Molecular technique for HIV drug resistance detection

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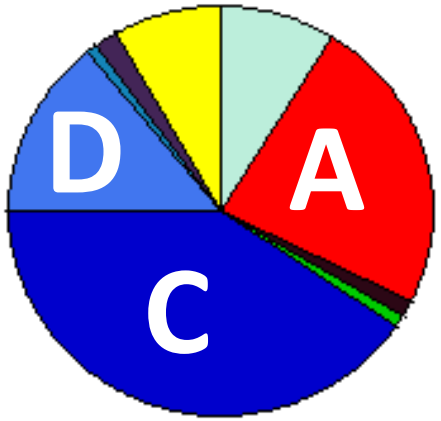
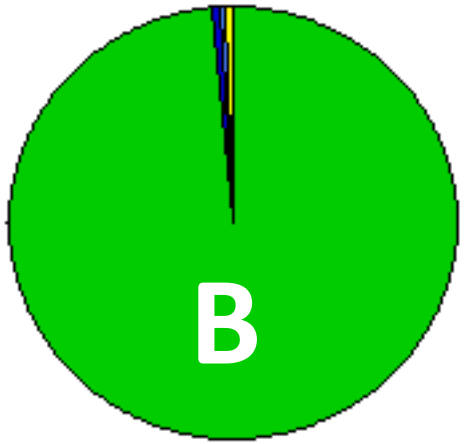
Human Immunodeficiency virus (HIV)

- HIV is a member of *Lentivirus* genus of the *Retroviridae* family.
- Infections with lentiviruses typically show a chronic course of the disease, with a long period of clinical latency, persistent viral replication.

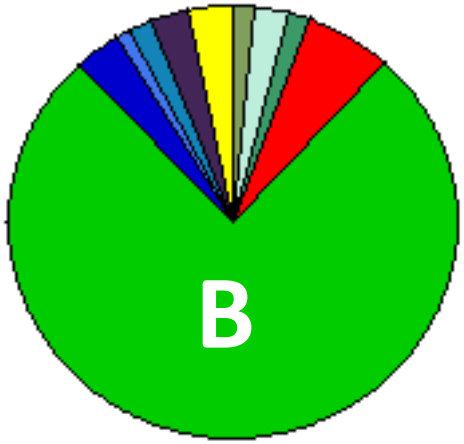
World Subtype distribution 2012



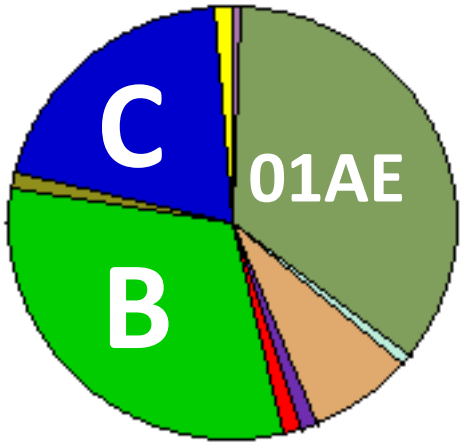
Africa



North America



Europe

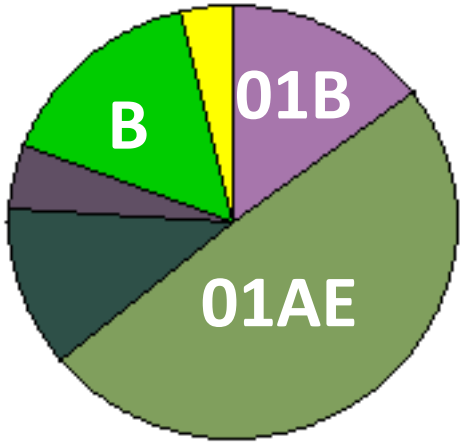


Asia

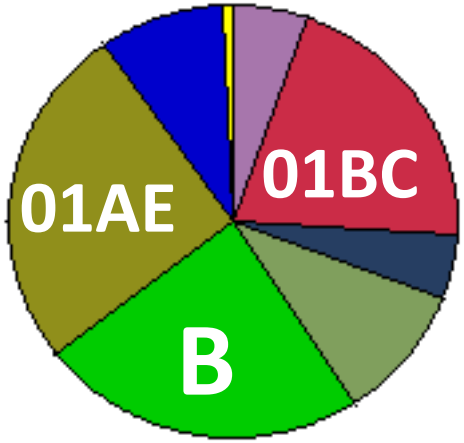
http://www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp

Regional Subtype distribution 2012

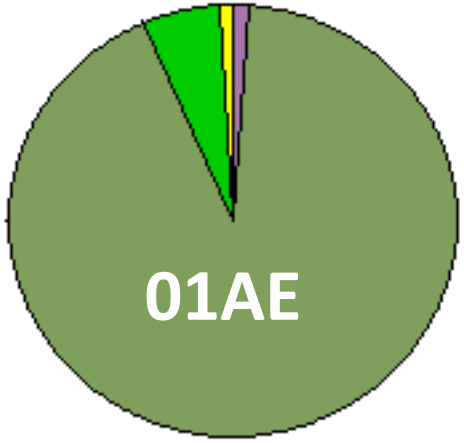
Vietnam



Malaysia



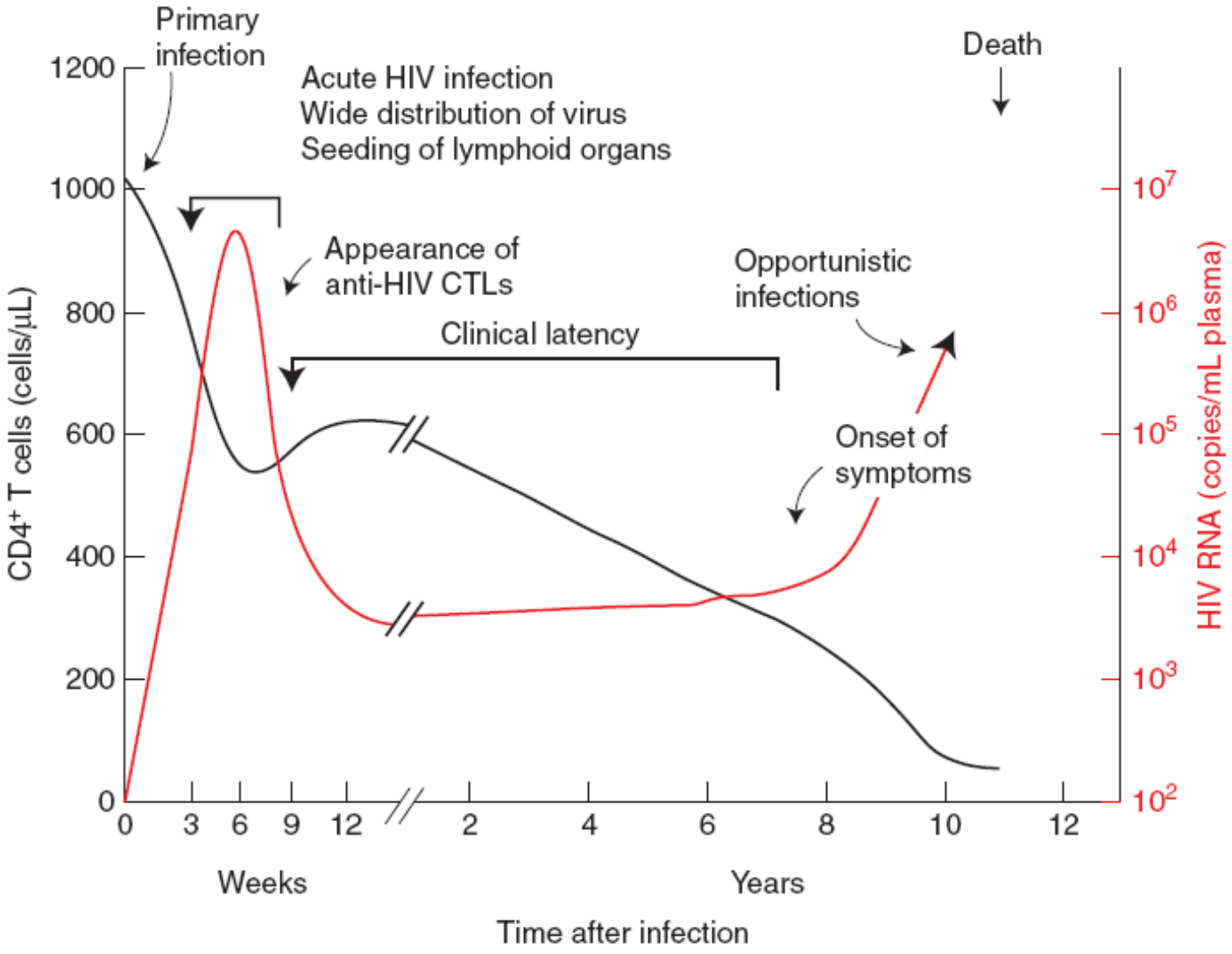
Myanmar



Thailand

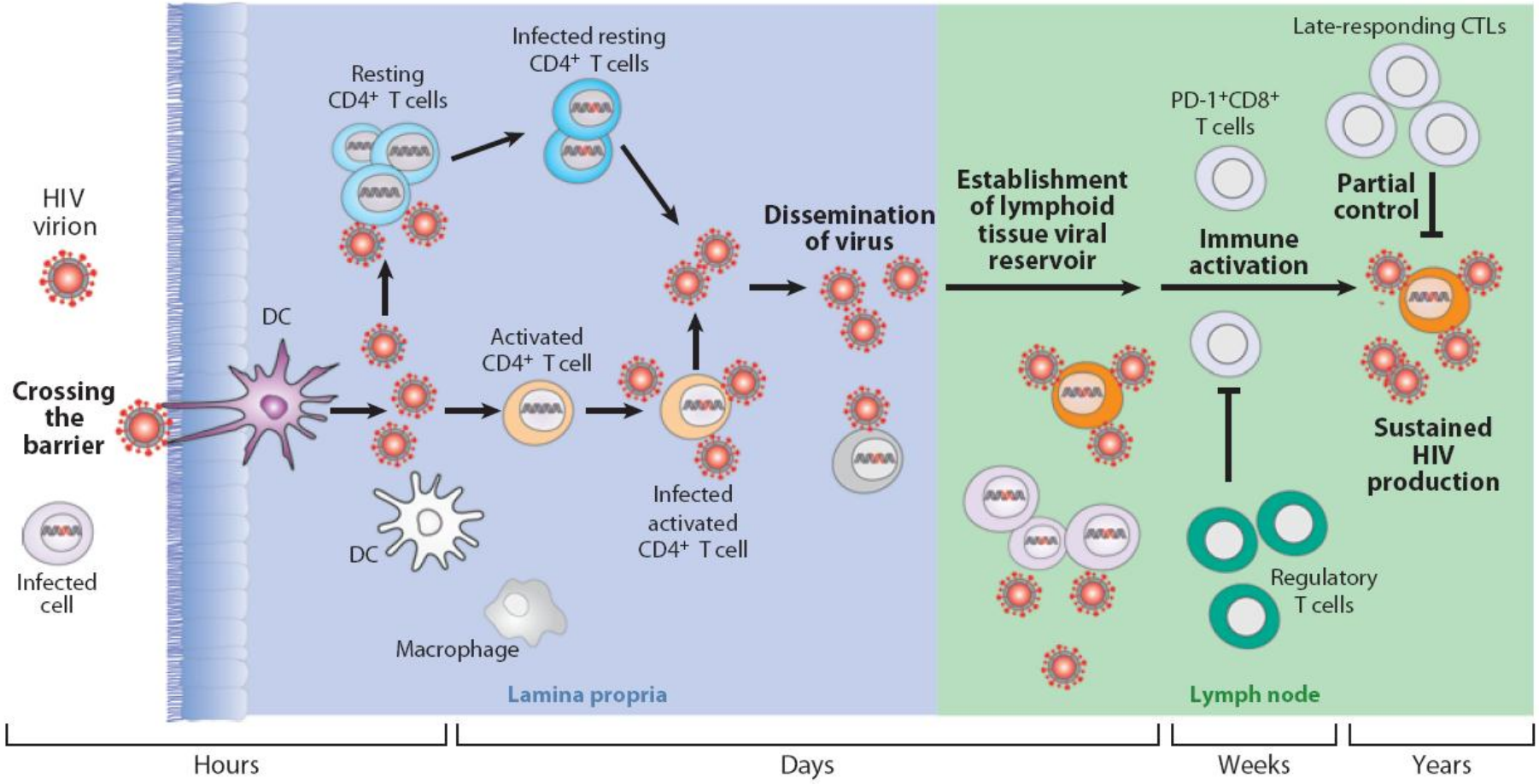
http://www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp

Time course of HIV infection



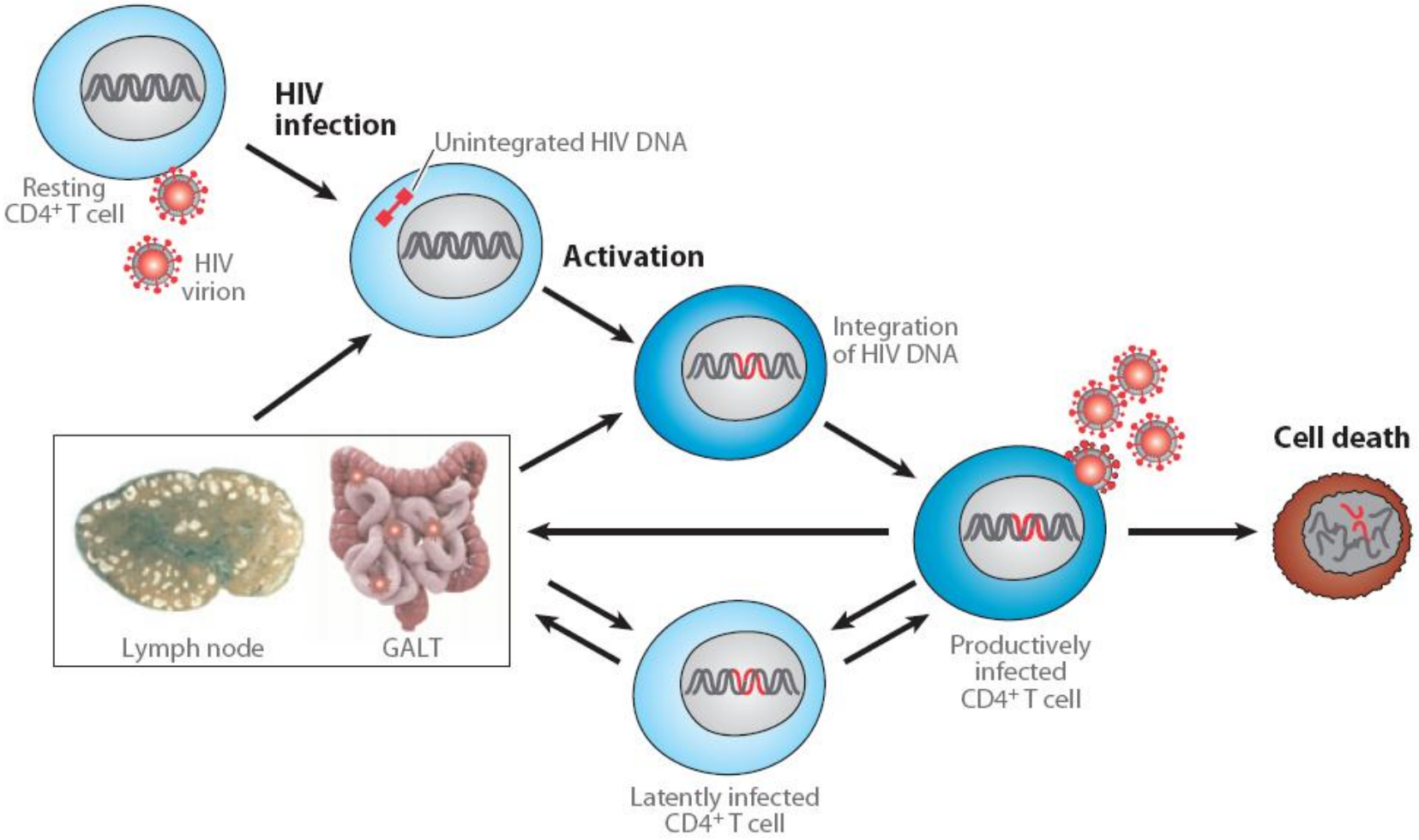
Cold Spring Harb Perspect Med 2013.1-16

Phases of infection following exposure to human immunodeficiency virus (HIV)



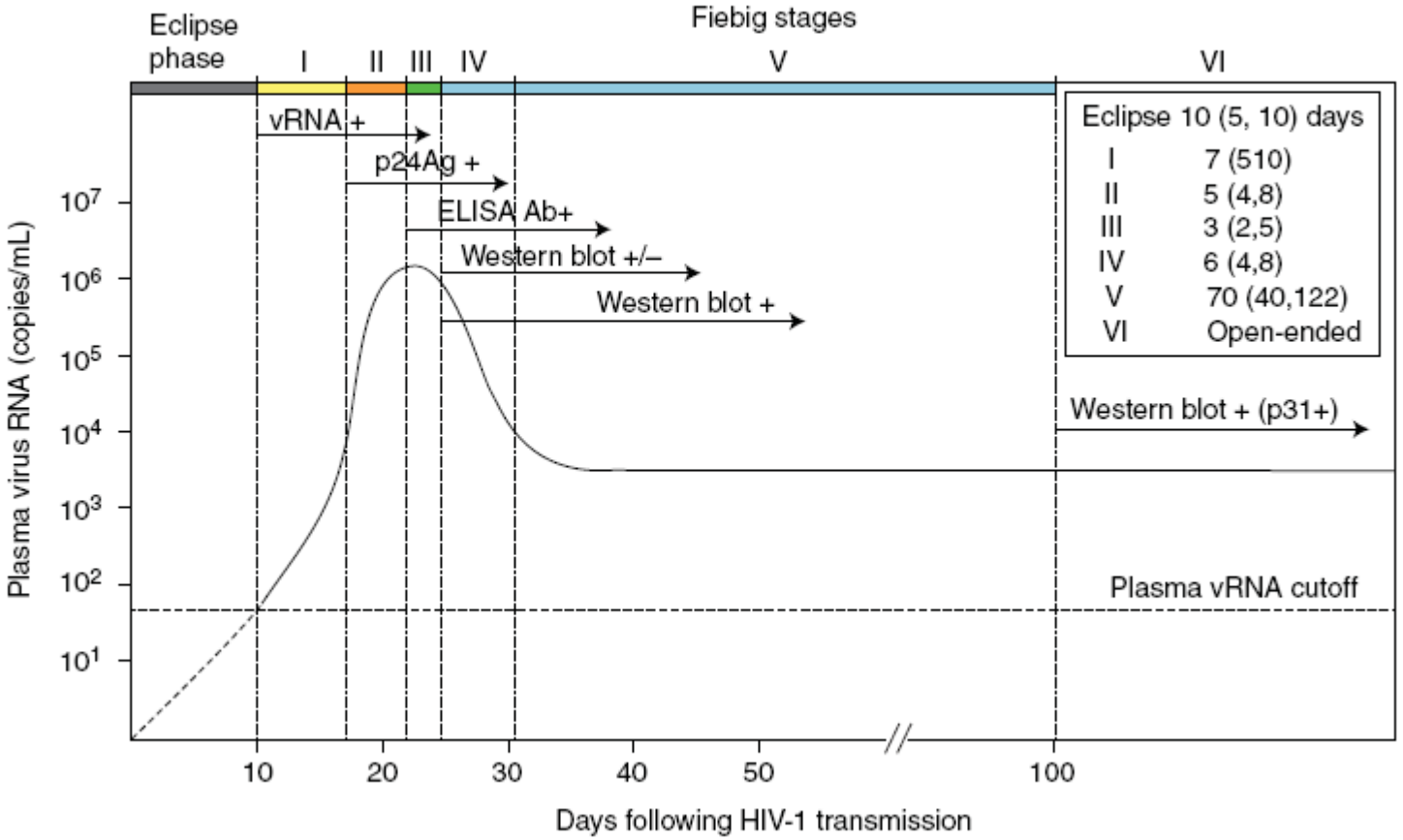
Annu. Rev. Pathol. Mech. Dis. 2011. 6:223–48

Establishment and maintenance of the resting CD4+ T cell reservoir in human immunodeficiency virus (HIV)-infected individuals



Annu. Rev. Pathol. Mech. Dis. 2011. 6:223–48

Laboratory staging and natural history of acute and early HIV-1 infection



http://www.docstoc.com/docs/71261433/Natural-history-and-laboratory-staging-of-HIV-infection

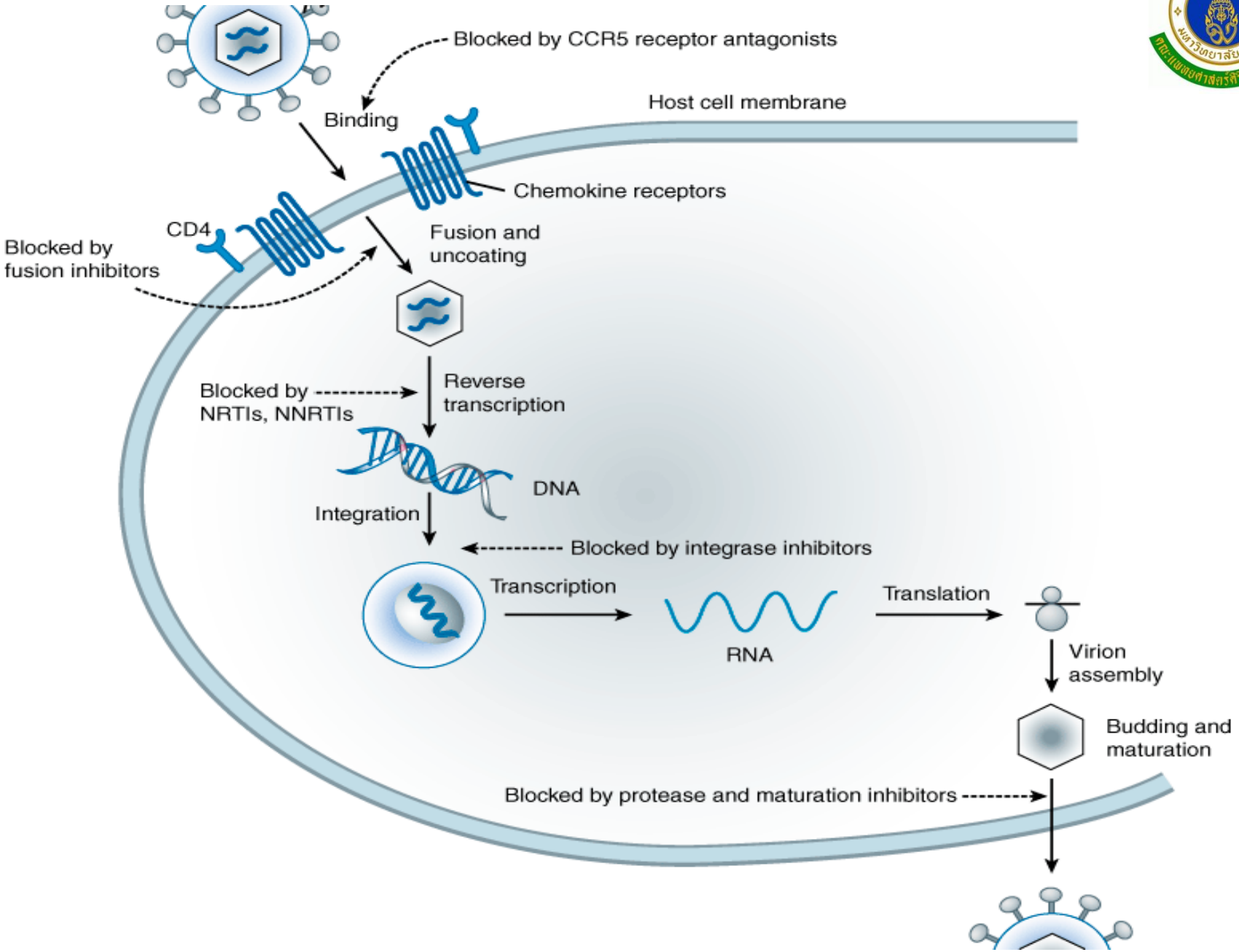
Current classes of antiretroviral drugs

Three main enzymatic targets:

- Reverse Transcriptase,
- Protease,
- Integrase

Six drug classes

1. Nucleoside Reverse Transcriptase Inhibitors (NRTIs)
2. Non Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
3. Protease inhibitors (PIs)
4. Entry inhibitors
5. CCR5 receptor antagonists
6. Integrase inhibitors


Blocked by CCR5 receptor antagonists
Host cell membrane
Binding
Chemokine receptors
CD4
Blocked by
fusion inhibitors
Fusion and
uncoating
Blocked by
NRTIs, NNRTIs
Reverse
transcription
DNA
Integration
Blocked by integrase inhibitors
Transcription
RNA
Translation
Virion
assembly
Budding and
maturation
Blocked by protease and maturation inhibitors

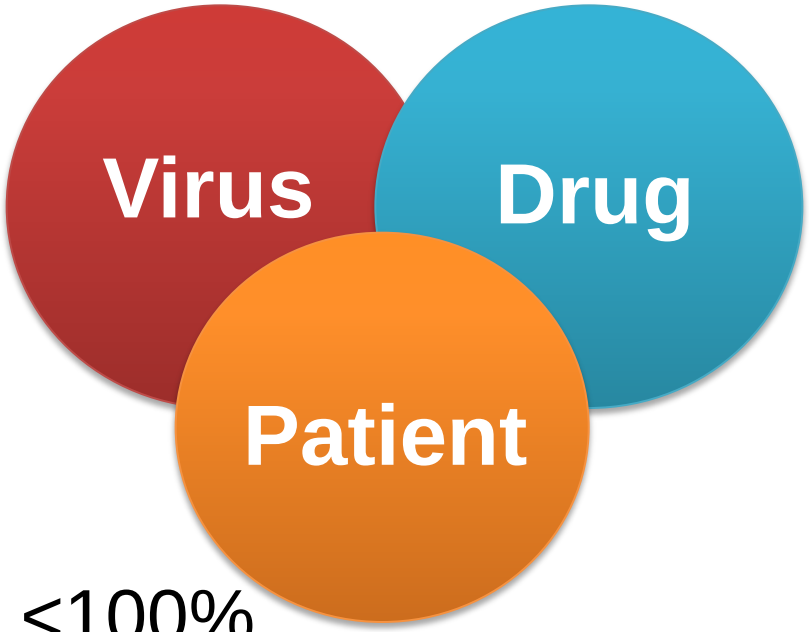
Factors Leading to Resistance

- VIRUS related
- DRUG related
- PATIENT related

One or more of these factors can lead to ARV resistance in a given patient.

Factors Leading to Resistance

- High replication rate
- High mutation rate – resistance
- Latent reservoirs of HIV



- Inadequate potency
- Inadequate durability
- Drug-drug interactions
- Poor tolerability
- Inconvenience

- Adherence <100%
- Toxicity or inconvenience

Latent Reservoirs and Resistance

- ARV resistance, once it develops, is probably life-long, since resistant HIV can hide in latent cellular reservoirs, which can be activated many years later.

- Once a patient is resistant to an ARV drug, that drug will probably be ineffective in the future. HIV does not “forgive” treatment errors or nonadherence.

Baby cured of HIV - what does it mean?

- A case presented at CROI meeting in Atlanta of a baby born in Mississippi who was infected with HIV at birth but is **now apparently free of the virus**.
- The mother was not known to be infected until the time of birth, and tests done when the baby was 2 days old showed that it was HIV positive.

Baby cured of HIV - what does it mean?

- Doctors decided to give the baby a full regimen of ART. By 29 days, no virus was detectable. (HIV Viral load, HIV proviral DNA)
- Treatment continued for 18 months, at which point mother and baby were lost to follow.
- When the child was next seen by doctors at 2 years old, it remained **free of functional HIV.**

How do to measure drug resistance?

- **Genotypic** Testing: Prediction of phenotype based on sequence
- **Phenotypic** Testing: Measure of susceptibility to specific drugs
  - Recombinant Assays: RT/PCR portion of patient virus and transfer into a vector
    - Several different versions commercialized, automated and regulated
  - PBMC Assay: Culture virus from patient
    - Largely replaced by recombinant assays due to difficulties in reproducibility and throughput

Sources and Types of Genotype Assays

- **Commercial kits**
  - US FDA-approved HIV-1 genotyping systems
    - TruGene
    - ViroSeq (Abbott/Celera)
- **In-house assays**
  - "home brew" assay performed at one site (clinical, hospital or research laboratory)
  - Must be validated and approved if used for patient management

How is resistance measured?

- Genotype:
  - Nucleotide sequences (A, C, G, T) which constitutes a *pol* gene
- Phenotype
  - Behavior of virus or
  - *pol* gene function In vitro drug sensitivity

Nucleotide sequencing
(Sanger Method)

- ✓ Using ‘terminators’, (dideoxi-nucleotides) inhibit chain elongation
- ✓ Requires a primer, DNA polymerase, a template, a mixture of nucleotides
- ✓ Incorporation of di-deoxynucleotides into growing strand terminates synthesis
- ✓ Synthesized strand sizes are determined for each di-deoxynucleotide by using gel or capillary electrophoresis
- ✓ Enzymatic methods

What to label for visualization?

- Primers?
- Disadvantages of primer-labels:
  - four reactions
  - tedious
  - limited to certain regions, custom oligos or
  - limited to cloned inserts behind ‘universal’ priming sites.
- Advantages: it works
- Solution:
  - labeled “terminators” - ddNTPs

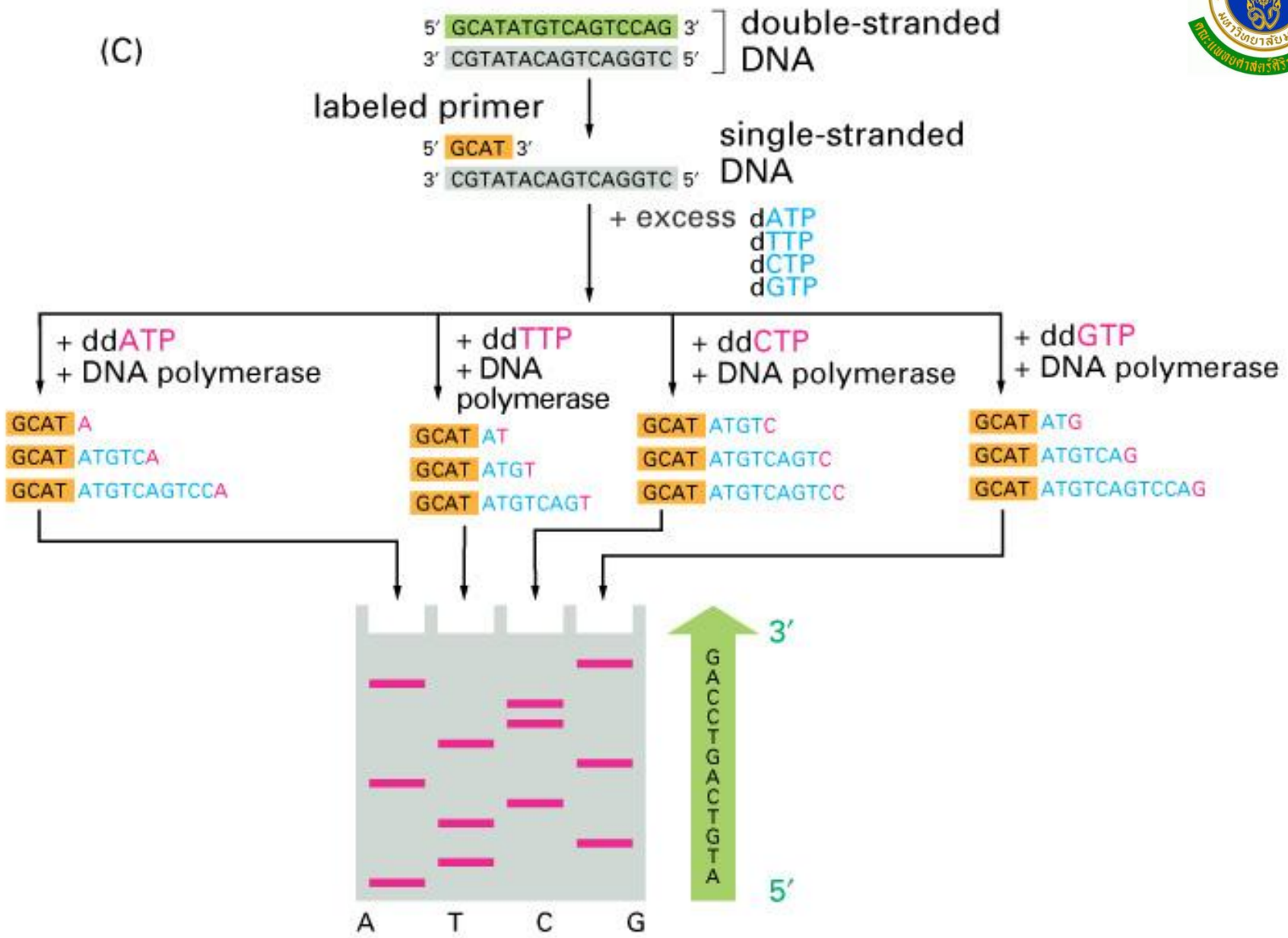


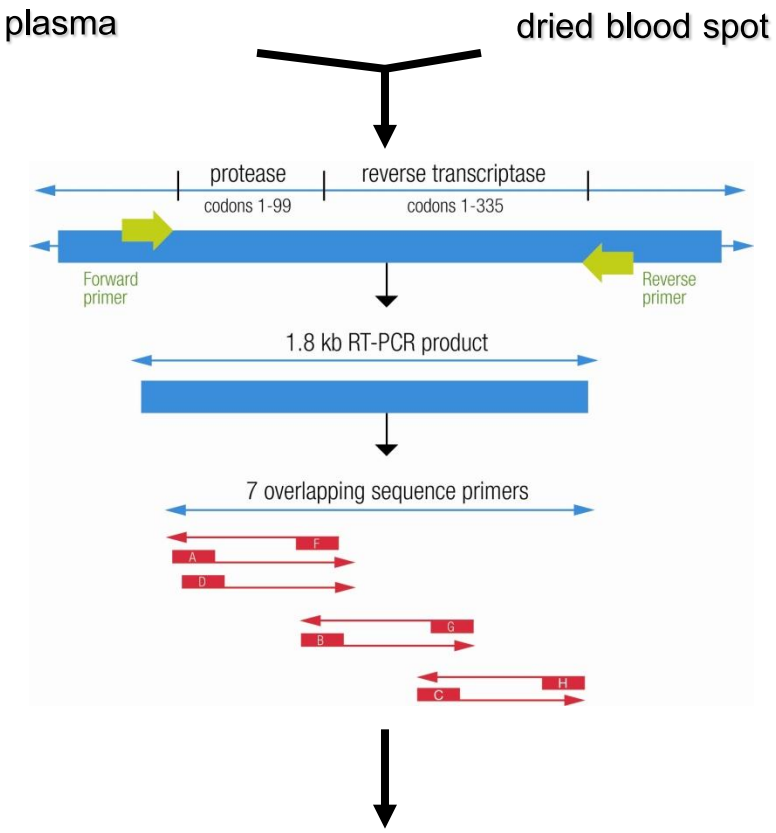
Figure 10-7 part 2 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

HIV drug resistance assay

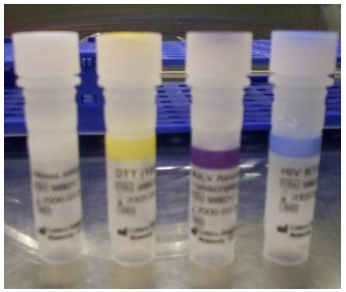
Genotypic assay

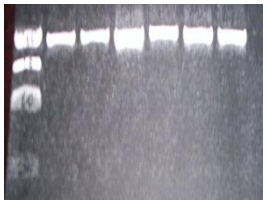
- EDTA blood 5-10 ml sent to laboratory within 6 hours
  - EDTA or ACD or heparin blood 5-10 ml. in vacuum tube sent to lab within 6 hours at room temperature
  - In case unable to send blood within 6 hours:
    - Plasma separation and frozen at -20^{0}C until shipment to lab (dry ice)
- HIV viral load $\geq$ 2000 copies/mL

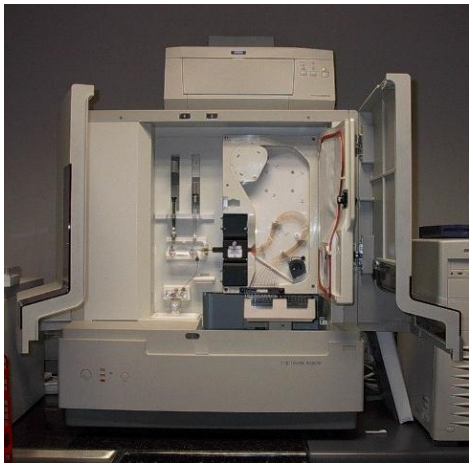
Genotyping using the Viroseq Kit



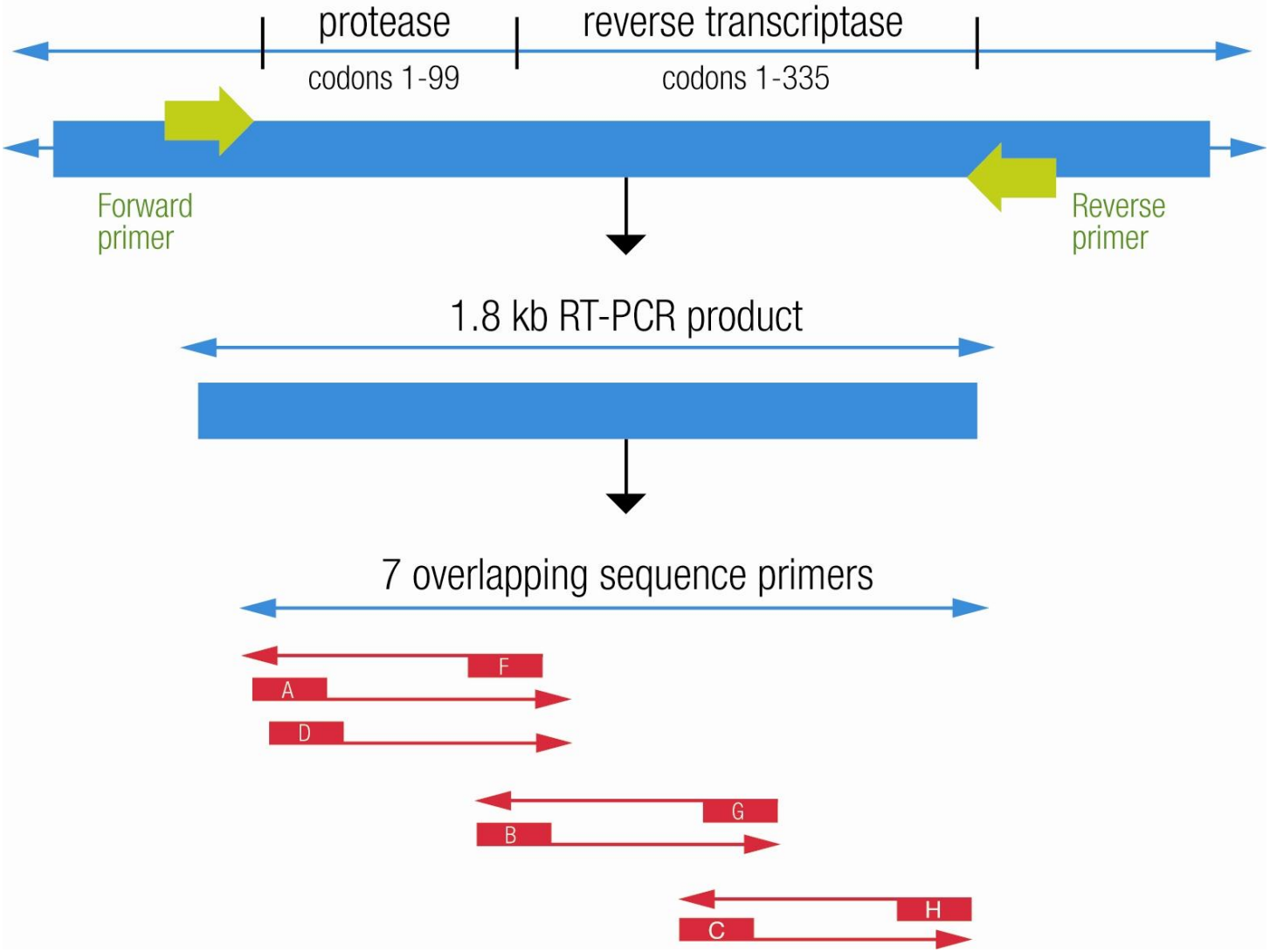
Load samples onto ABI 3130



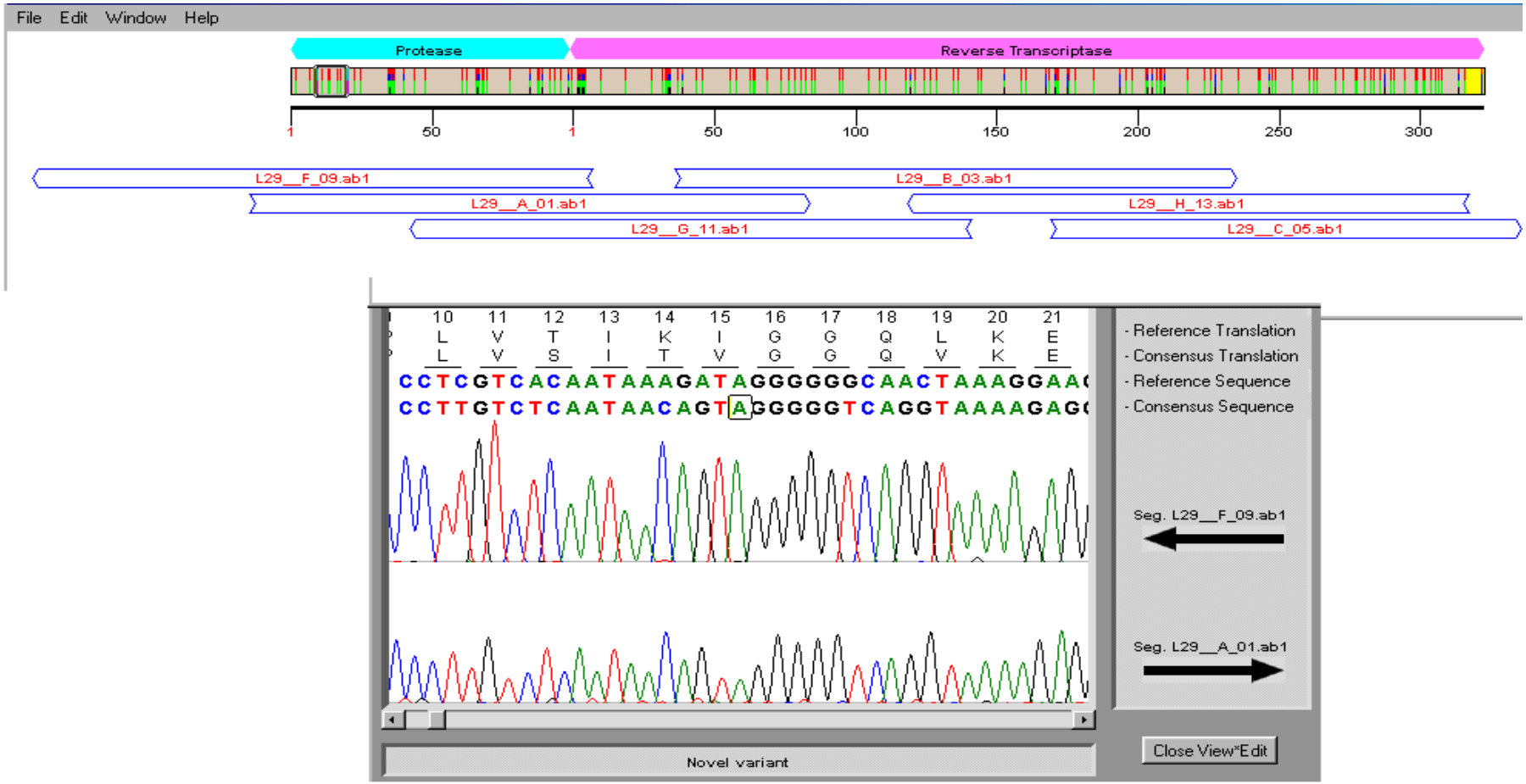




Genotyping using the Viroseq Kit



Sequence Analysis



Genotype Report (ViroSeq)

ViroSeq™ HIV-1 Antiretroviral Drug Resistance Report

Patient ID	---	Testing Laboratory	DEPT.MICROBIOLOGY SIRIRAJ HOSPITAL
Patient Name Last	---	Lab Director	
Patient Name First MI	GEN018DR.02A -	Department ID	
Accession Number	---	Mailstop	
Patient Gender	Not Available	Street Address1	
Patient Birthdate & Age		Street Address2	
Report Generated By	admin	City	
Report Date & Time	16 Jan 2010, 11:13:44 AM, ICT	State/Province	
Ordering Physician	---	Postal Code	
Institution	---	Country	
Date Drawn		Telephone/Fax	
Assay Operator	DR.NAVIN HORTHONGKHAM	E-mail	
Field1	---	Web Site	
Field2	---		

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

Drug Class	Drug Resistance Mutations Identified
NRTI	M41L, V75T
NNRTI	V106M, V179D
PI	M36I, H69K

* NOTE: At least one mutation used to determine Evidence of Resistance for this drug has not been fully validated.
** NOTE: At least one mutation used to determine Evidence of Resistance for this drug has not been clinically verified.
*** NOTE: For at least one mutation used to evaluate Evidence of Resistance for this drug, both notes above apply.
+ Evidence of Resistance for Protease Inhibitors estimates response to ritonavir-boosted regimens. Refer to section titled "Notes on Evidence of Resistance".

Review & Release of Results

Signature / Date: Name(Print) / Title:

Notes:

FOR IN VITRO DIAGNOSTIC USE
Project ID: [illegible]

ViroSeq™ HIV-1 Antiretroviral Drug Resistance Report

Patient ID	---	Testing Laboratory	DEPT.MICROBIOLOGY SIRIRAJ HOSPITAL
Patient Name Last	---	Lab Director	
Patient Name First MI	GEN018DR.02A -	Department ID	
Accession Number	---	Mailstop	
Patient Gender	Not Available	Street Address1	
Patient Birthdate & Age		Street Address2	
Report Generated By	admin	City	
Report Date & Time	16 Jan 2010, 11:13:44 AM, ICT	State/Province	
Ordering Physician	---	Postal Code	
Institution	---	Country	
Date Drawn		Telephone/Fax	
Assay Operator	DR.NAVIN HORTHONGKHAM	E-mail	
Field1	---	Web Site	
Field2	---		

Novel Mutations: Additional mutations identified and defined as differences from the reference (HXB-2, accession number K03455) that have not been associated with drug resistance. The performance characteristics of the additional mutations have not been established.

Protease:

V3I, I15V, L19I, S37D, R41K, L89M, I93L

Reverse Transcriptase:

E6D, K11Q, V35T, T39E, S48T, S68G, V90I, D121Y, I142T, K173A, D177E, I178M, T200A, Q207E, R211K, V245Q, P272R, R277K, T286A, E291D, V292I, I293V, D324E, Q334H, G335D

Comments

Software Version: ViroSeq® HIV-1 System v2.8 Software v2.7 Build 20

Profile Version: [illegible]

FOR IN VITRO DIAGNOSTIC USE
Project ID: [illegible]

TRUGENE® HIV-1: Genotyping Procedure

Preparation
Sample preparation: Viral RNA extraction using QIAamp viral RNA Mini Kit or other commercial kits

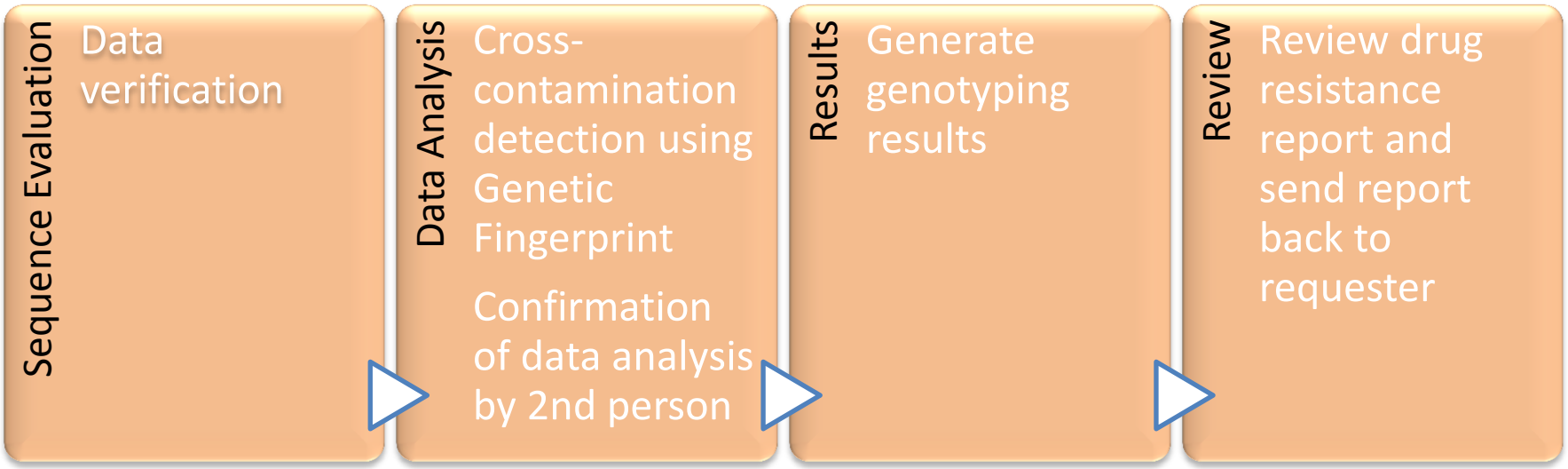
RT-PCR
RT-PCR transcription and amplification using an ABI 9700 thermocycler

Sequencing
CLIP™ Sequencing using an ABI 9700 thermocycler

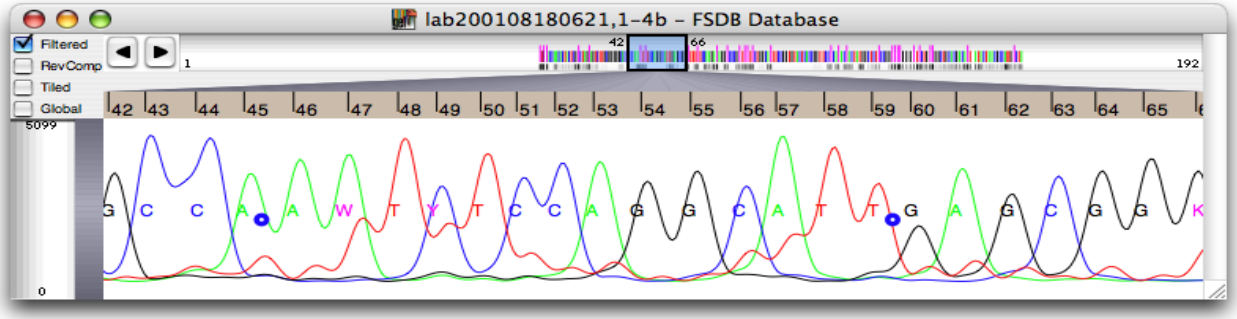
Electrophoresis
Detection using Long-Read Towers

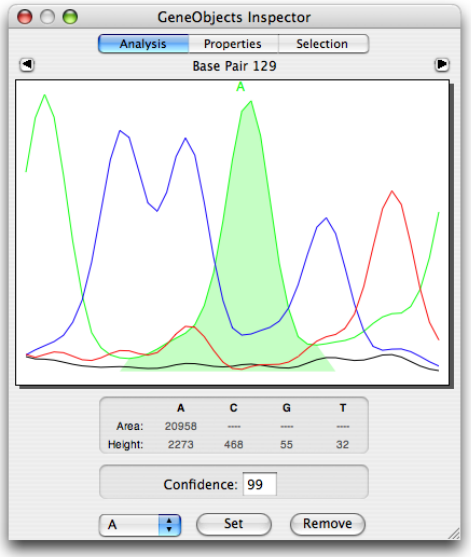
- Cast acrylamide gels
- Prepare MicroCel™ cassette and loading gel onto Long-Reader Tower

TRUGENE® HIV-1: Genotyping Procedure



Data Analysis (TruGene)





Limitations of genotyping

- Need ≥2000 copies/ml
- Subpopulation (<20%) not detected
- May not detect resistance to previous therapy

STANFORD UNIVERSITY

HIV DRUG RESISTANCE DATABASE

A curated public database designed to represent, store, and analyze the divergent forms of data underlying HIV drug resistance.

HOME GENOTYPE-RX GENOTYPE-PHENO GENOTYPE-CLINICAL HIVdb PROGRAM

HIVdb Program Integrase Update
Mutation classification, Scores, Comments and References.

HIVdb User Guide (link to PDF)
Database query and reference pages, Interactive program, Educational resources

Crystallographic Structures
RT, protease, and integrase

More news »

GENOTYPE-TREATMENT CORRELATIONS

- Retrieve sequences (and/or mutations) from persons receiving selected HIV drugs
- Retrieve sequences and treatments from viruses with specific mutations

GENOTYPE-CLINICAL CORRELATIONS

- Summaries of genotype-clinical outcome studies
- Genotype-clinical outcome datasets (download)

GENOTYPE-PHENOTYPE CORRELATIONS

- Retrieve drug susceptibility data for isolates with selected mutations
- Download genotype-phenotype research datasets

REFERENCES

- Published drug resistance studies in HIVRT&PrDB
- Published studies by Stanford database group

NEW SUBMISSIONS

- Fujisaki, et al. 11-year surveillance of HIV subtypes in Japan

SURVEILLANCE MUTATIONS

- World Health Organization 2009 Mutation List

HIVdb PROGRAM Genotype Resistance Interpretation

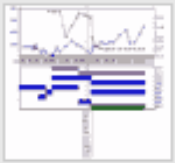
This program interprets user-entered mutations to infer the level of resistance to NRTIs, NNRTIs, PIs. Web Service is available.

MARVEL

MARVEL (Mutation ARV Evidence Listing) » Go To Program

ART-AiDE

Antiretroviral Therapy - Acquisition & Display Engine
» Go To Program



HIVseq Program

Provides mutation frequencies by subtype. » Go To Program

HIValg Program

Compare HIVdb, ANRS, Rega, or create

Standford database informations

HIVdb: Genotypic Resistance Interpretation Algorithm

Date: 30-Aug-2010 20:24:21 PDT

Seq ID:

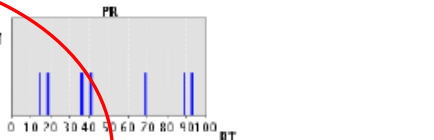
Summary Data

Sequence includes PR: codons: 1 - 99
Sequence includes RT: codons: 1 - 333
There are no insertions or deletions
Subtype and % similarity to closest reference isolate:

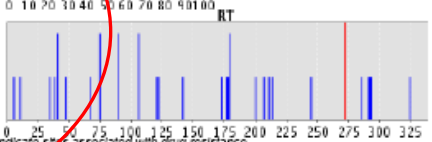
1. *PR: C (93.9%)*
2. *RT: C (93.9%)*

Sequence Quality Assessment

Gene	QA Problem	Codon
PR	Stop Codons, Frame Shifts:	None
PR	B,D,H,V,N:	None
PR	Unusual Residues:	None



Gene	QA Problem	Codon
RT	Stop Codons, Frame Shifts:	None
RT	B,D,H,V,N:	None
RT	Unusual Residues:	272



Blue lines indicate differences from consensus B; tall blue lines indicate sites associated with drug resistance.
Red lines indicate QA problems.

Drug Resistance Interpretation: PR

PI Major Resistance Mutations:	None
PI Minor Resistance Mutations:	None
Other Mutations:	I15V, L19I, M36I, N37D, R41K, H69K, L89M, I93L

Protease Inhibitors	
atazanavir/r (ATV/r)	Susceptible
darunavir/r (DRV/r)	Susceptible
fosamprenavir/r (FPV/r)	Susceptible
indinavir/r (IDV/r)	Susceptible
lopinavir/r (LPV/r)	Susceptible
nelfinavir (NFV)	Susceptible
saquinavir/r (SQV/r)	Susceptible
tipranavir/r (TPV/r)	Susceptible

PR Comments

Special

Drug Resistance Interpretation: RT

NRTI Resistance Mutations:	**M41L, V75T**
NNRTI Resistance Mutations:	**V90I, V106M, V179D**
Other Mutations:	E6D, K11Q, V35T, T39E, S48T, S68G, D121Y, K122E, I142T, K173A, D177E, I178M, T200A, Q207E, R211K, F214L, V245Q, A272R, T286A, E291D, V292I, I293V, D324E

Nucleoside RTI		Non-Nucleoside RTI	
lamivudine (3TC)	Susceptible	**delavirdine (DLV)**	High-level resistance
abacavir (ABC)	Low-level resistance	**efavirenz (EFV)**	High-level resistance
zidovudine (AZT)	Low-level resistance	**etravirine (ETR)**	Low-level resistance
stavudine (D4T)	High-level resistance	**nevirapine (NVP)**	High-level resistance
didanosine (DDI)	Intermediate resistance		
emtricitabine (FTC)	Susceptible		
tenofovir (TDF)	Low-level resistance		

RT Comments

NRTI

- M41L usually occurs with T215Y. Together these mutations confer intermediate-to-high level resistance to AZT and d4T and a lower level of resistance to ddI, ABC, and TDF.
- V75T/M/A/S reduce d4T and possibly ddI susceptibility.

NNRTI

- V90I is a common polymorphism that was weakly associated with decreased ETR response in the DUET studies. However, it has minimal if any effect on NNRTI susceptibility.
- V106M causes high-level resistance to NVP, EFV, and DLV but does not appear to decrease ETR susceptibility except as a marker of past NNRTI therapy.
- V179D/E cause low-level reductions in susceptibility to NVP, EFV, and DLV. V179D occurs in about 1% of untreated persons and reduces the susceptibility of each NNRTI by about 2-fold. The combination of K103R + V179D reduces the susceptibility of NVP, DLV, and EFV by about 15-fold; the combination's effect on ETR is not known. V179D was associated with a decreased response to ETR in the DUET studies.

Special

- The following 2 of the 13 etravirine DUET study mutations were present: V90I, V179D (Katlama C et al, IAS 2007).

Mutation Scoring

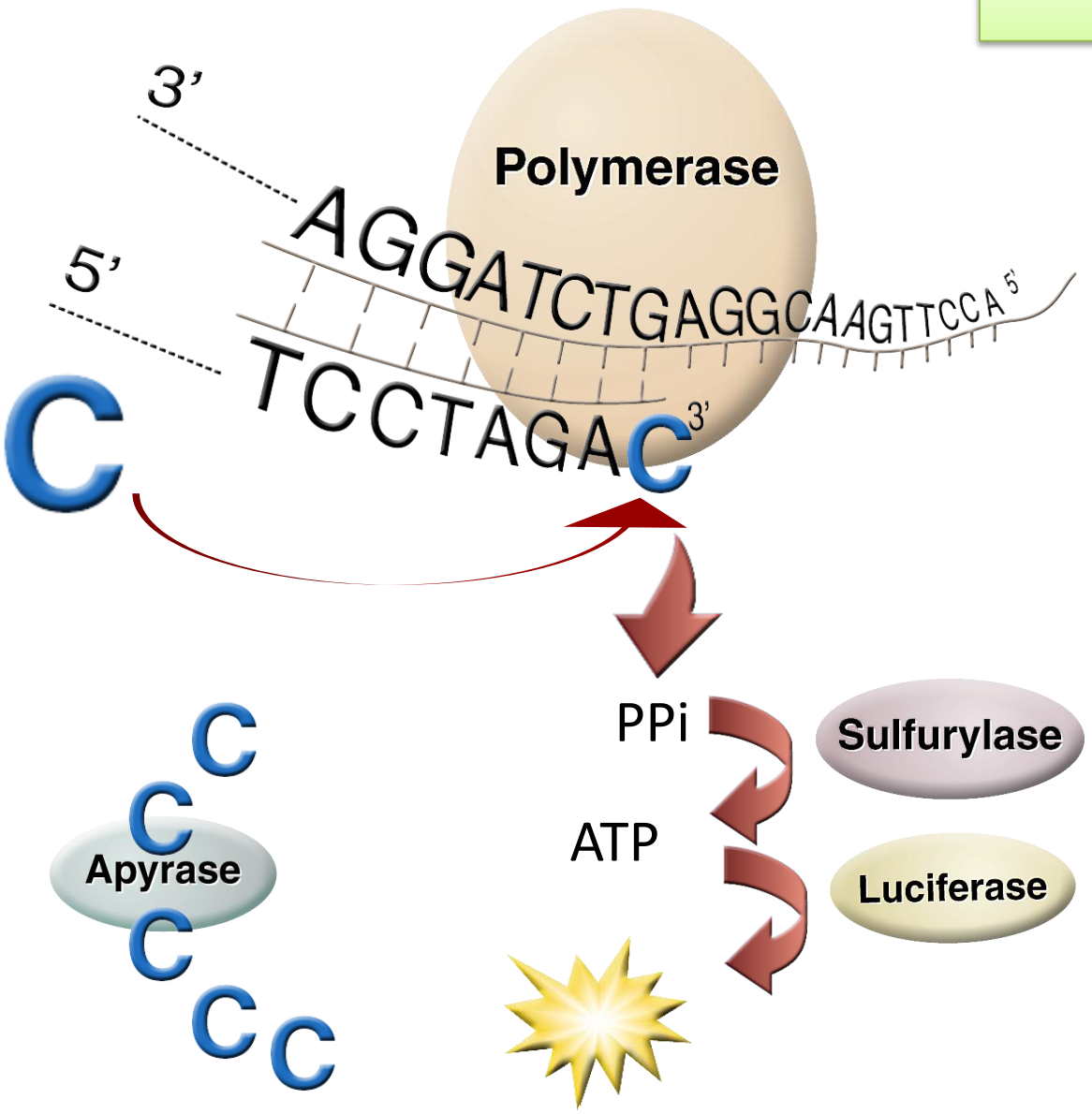
PR	ATV/r	DRV/r	FPV/r	IDV/r	LPV/r	NFV	SQV/r	TPV/r
Total:	0	0	0	0	0	0	0	0

Pyrosequencing

- solution for applied DNA analysis

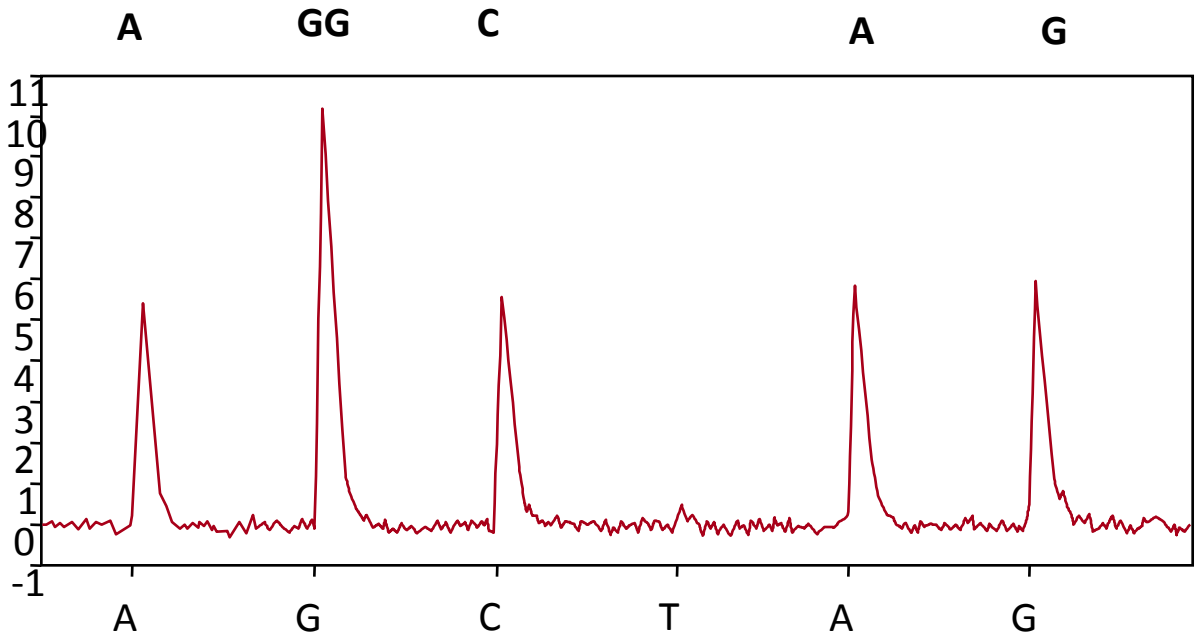
- Sequence based technology
- Accurate
- Simple and robust
- No labels or gels
- Real-time results

Pyrosequencing



Pyrosequencing

- nucleotides dispensed sequentially



the sequence in this pyrogram™ is AGGCAG

Four enzymes are crucial for the accuracy of this DNA sequencing technology

- Klenow DNA Polymerase : extension of the primer and simultaneous release of PPi
- ATP Sulfurylase : catalyze ATP from PPi
- Luciferase : catalyses the light production from ATP
- Apyrase : degradation of unincorporated nucleotides and excess ATP between base additions

Result analysis

2010-08-18

IdentiFire

Detailed Report

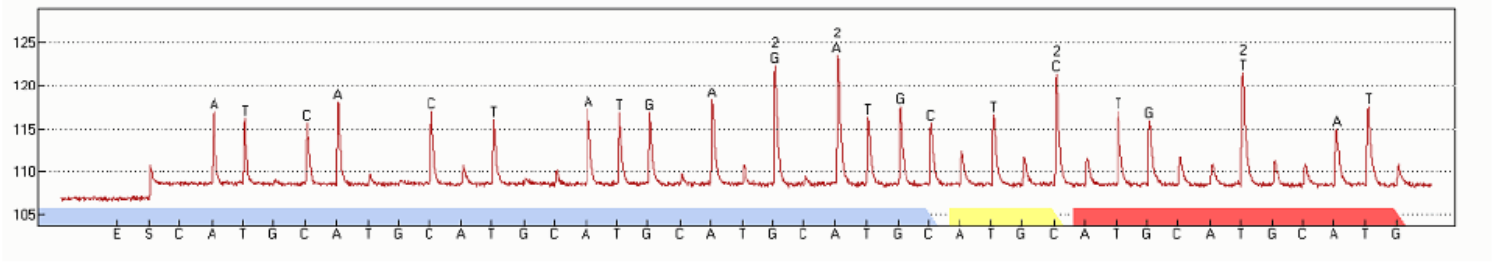
Biotage

Sample ID: **ICRCNPASC01/09**

Well: A1
PSQ run: 160453
Entry ID: NA modified

Sequence library: NA drug resis (2009-08-20, 9:24:18 AM)
Query sequence: ATCACTATGAGGAATGCTCCTGTTAT

Result: H274Y_A_Yamaguchi_22_2009H1N1 **Score:** 92.7
Quality: Uncertain
Warnings: Low homology between query and best hit.

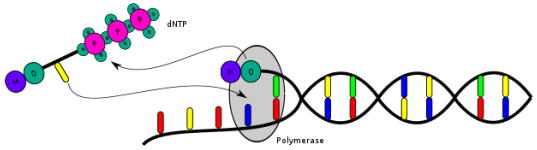


Hit 1: **H274Y_A_Yamaguchi_22_2009H1N1**

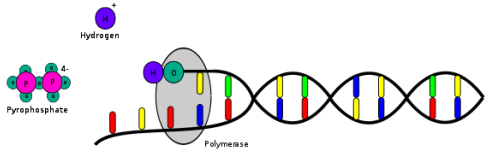
Score: 92.7
Identities: 25/26 (96%)
Gaps: 0/26 (0%)
E-value: 6.83e-023

```
Query    1  ATCACTATGAGGAATGCTCCTGTTAT 26
           ||  ||||||||||||||||||||||||
Library  1  ATTACTATGAGGAATGCTCCTGTTAT 26
```

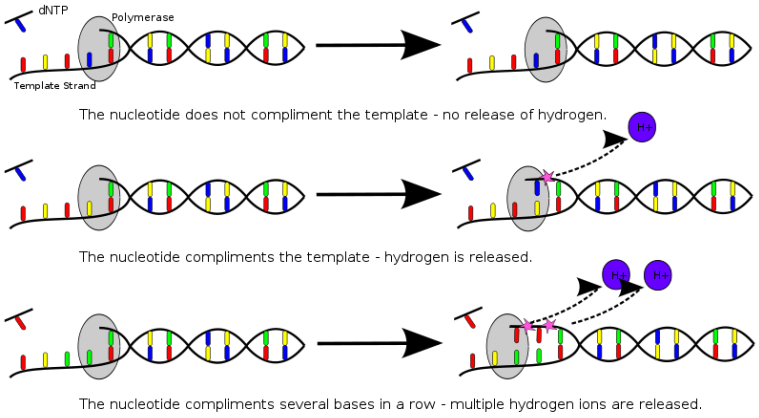
Next generation of sequencing

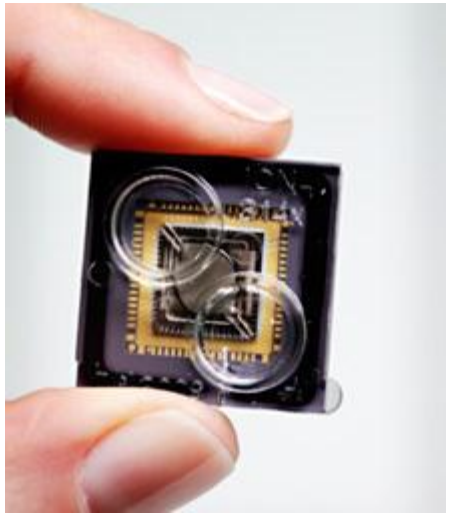


Polymerase integrates a nucleotide.

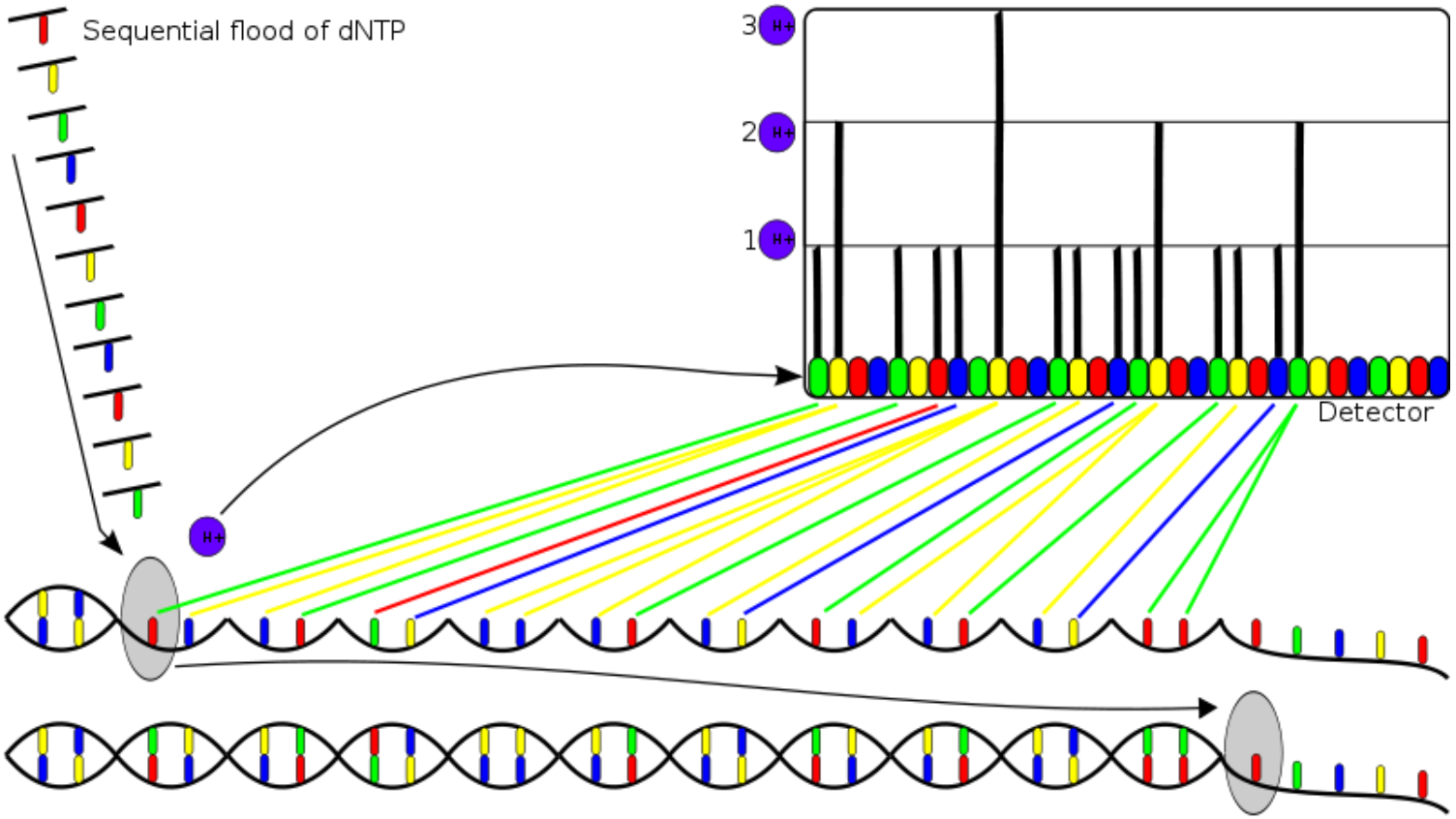


Hydrogen and pyrophosphate are released.





Next generation of sequencing



	Maximum Throughput Mb/run	Mean Length (nucleotide)	Error rate *	Applications	Main source of errors
Illumina	6,000	~100	10^{-2}–10^{-3}	Genome resequencing, quantitative transcriptomics, genotyping, metagenomics	Signal interference among neighboring clusters, homopolymers, phasing, nucleotide labeling, amplification, low coverage of AT rich regions
Ion Torrent PGM	1,000	~200	3×10^{-2}	De novo genome sequencing and resequencing, target resequencing, genotyping, RNA-seq on low-complexity transcriptome, metagenomics	Homopolymers, amplification
GS Junior	~35	~400	10^{-3}–10^{-4}	Target resequencing (amplicons), genotyping	Intensity cutoff, homopolymers, signal cross-talk interference among neighbors, amplification, mixed beads

Int. J. Mol. Sci. 2011, *12*, 7861-7884

Bansode et al. BMC Infectious Diseases 2013, 13:52
http://www.biomedcentral.com/1471-2334/13/52

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Characterizing the emergence and persistence of drug resistant mutations in HIV-1 subtype C infections using 454 ultra deep pyrosequencing

Vijay Bansode[1], Grace P McCormack[1], Amelia C Crampin[2,3], Bagrey Ngwira[2,3], Ram K Shrestha[4], Neil French[3,5], Judith R Glynn[3] and Simon A Travers[4*]

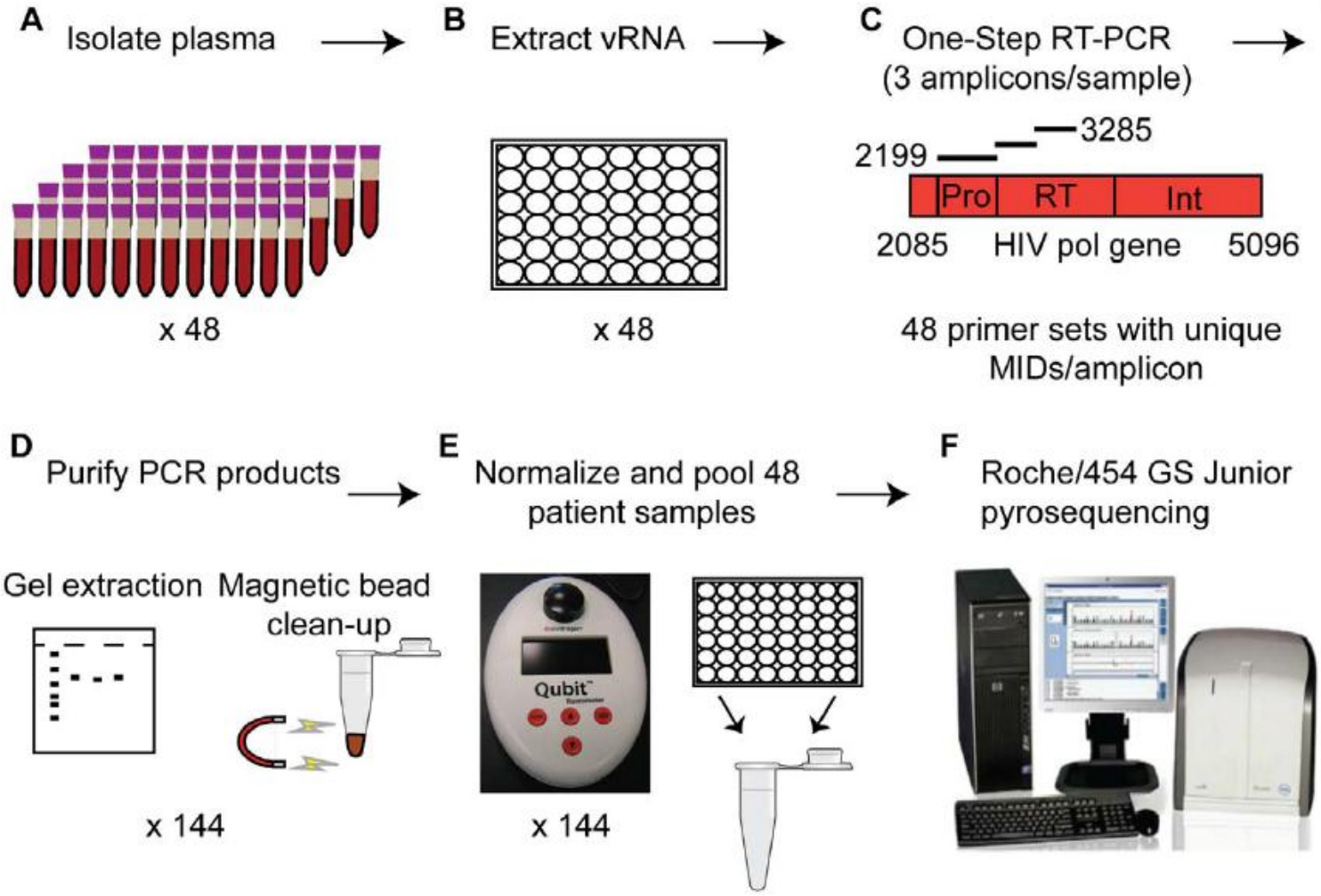
OPEN ACCESS Freely available online

PLoS one

Low-Cost Ultra-Wide Genotyping Using Roche/454 Pyrosequencing for Surveillance of HIV Drug Resistance

Dawn M. Dudley[1], Emily N. Chin[2], Benjamin N. Bimber[1], Sabri S. Sanabani[4], Leandro F. Tarosso[5], Priscilla R. Costa[5], Mariana M. Sauer[5], Esper G. Kallas[5], David H. O.'Connor[1,3]*

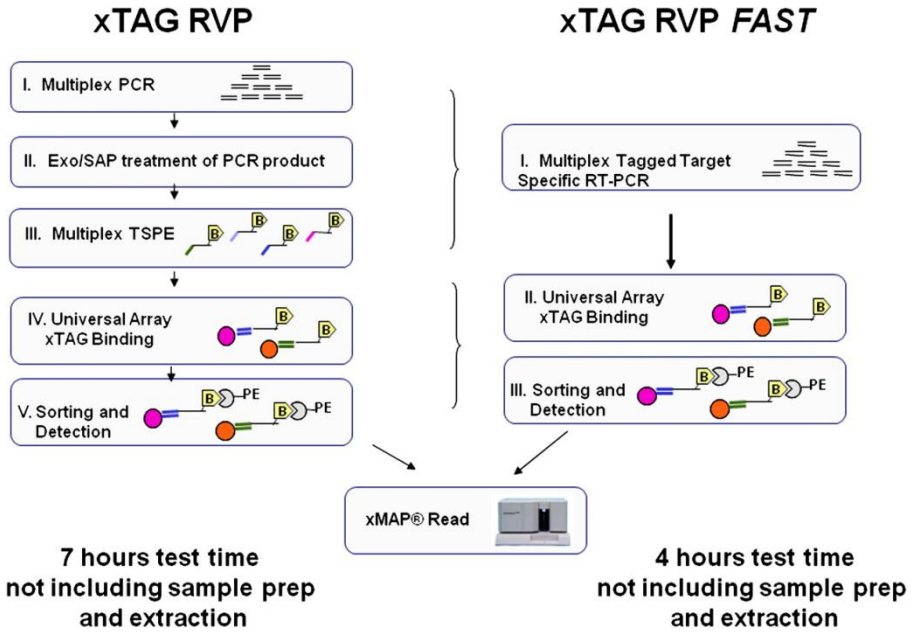
1 Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, Wisconsin, United States of America, **2** Department of Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, Wisconsin, United States of America, **3** Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, Wisconsin, United States of America, **4** São Paulo Institute of Tropical Medicine, University of São Paulo, São Paulo, Brazil, **5** Division of Clinical Immunology and Allergy, University of São Paulo, São Paulo, Brazil

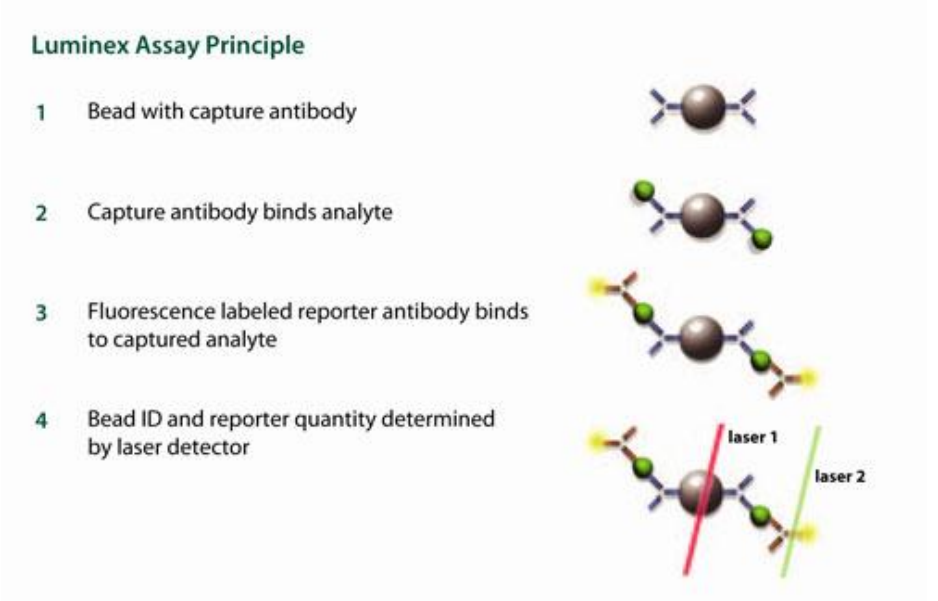


PLoS One. 2012;7(5):e36494

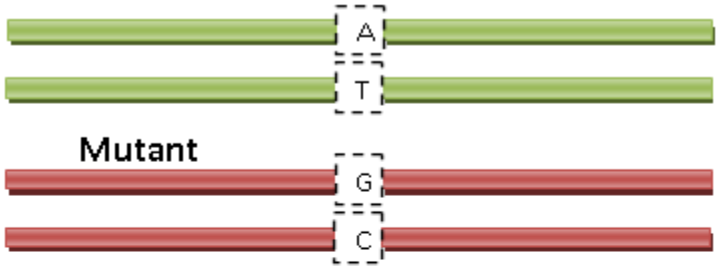
- This method is also 4-fold more sensitive (5% minimal detection frequency vs. 20%) at a cost 3–56x less than the traditional Sanger-based genotyping method.
- A Roche/454 GS Junior run costs about $1000 or ,$20 per sample when 48 samples are multiplexed together.

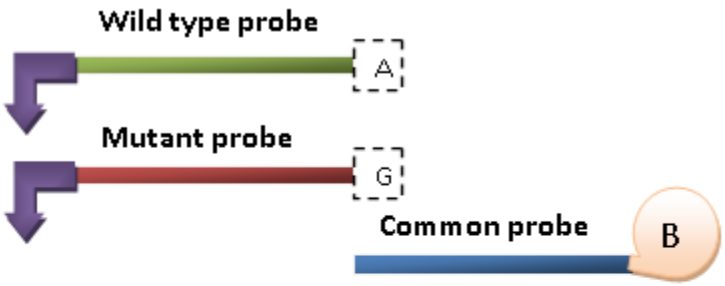
Luminex


xTAG RVP
I. Multiplex PCR
II. Exo/SAP treatment of PCR product
III. Multiplex TSPE
IV. Universal Array xTAG Binding
V. Sorting and Detection
PE
xTAG RVP FAST
I. Multiplex Tagged Target Specific RT-PCR
II. Universal Array xTAG Binding
III. Sorting and Detection
xMAP® Read
7 hours test time not including sample prep and extraction
4 hours test time not including sample prep and extraction

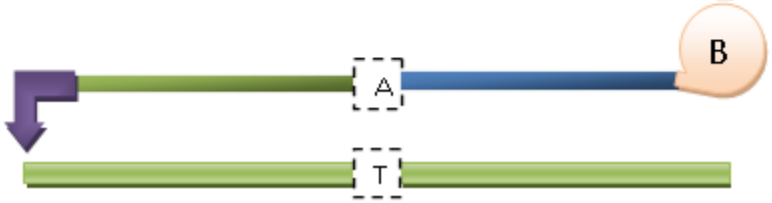

Luminex Assay Principle
1 Bead with capture antibody
2 Capture antibody binds analyte
3 Fluorescence labeled reporter antibody binds to captured analyte
4 Bead ID and reporter quantity determined by laser detector
laser 1
laser 2

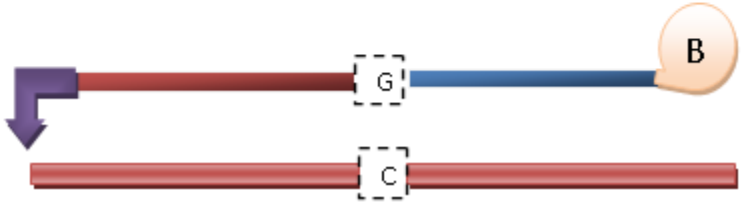
Luminex



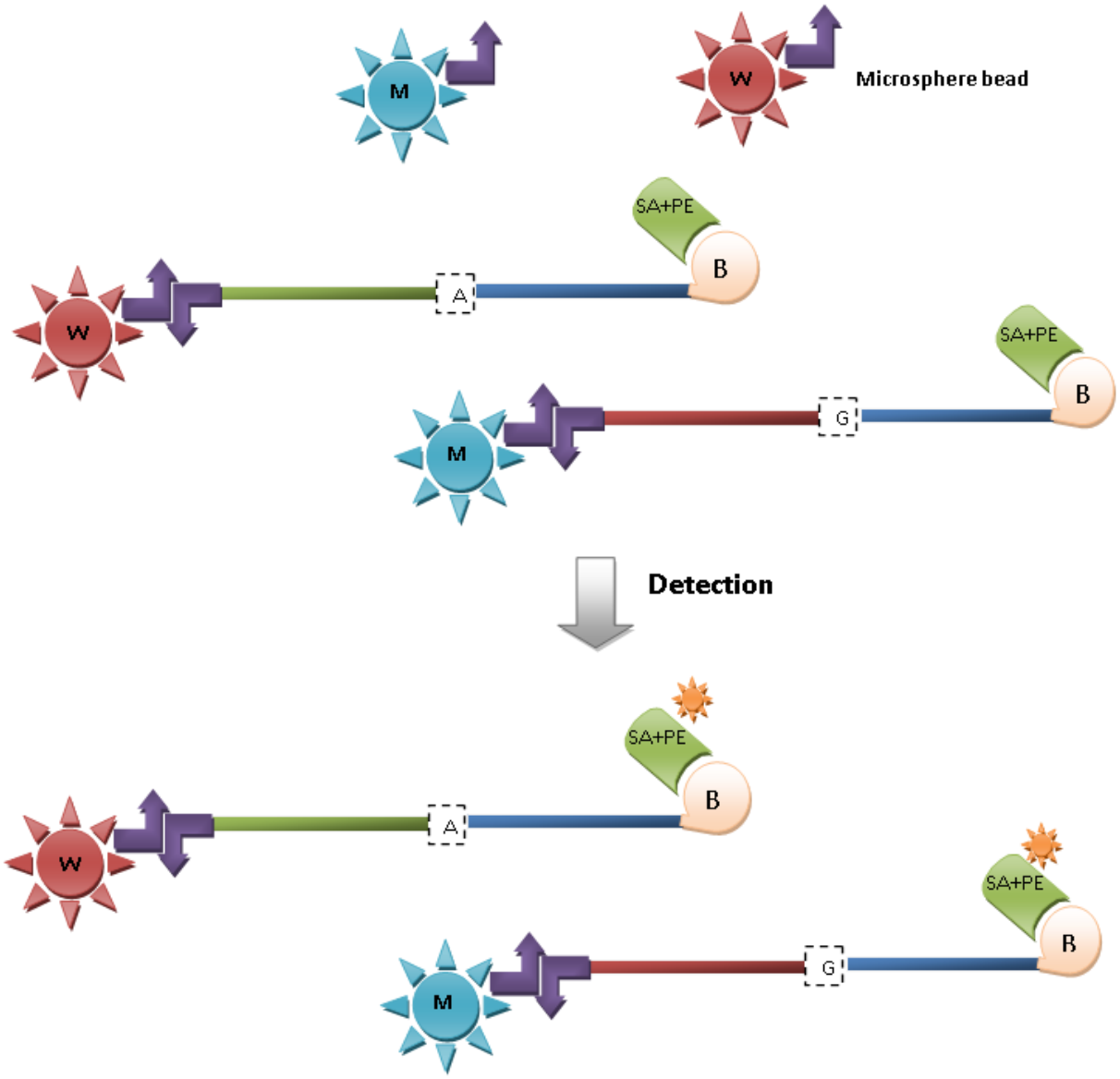


Ligation

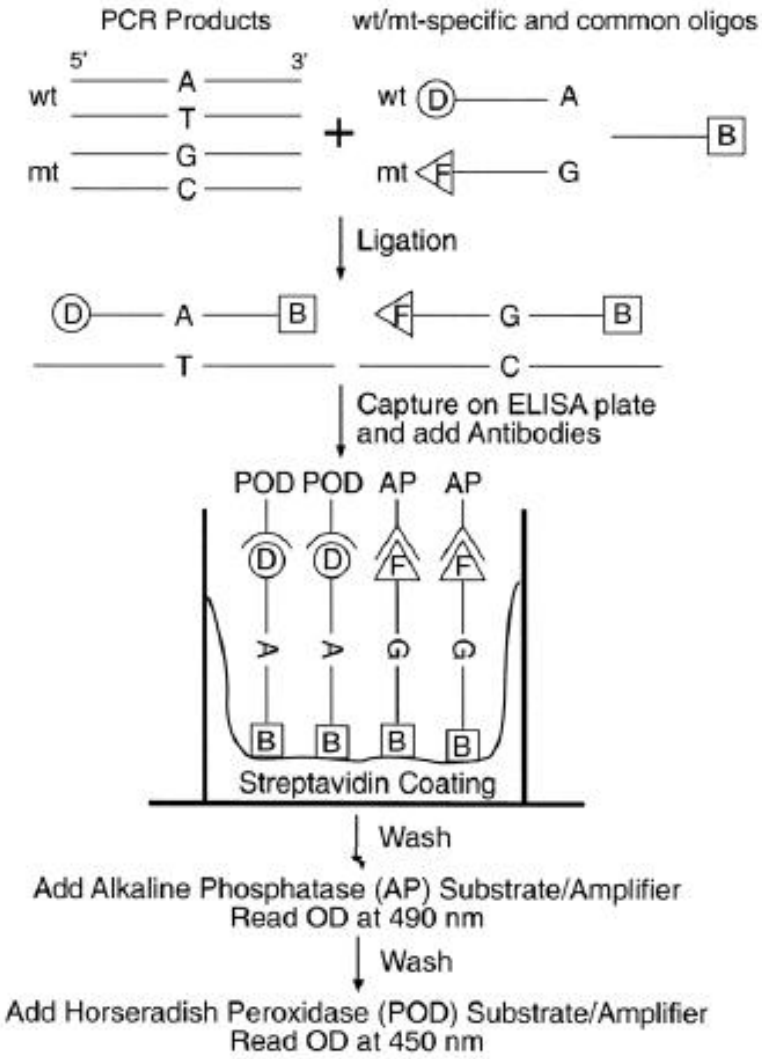


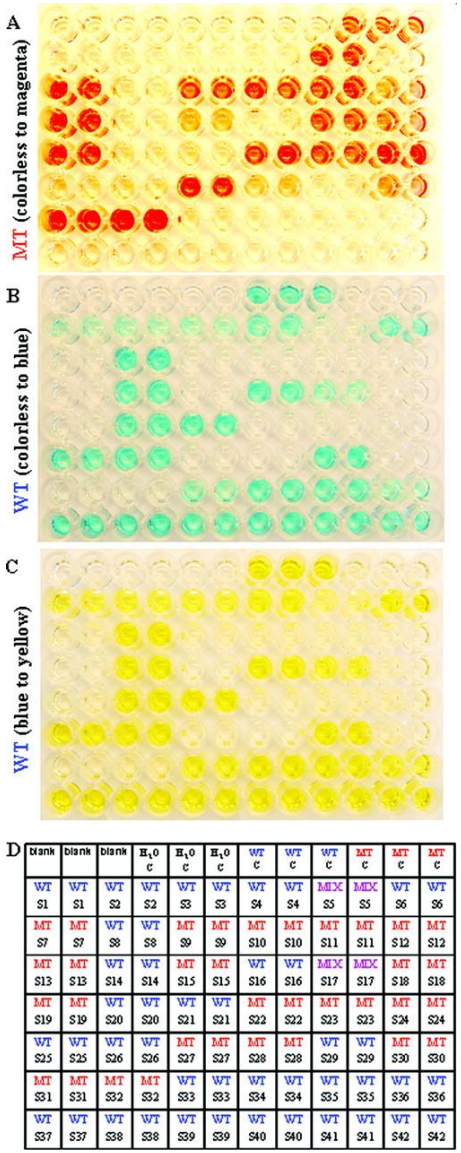


Luminex

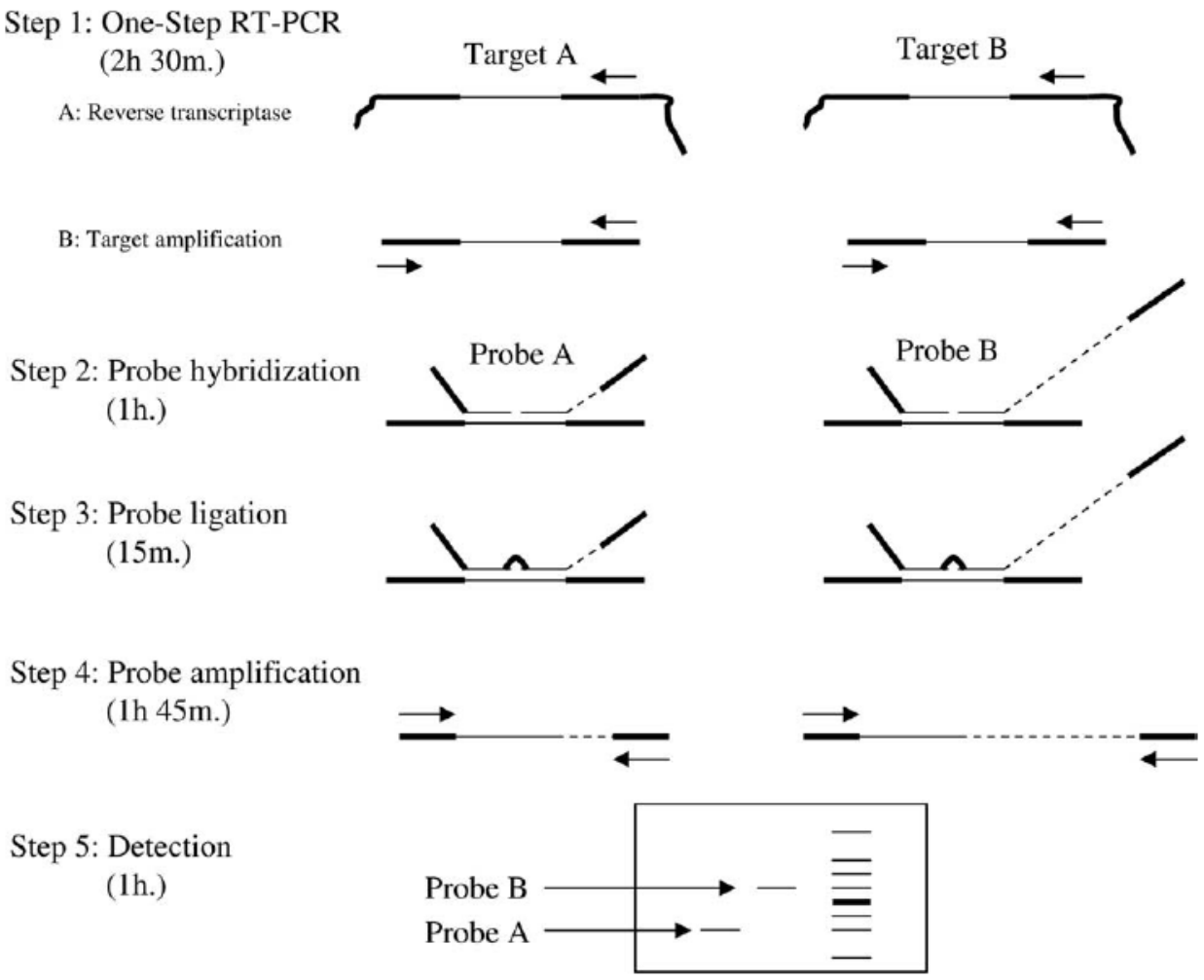

M
W
Microsphere bead
SA+PE
B
W
A
SA+PE
B
M
G
Detection
SA+PE
B
W
A
SA+PE
B
M
G

Oligonucleotide Ligation assay

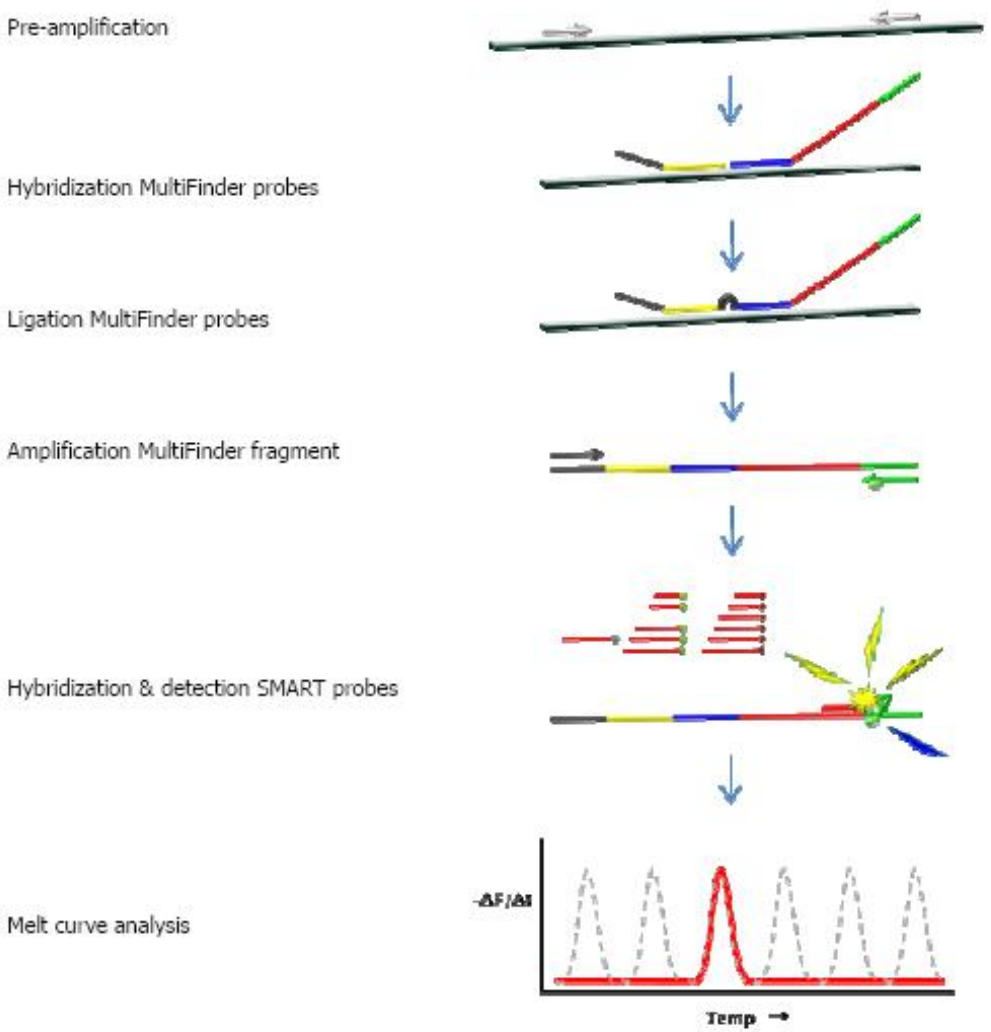




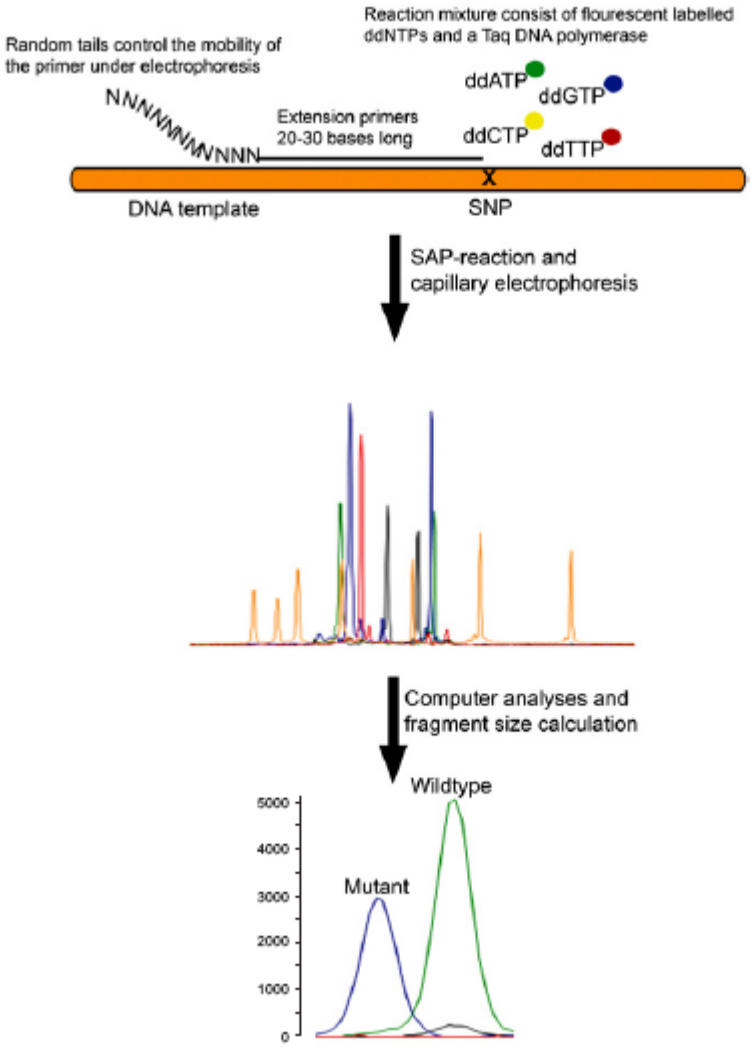
Multiplex ligation-dependent probe amplification

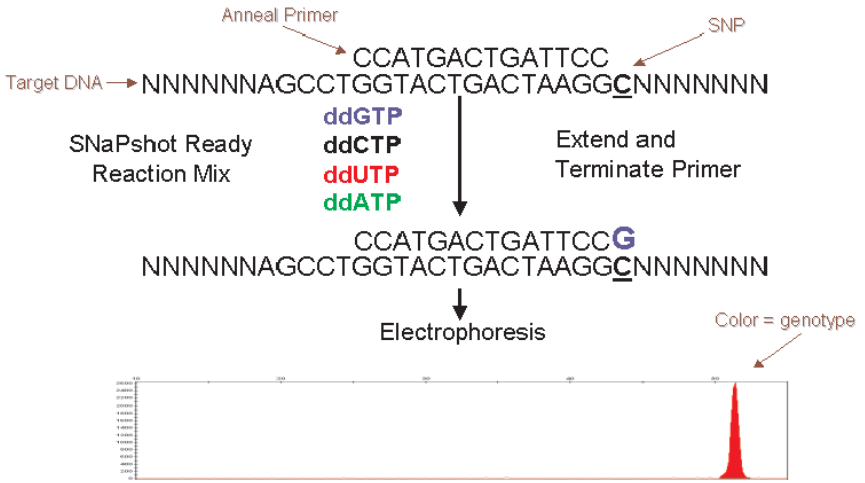


Multiplex ligation-dependent probe amplification



Snapshot assay





Snapshot assay

(A) Primers Used for PCR Amplification of the HIV-1 RT-Region from Proviral DNA

Name	Target Area[5]	Sequence
FW-1	1923 - 1954	GGGAACCAAAAATGATAGGGGGAATTGGAGG
BW-1	3056 - 3086	CTGTATTTCTGCTATTAAGTCTTTTGATGG
FW-2	2033 - 2055	CCTACACCTGTCAACATAATTG
BW-2	2945 - 2965	GTTAGTGCTTTGGTTCCTCT

[5]Reference sequence HXB-2 complete genome (9181 bp; AF033819).

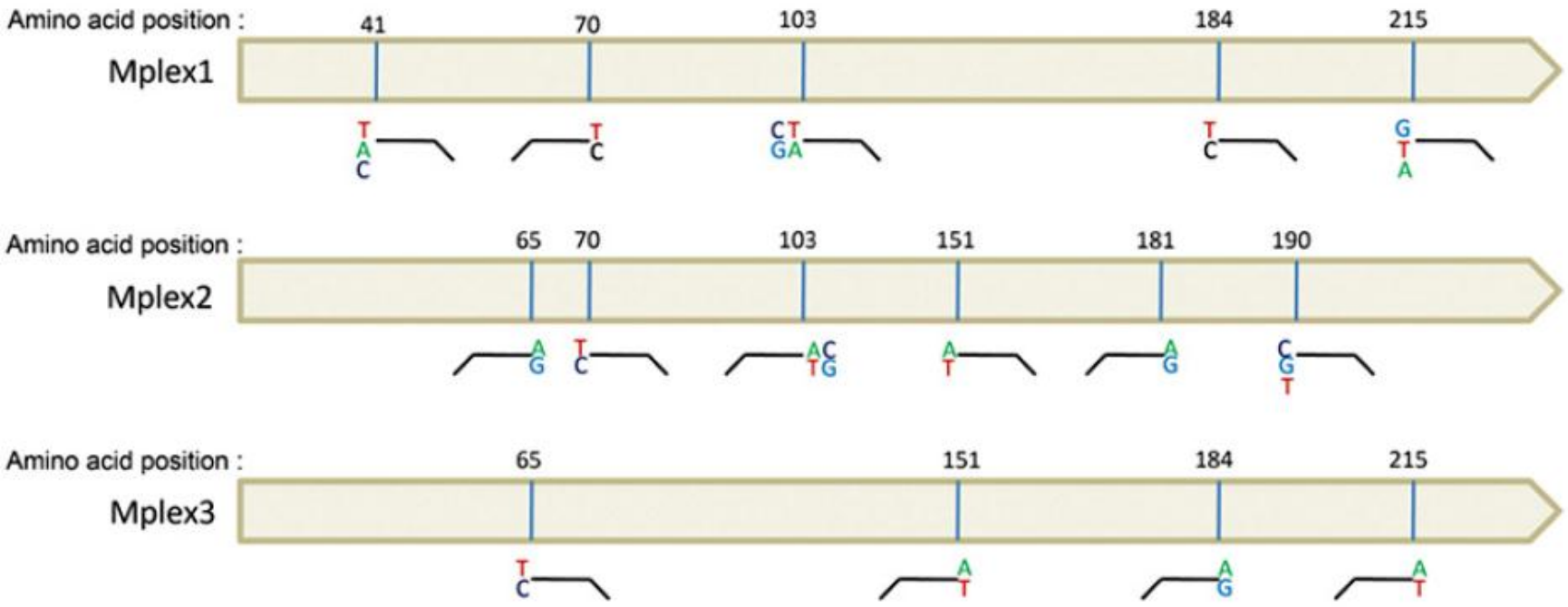
(B) Extension Primer Used in the Multiplex Assay

Target	Direction[1]	Extension Probe Sequence	SNP[2]	Length	w/ Tail	[pmol][3]	Mplex[4]
M41L	R	$(N)_3$-CAATTTTTGAAATTTTCCCTTCCTTTTCCA	T > A/C	30	33	0.3	1
K65R	F	CAATACTCCAGTATTTGCCATAAAGA	A > G	26	26	0.2	2
K65R	R	$(N)_{16}$-CTACTAATTTTCTCCATTTAGTACTGTCTTTT	T > C	32	48	0.7	3
K70R	F	$(N)_{16}$-TGCCATAAAGAAAAAAGACAGTACTA	A > G	26	42	0.2	1
K70R	R	$(N)_{17}$-GTTCTCTGAAATCTACTAATTTTCTCCAT	T > C	29	46	0.3	2
K103N	F	ACATCCCGCAGGGTTAAAAAAGAA	A/G > G/A	24	24	0.5	2
K103N	R	CCCACATCCAGTACTGTTACTGATTT	T/C > G/A	26	26	0.2	1
Q151M	F	$(N)_{12}$ -ATA TCA GTA CAT TGT GCT TCC A	C > A	22	34	0.2	3
Q151M	R	$(N)_2$-GAATATTGCTGGTGATCCTTTCCATCCC	T > A	28	30	0.4	2
Y181C	F	$(N)_{25}$-GAAAACAAAATCCAGACATAGTTATCT	A > G	27	52	0.3	2
M184V	F	CCAGACATAGTTATCTATCAATAC	A > G	24	24	0.2	3
M184V	R	$(N)_{20}$-GTCAGATCCTACATACAAATCATCCA	T > C	26	46	0.5	1
G190A/E	R	$(N)_{12}$-CTATGCTGCCCTATTTCTAAGTCAGAT	C > G/T	27	39	0.3	2
T215Y/F	F	$(N)_{17}$-CATCTGTTGAGGTGGGGATTT	A > T	21	38	0.3	3
T215Y/F	R	$(N)_{31}$-CTGATGTTTTTTGTCTGGTGTG	G > A/T	22	53	0.8	1

[1] Direction of primer-extension, either forward (F) or reverse (R).
[2] Possible nucleotides present at the mutation site.
[3] Balanced concentration of primers used in the different multiplex reaction.
[4] Mplex reaction where the primer is used in.

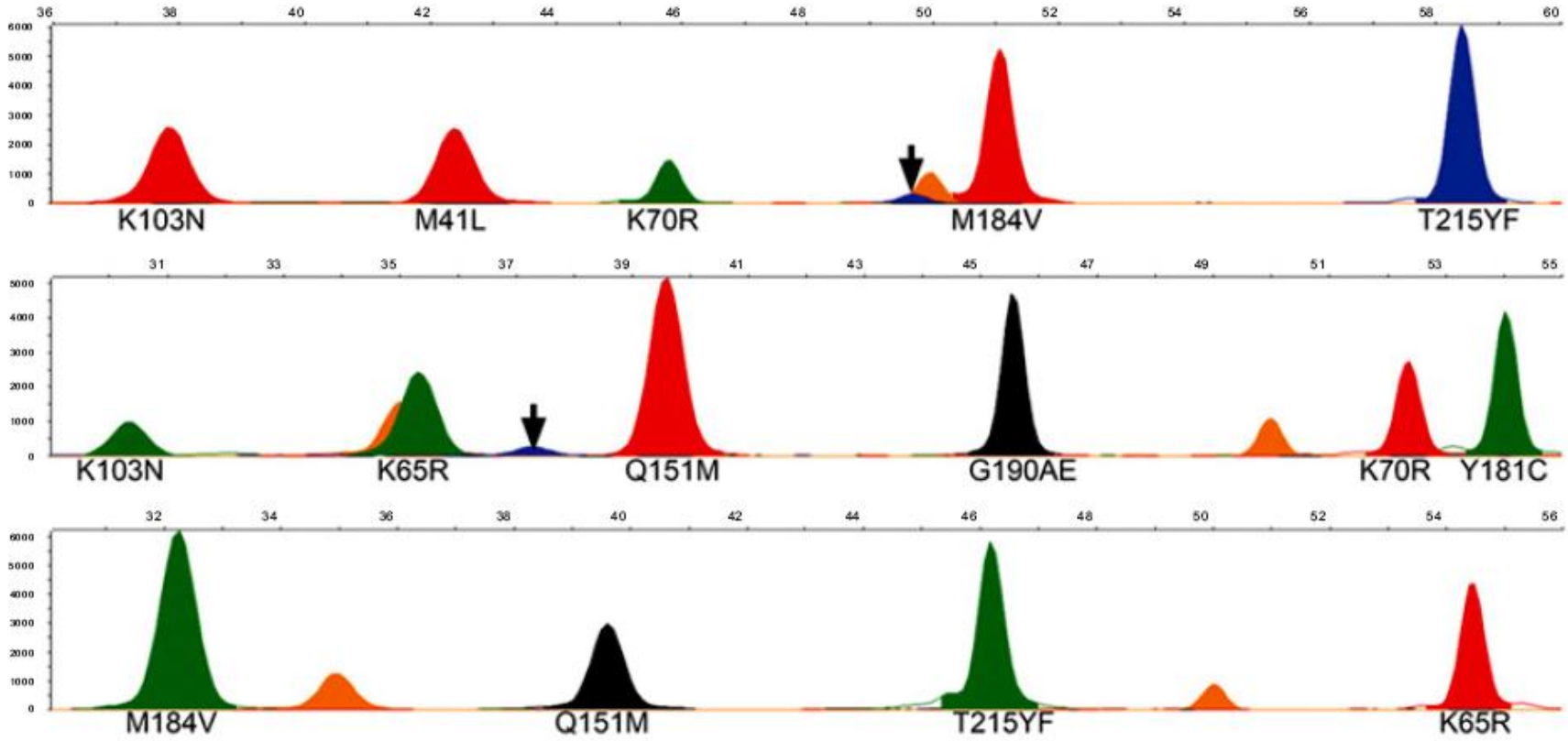
Current HIV Research, 2009, *7*, 398-409

Snapshot assay



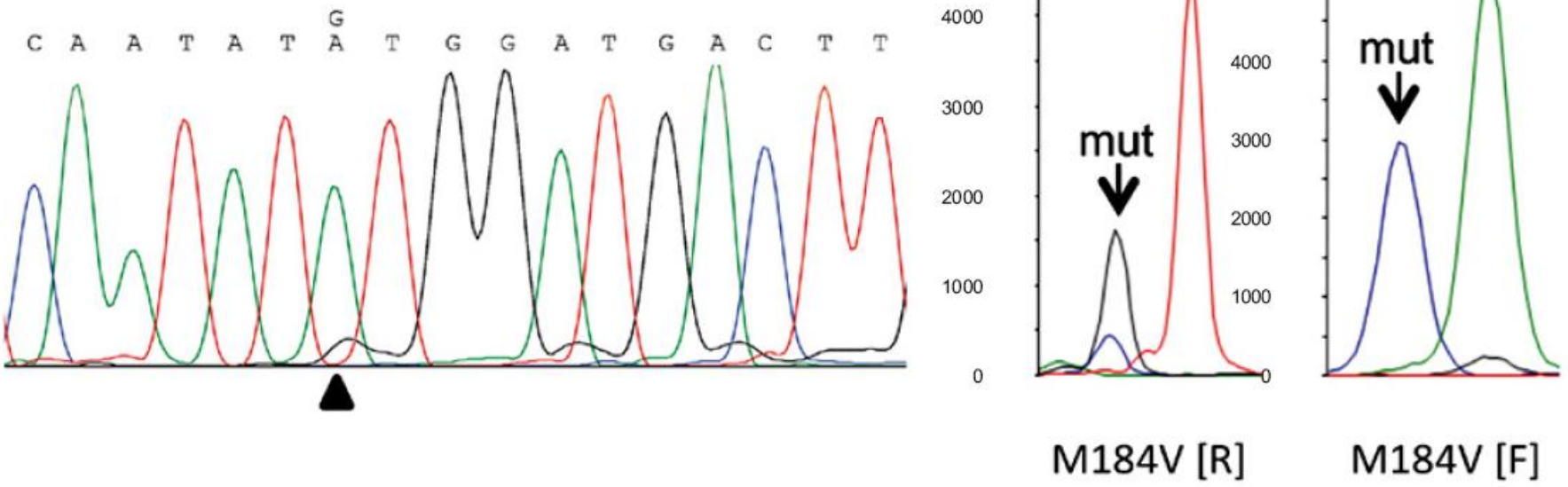
Current HIV Research, 2009, *7*, 398-409

Snapshot assay



Current HIV Research, 2009, *7*, 398-409

Snapshot assay

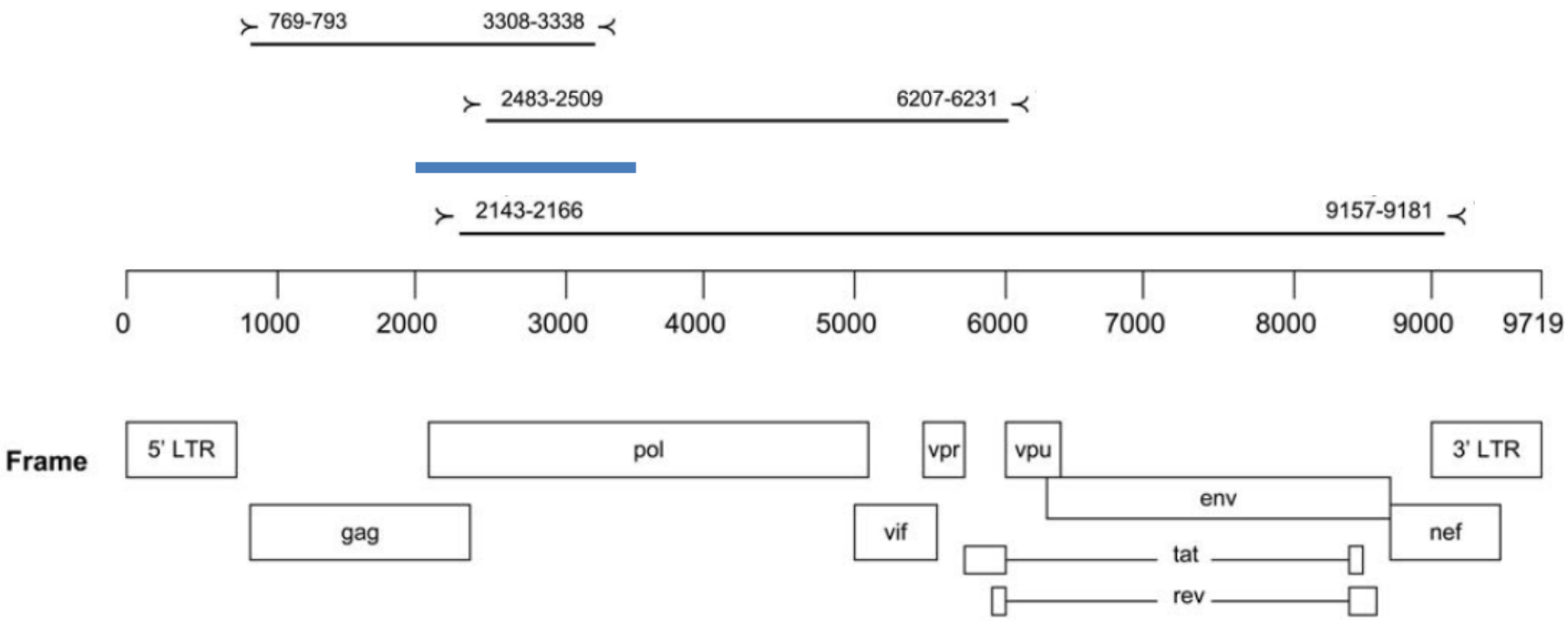


Current HIV Research, 2009, *7*, 398-409

New targets for resistance testing

- **Integrase**
  - Raltegravir (RAL), an integrase inhibitor
- **Gag**
  - may also influence susceptibility to protease inhibitors and compensatory mutations which restore fitness have been identified.
- **Tropism testing**
  - Maraviroc : CCR5 antagonist, and determination of viral tropism is essential prior to its use.

New targets for resistance testing



Technological advances

- **Resistance testing at low viral loads**
  - Major resistance mutations were as likely to be detected at viral loads of less than 1000 copies/ml
- **Minority species detection**
  - Allele specific PCR
  - single-genome sequencing
  - ultra-deep sequencing

Phenotype

- Measures the ability of the virus to grow in different concentrations of ARVs
- PCR amplicification of the reverse transcriptase, integrase, or protease gene sequences from patient plasma and insertion into backbone of lab strain of HIV (compared to reference HIV strain and reported as fold resistance change)
- Preferred test for more treatment-experienced patients with complex drug resistance mutation patterns
- More expensive than genotypic assays; takes longer to perform and obtain results

Phenotype Assays: Generic Procedure

Patient virus

↓ **RT-PCR**

PR-RT DNA

↓ **(Vector Assembly)**

(Resistance Test Vector)

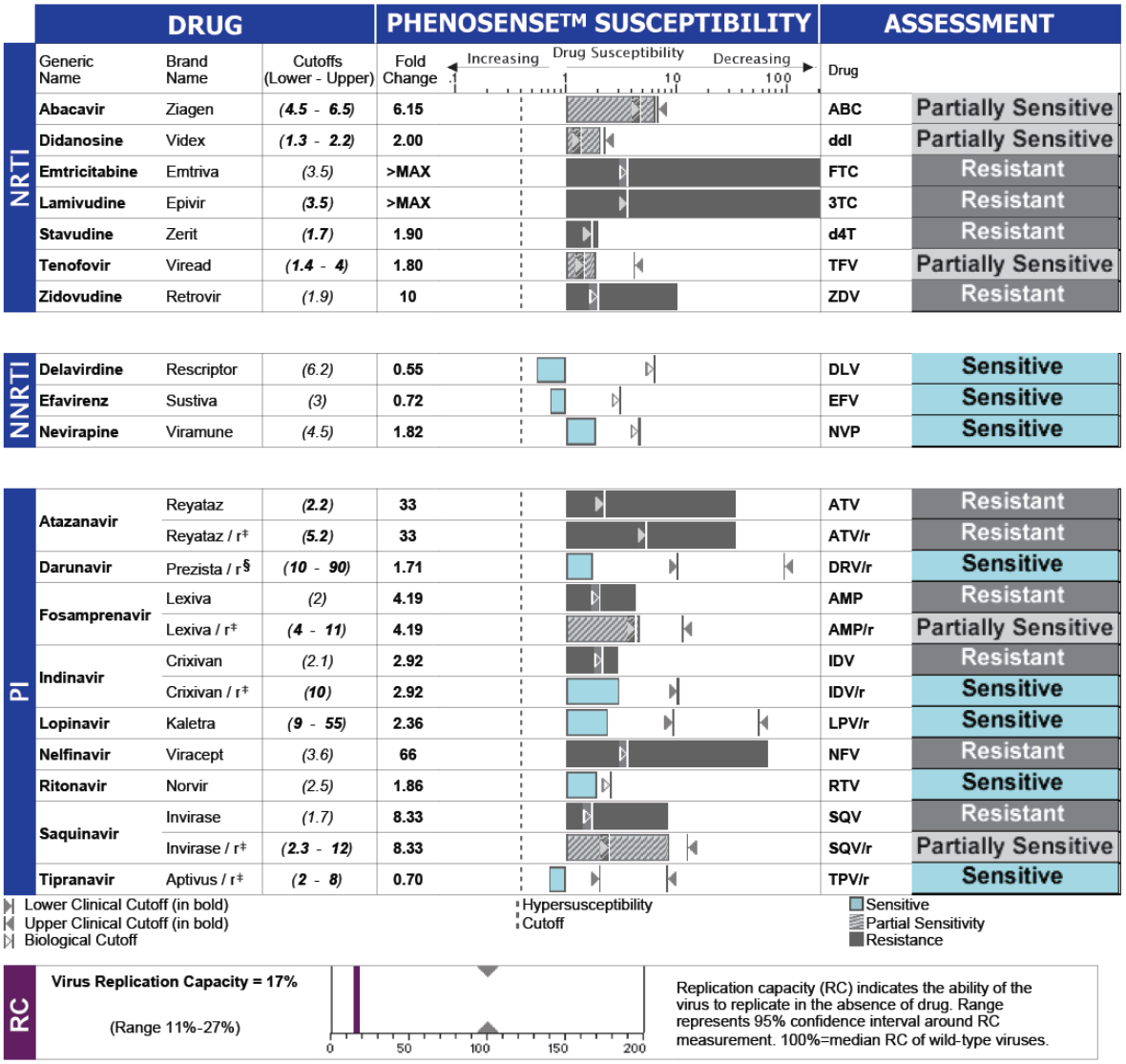
↓ **Transfection**

Recombinant Virus

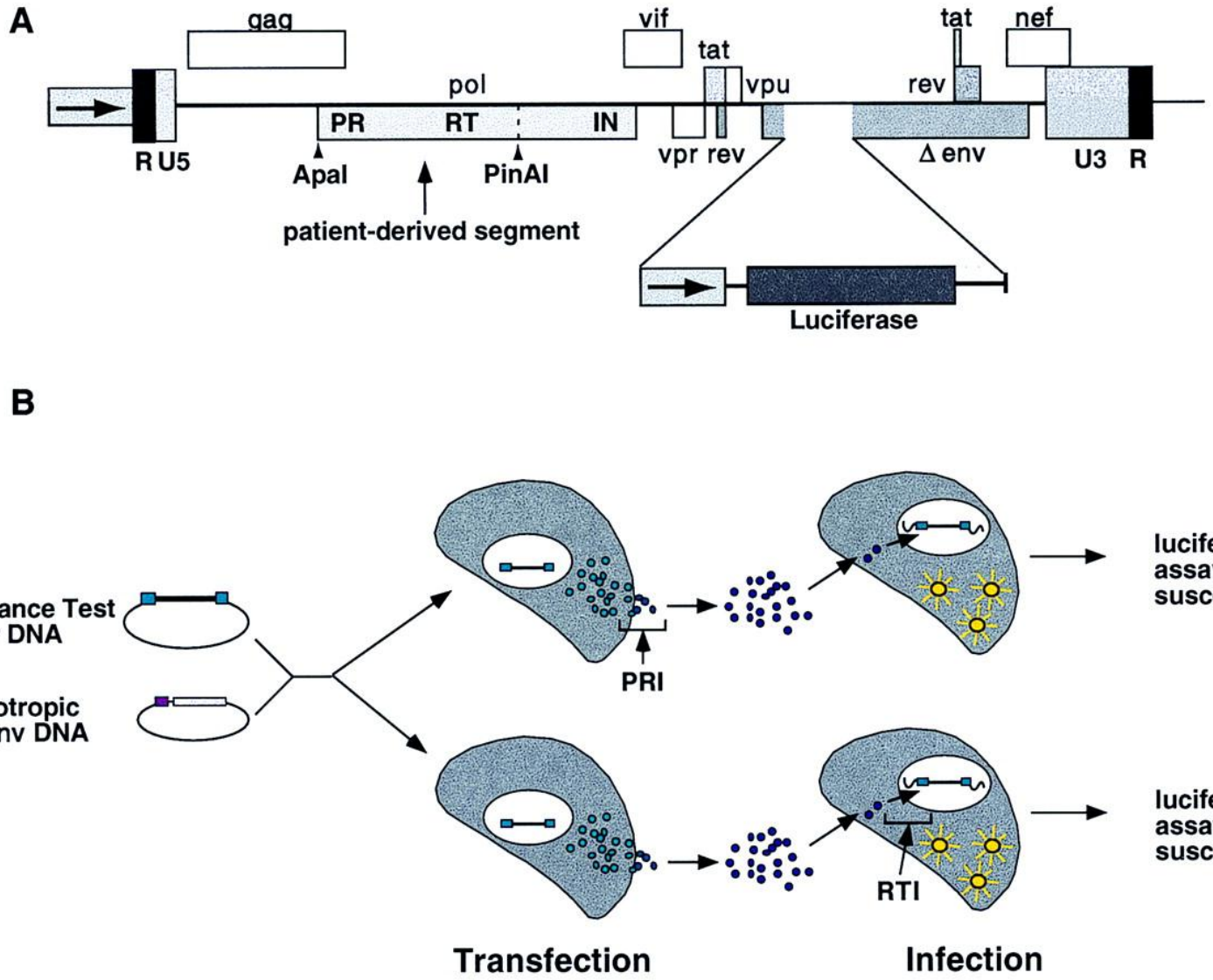
↓ **Infection**

Measure of Drug Susceptibility

Phenotype Report (Monogram)

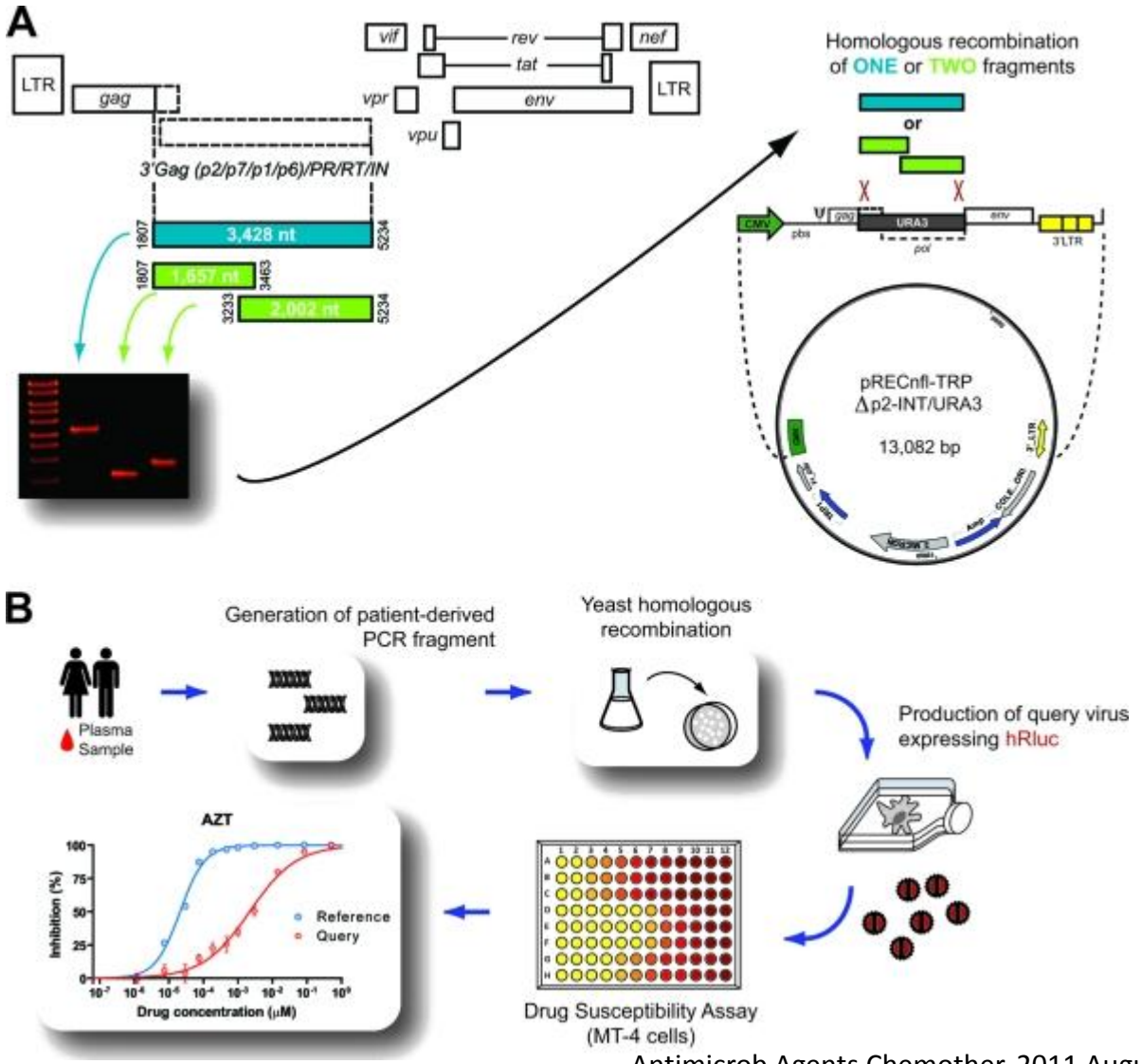


A Novel Phenotypic Drug Susceptibility Assay for Human Immunodeficiency Virus Type 1



Antimicrob Agents Chemother. 2000 April; 44(4): 920–928.

A Novel Phenotypic Drug Susceptibility Assay for Human Immunodeficiency Virus Type 1



Antimicrob Agents Chemother. 2011 August; 55(8): 3729–3742

Thank you