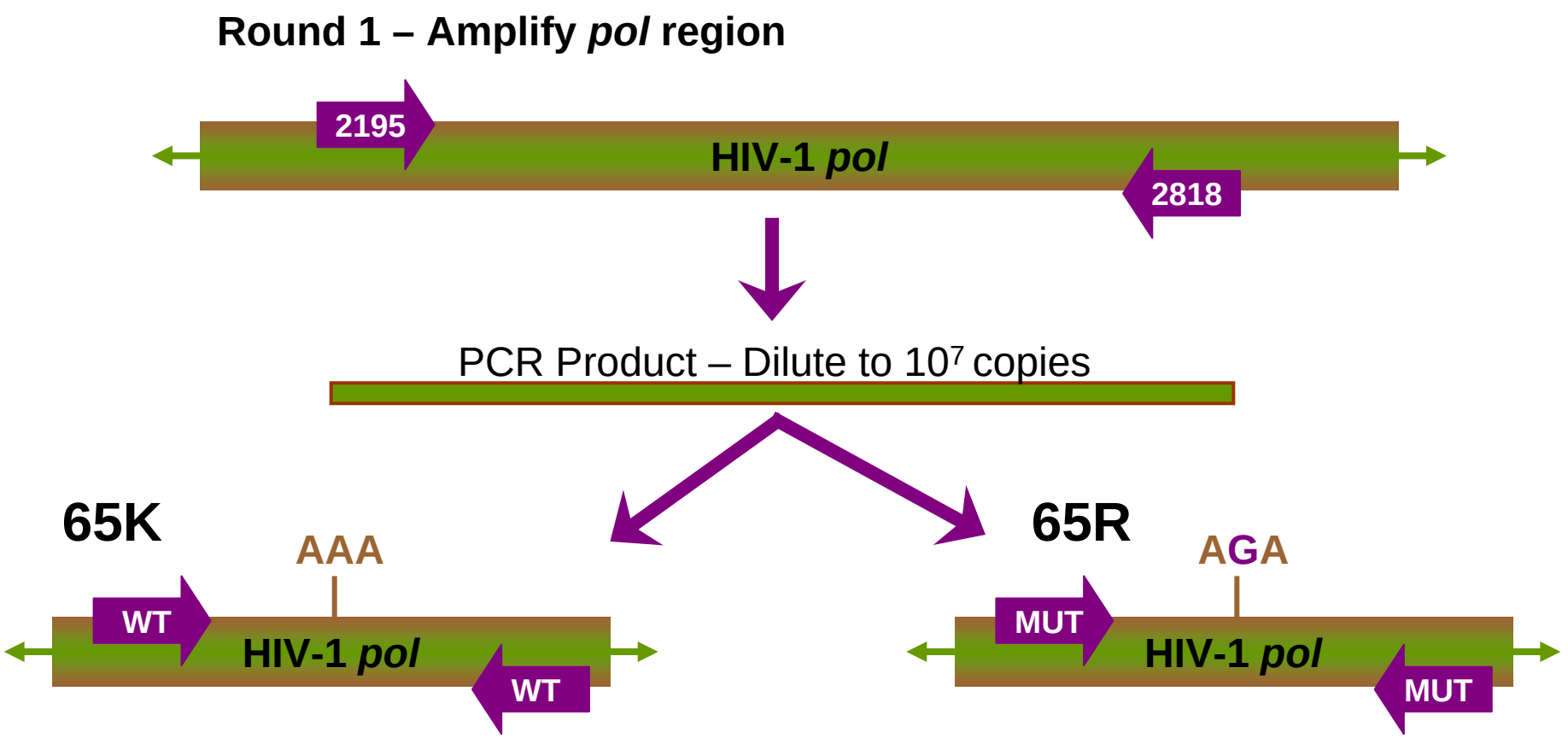


Amplify and Quantify with CYBR green

Discriminatory Primer
Detects % Mutant

Total Primer
Detects ALL

Allele-specific PCR (ASPCR)



Detection Limit: 0.1% mutant

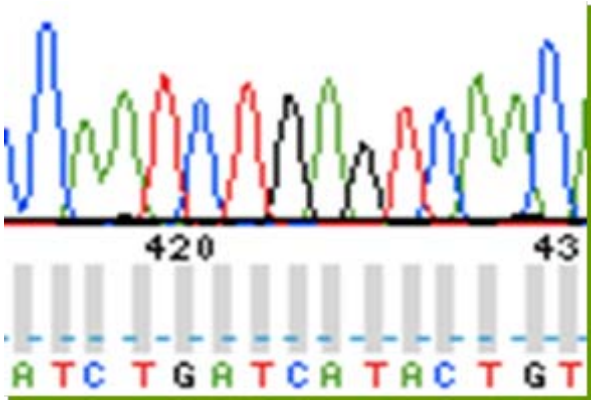
(Halvas, J Clin Micro 2006)

Single Genome Sequencing (SGS)

Sensitivity depends on number of genomes sequenced

20 sequences detects mutations present at 10%

SINGLE GENOME SEQUENCE (SGS)



Ref: Palmer et al., J Clin Micro 2005

Single Genome Sequencing (SGS)

Certainty of Detection	# Sequences needed to detect mutation present at				
	1%	5%	10%	25%	50%
90%	230	45	**22**	8	4
95%	298	59	29	11	5
99%	459	90	44	16	7

Table from L.Halvas

What is the difference?

Method	Type	Description
VIROSEQ	CLINICAL (USA FDA-approved)	Population genotype – major mutations
ASPCR	RESEARCH ONLY	% of a specific mutant
SGS	RESEARCH ONLY	All mutations, major and minor polymorphisms

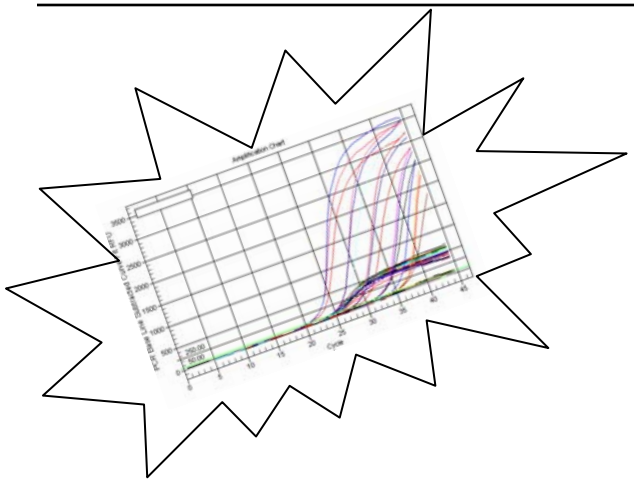
How will we use the data?

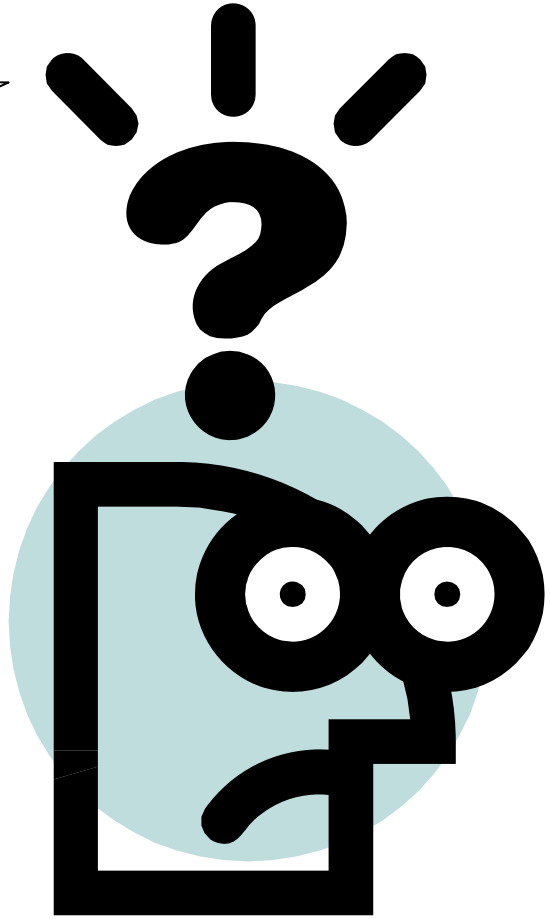
Method	What we learn
VIROSEQ	If patient has virus with resistance mutations, can help decide what therapy to put her on
ASPCR	Gives an idea if patient has “undetected” resistance, and to what extent
SGS	Gives a picture of the diversity of virus in the patient to help better understand how resistance occurred

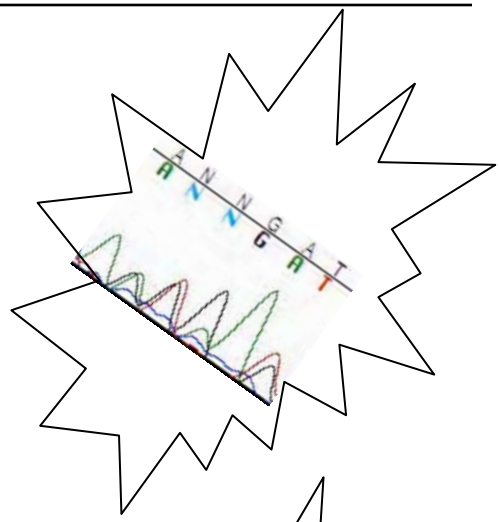
Finally, remember…

- If the microbicide **PROTECTS** against HIV, drug resistance is **not** an issue!!!
- Drug resistance is a concern if:
  - A positive person uses a microbicide
  - The microbicide does not protect and the participant becomes infected
- **MONITORING** for drug resistance can assure us that resistance is not occurring, or help identify the correct drugs for treatment

Questions?







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GGACCAGCAGCTACACTAGAAGAAATGA
TGACAGCATGTCAGGGAGTGGGAGGACC
CGGCCATAAGGCAAGAGTTTTGGCTGAA
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