

Antivirogram™ & VircoGEN™

Virco's HIV-1 Resistance Assays

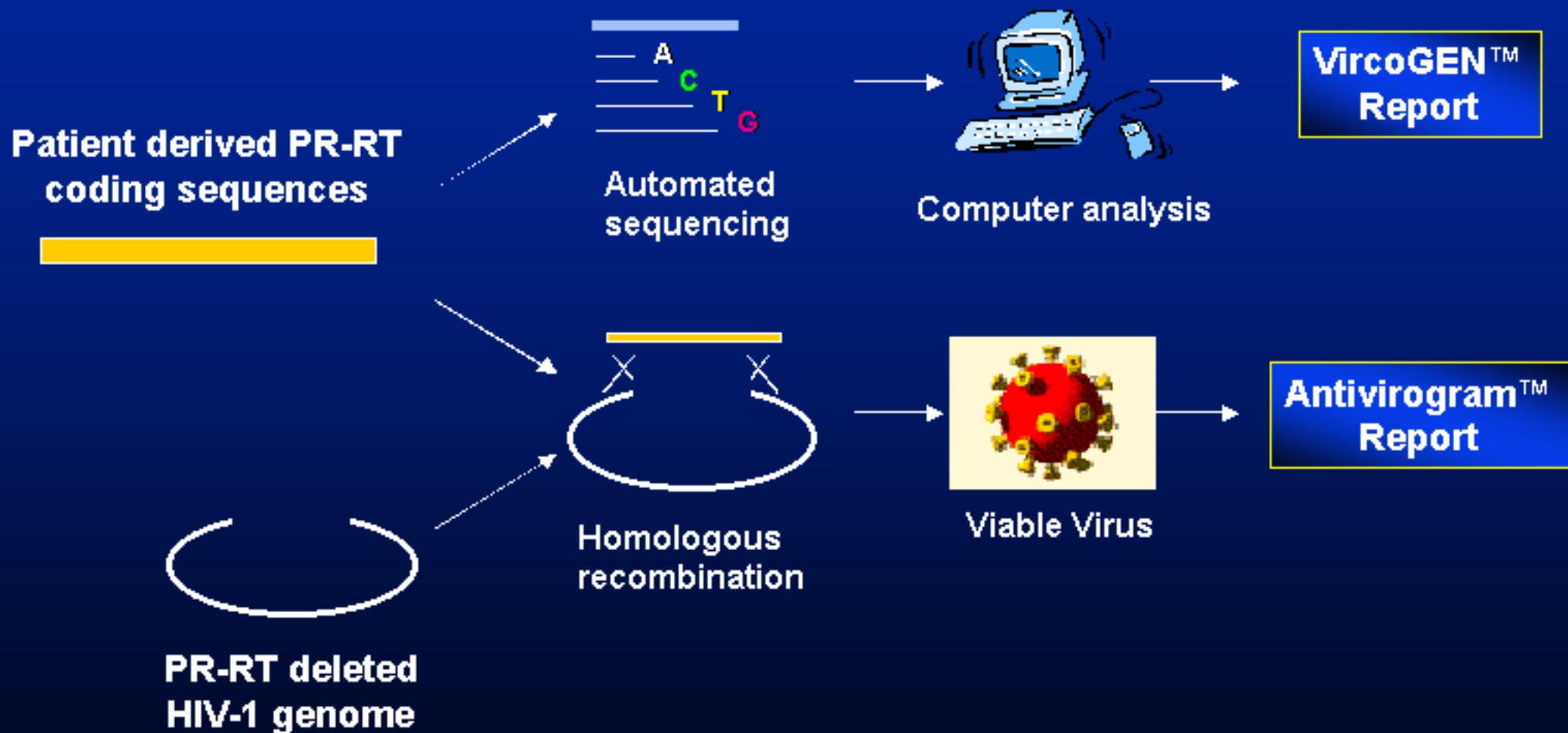
Brendan Larder



Utility of Resistance Monitoring in HIV Disease Management

- **Guide choice of initial regime**
 - Transmitted resistance is increasing
- **Understand reason for failure**
 - Resistant virus, adherence problem etc.
- **Guide choice of next therapy**
 - *Rule out* drugs where resistance has developed
 - *Rule in* drugs to which virus is sensitive
 - *Protect* new drugs by identifying most active partners

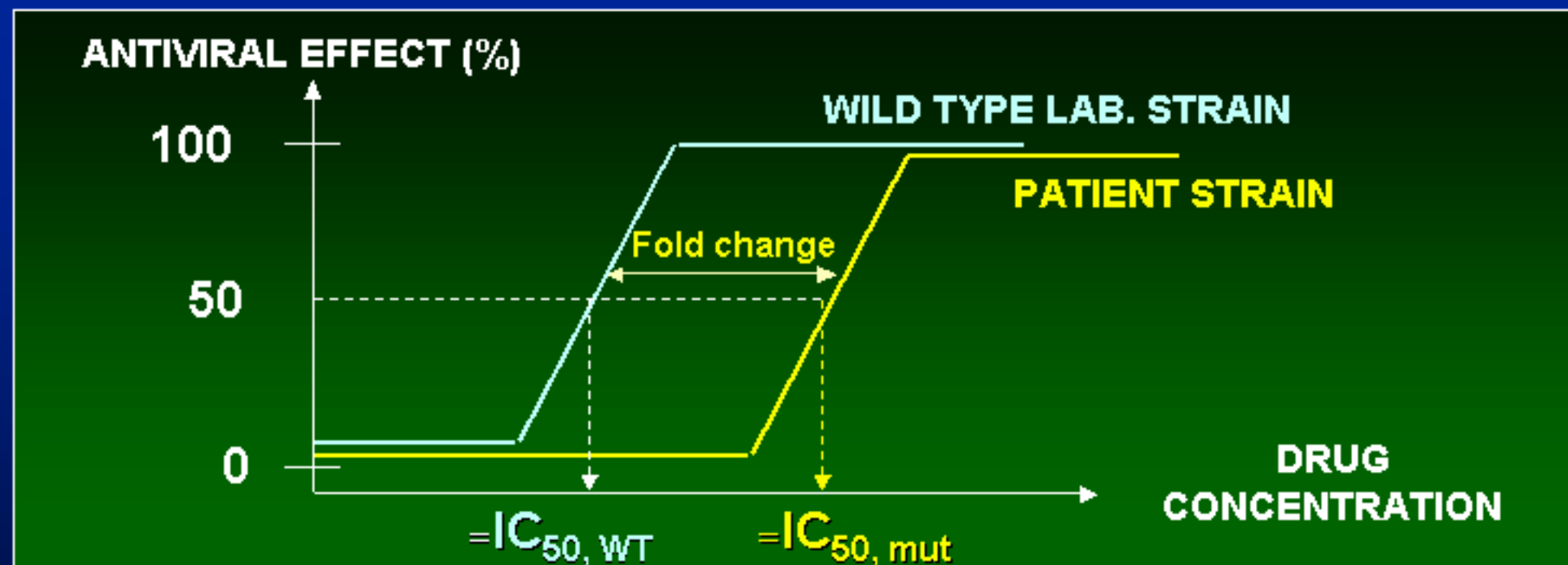
Genotyping & Phenotyping: Assay Principles



HIV-1 Phenotyping Assays

Antivirogram™

Antiviral Definitions



- IC_{50} = 50% Inhibitory Concentration
- $IC_{50, mut} / IC_{50, WT} = \text{Fold change}$
- e.g., $IC_{50, mut} 5\mu M$, $IC_{50, WT} 0.5\mu M$,
⇒ **Fold change = 10**

Antivirogram™ Report

Drugs		Susceptibility of patient sample						
Trade name	Generic name	Fold Resistance						
		0.1	1	10	100	1,000	10,000	Resistance Class
Retrovir®	Zidovudine	Green	Green	Yellow	Red	Red	Red	R
Epivir®	Lamivudine	Green	Green	Yellow	Red			R
Videx®	Didanosine	Green	Green	Yellow	Red			S
Hivid®	Zalcitabine	Green	Green	Yellow	Red			S
Zerit®	Stavudine	Green	Green	Yellow	Red			S
	Abacavir	Green	Green	Yellow	Red			S
Viramune®	Nevirapine	Green	Green	Yellow	Red	Red	Red	S
Crixivan®	Indinavir	Green	Green	Yellow	Red	Red	Red	S
Norvir®	Ritonavir	Green	Green	Yellow	Red	Red		S
Viracept®	Nelfinavir	Green	Green	Yellow	Red	Red	Red	R
Invirase®	Saquinavir	Green	Green	Yellow	Red	Red	Red	S

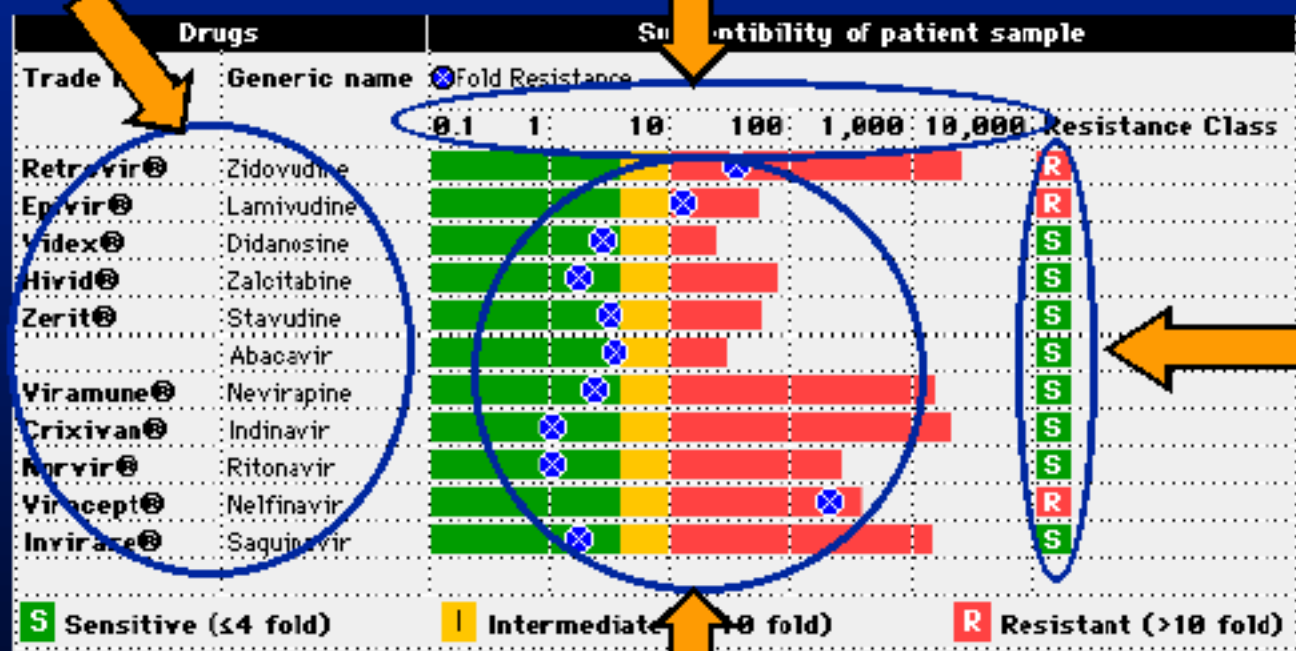
S Sensitive (≤4 fold)
 I Intermediate (4–10 fold)
 R Resistant (>10 fold)

All 14 approved NRTIs, NNRTIs & PIs reported (plus adefovir)

Antivirogram™ Report

1. Drugs tested
(trade & generic names)

2. Assay range
(fold resistance)



4. Assay
summary

3. Sensitivity to each drug
(position of blue dot)

HIV-1 Genotyping Assays

VircoGEN™

HIV-1 Drug Resistance Mutations

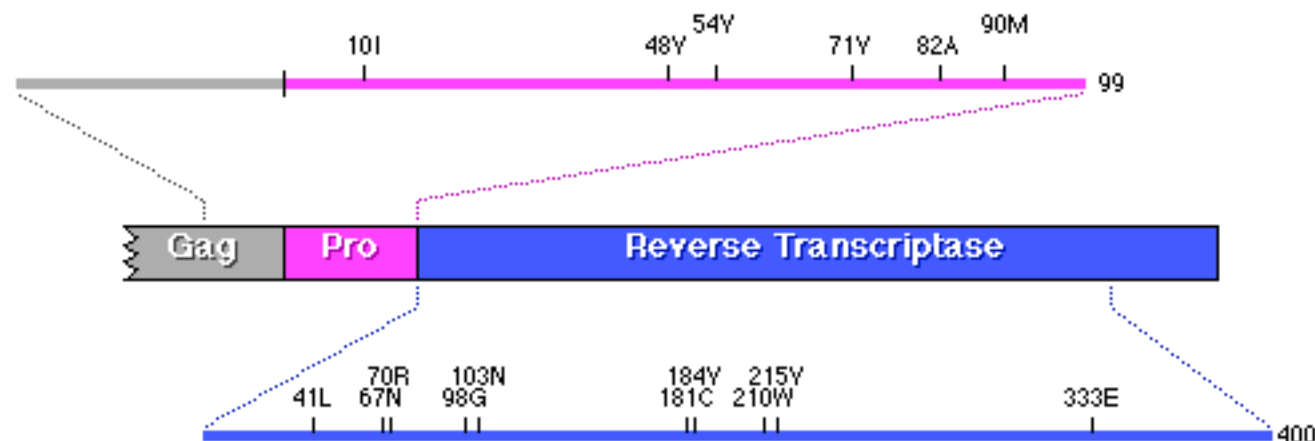
(changes relative to HXB2-D)

NRTI		NNRTI		Protease Inhibitor		<i>gag</i>
Mutation	Drug	Mutation	Drug	Mutation	Drug	Mutation
M41L	AZT	A98G	NVP	L10I/M/R	IDV, SQV	p7/p1:
A62V	MDR**	L100I	NVP, DMP	K20M/R	IDV, RTV	
K65R	ddl, ddC, 1592	K103N	NVP, DMP, DLV	L24I	IDV	A431V
D67N	AZT	V106A	NVP	D30N	NFV	Q430R
T69D	ddC	V108I	NVP, DMP	V32I	IDV, RTV	
T69S[XX]	MNR	V179D	DMP	L33F	RTV	
K70R	AZT	Y181C/I	NVP, DMP, DLV	M36I	RTV, NFV	p1/p6:
L74V	ddl, ddC, 1592	Y188C/L/H	NVP, DMP	M46I/L	IDV, RTV, NFV	
V75T/I	d4T / MDR**	G190A	NVP	I47V	141W94	L449F
F77L	MDR**	E233V	DLV	G48V	SQV	
Y115F	1592	P236L	DLV	I50V	141W94	
F116Y	MDR**	K238T	DLV	I54V	IDV, RTV, SQV	
Q151M	MDR**			A71T/M	IDV, RTV, NFL	
M184V/I/T	3TC, ddl, ddC, 1592			G73S	SQV	
L210W	AZT			V77I	IDV, NFV	
T215Y/F	AZT			V82A/F/I/S/T	IDV, RTV, SQV, NFV	
K219Q/E	AZT			I84V	IDV, RTV, SQV, NFV	
G333E/D	AZT			N88D	NFV	
				L90M	IDV, RTV, SQV, NFV	



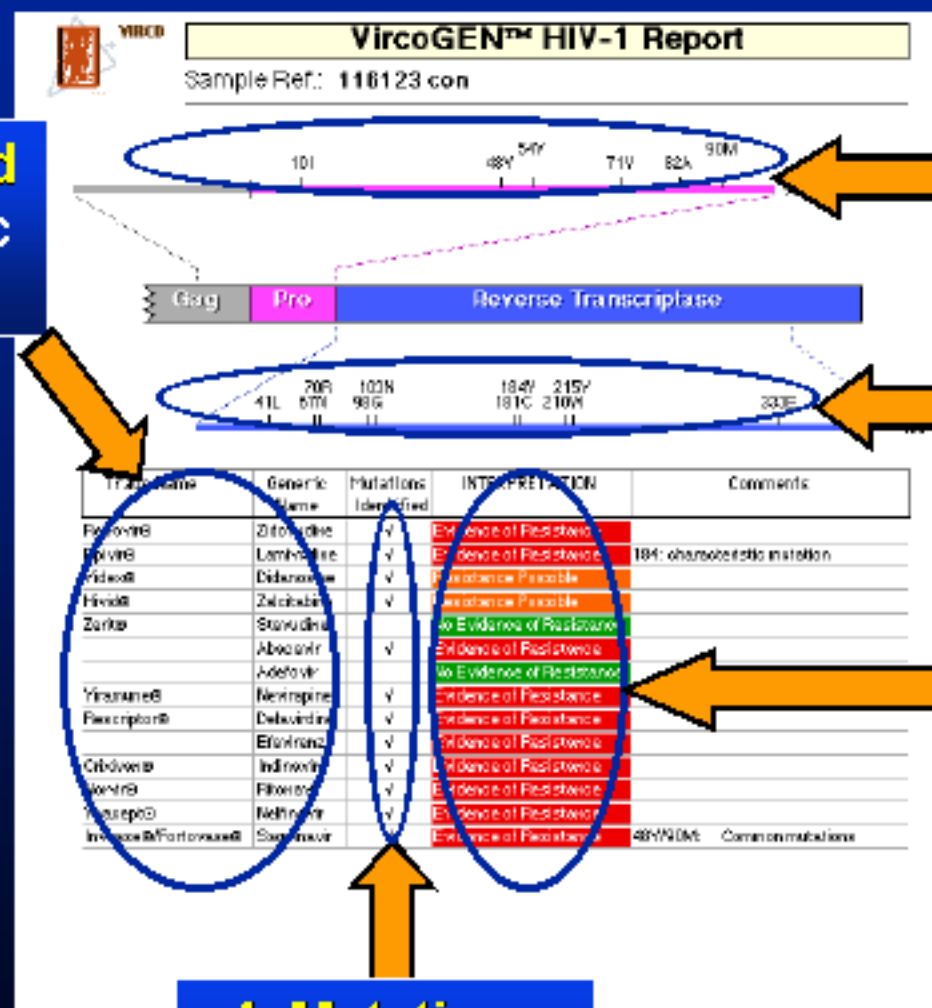
VircoGEN™ HIV-1 Report

Sample Ref.: 116123 con



Trade Name	Generic Name	Mutations Identified	INTERPRETATION	Comments
Retrovir®	Zidovudine	✓	Evidence of Resistance	
Epivir®	Lamivudine	✓	Evidence of Resistance	184: characteristic mutation
Yidex®	Didanosine	✓	Resistance Possible	
Hivid®	Zalcitabine	✓	Resistance Possible	
Zerit®	Stavudine		No Evidence of Resistance	
	Abacavir	✓	Evidence of Resistance	
	Adefovir		No Evidence of Resistance	
Viramune®	Nevirapine	✓	Evidence of Resistance	
Rescriptor®	Delavirdine	✓	Evidence of Resistance	
	Efavirenz	✓	Evidence of Resistance	
Crixivan®	Indinavir	✓	Evidence of Resistance	
Norvir®	Ritonavir	✓	Evidence of Resistance	
Viracept®	Nelfinavir	✓	Evidence of Resistance	
Invirase®/Fortovase®	Saquinavir	✓	Evidence of Resistance	48Y/90M: Common mutations

VircoGEN™ Report



1. Drugs tested
(trade & generic
names)

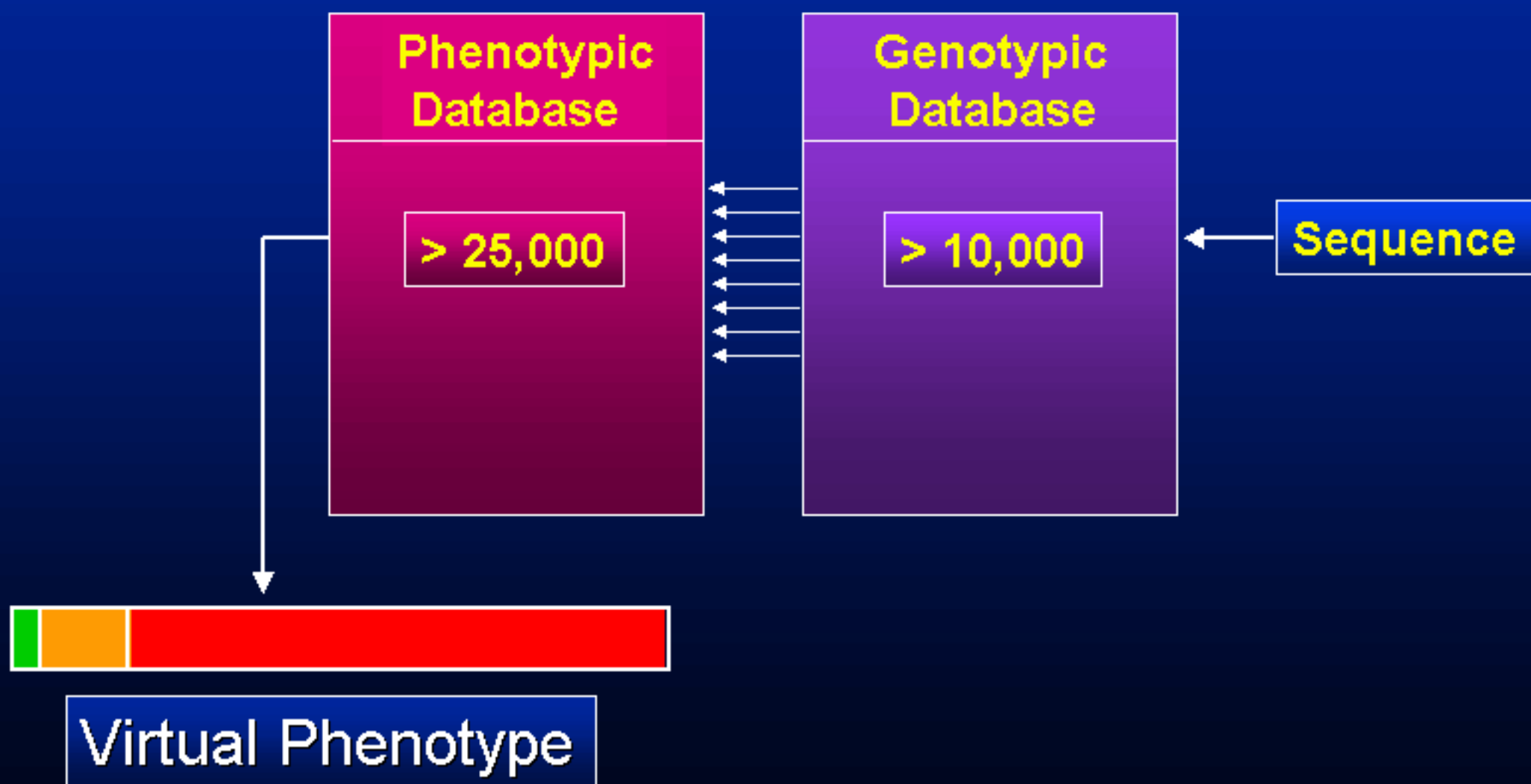
2. PI mutations

3. RT mutations

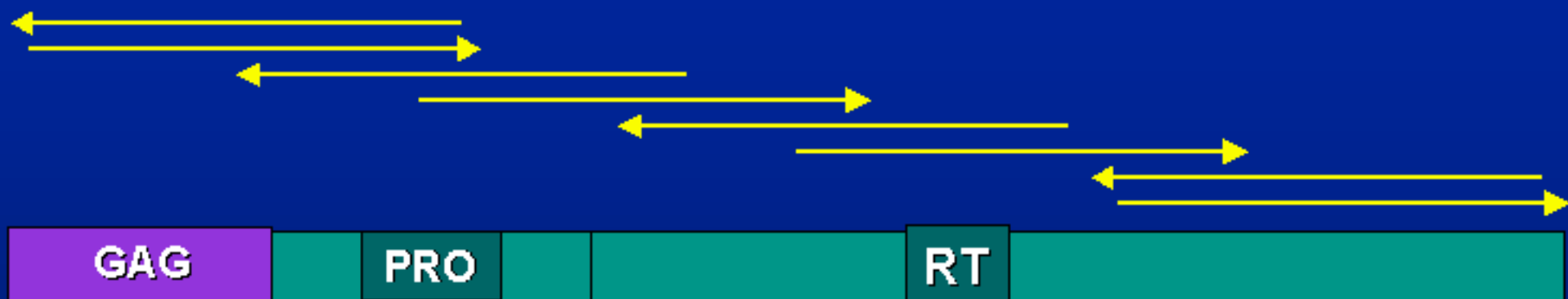
5. Interpretation

4. Mutations
found/drug

Linking Genotypes & Phenotypes: Resistance Database



DNA Sequence



```
CAAATGAAAGATTGTACTGAGAGACAGGCTAATTTTTTGTAGGGAAGATCTGGCCTTCCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAG
CCAACAGCCCCACCCAGAAAGAGAGCTTCAGGTCTGGGGTAGAGACAACAACCTCCCCCTCAGAAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAAC
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GGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAATATGCAAGAATGAGGGGTGCCACACTAATGATGTA
AAACAATTAACAGAGGCAGTGCAAAAAATAACCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAACTGCCCATACAAAAGGAAACATGGG
AAACA
```

Sequence text → import into VircoGEN II

Example of Search

- Sequence with protease inhibitor mutations:
- 10I, 46I, 71V, 77I, 82A, 84V, 90M

82

(2150)

90

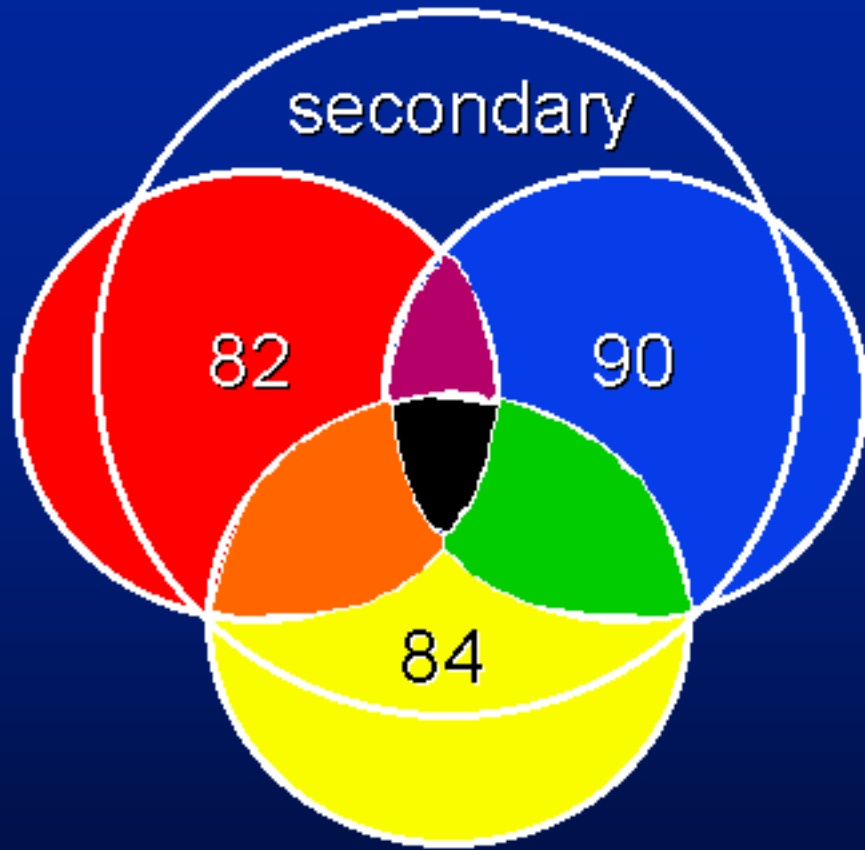
(3308)

secondary

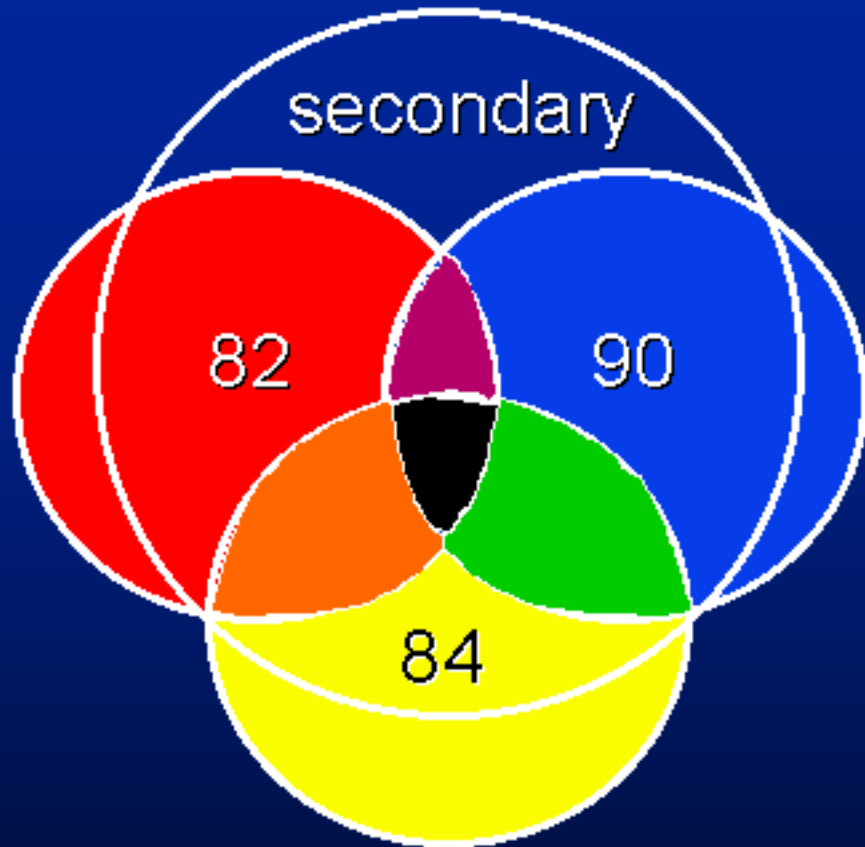
(5056)

84

(1282)



**82 & 84 & 90 &
secondary
(112)**



**82 & 84 & 90 &
secondary
(112)**

Find phenotypes



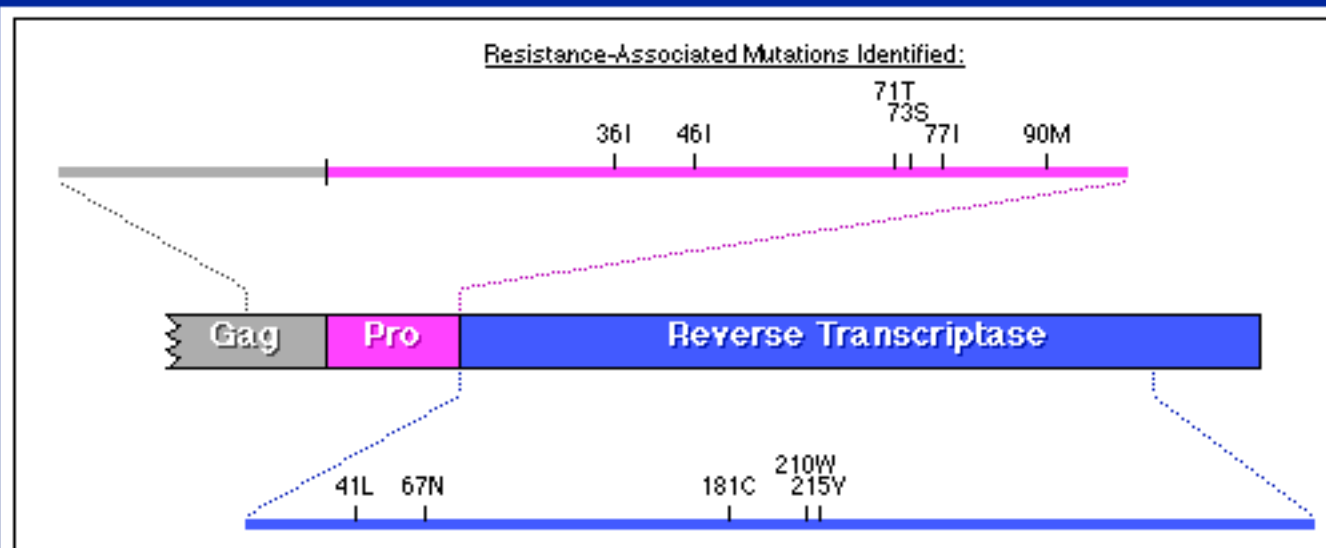
phenotype spread (%)

25 50 75



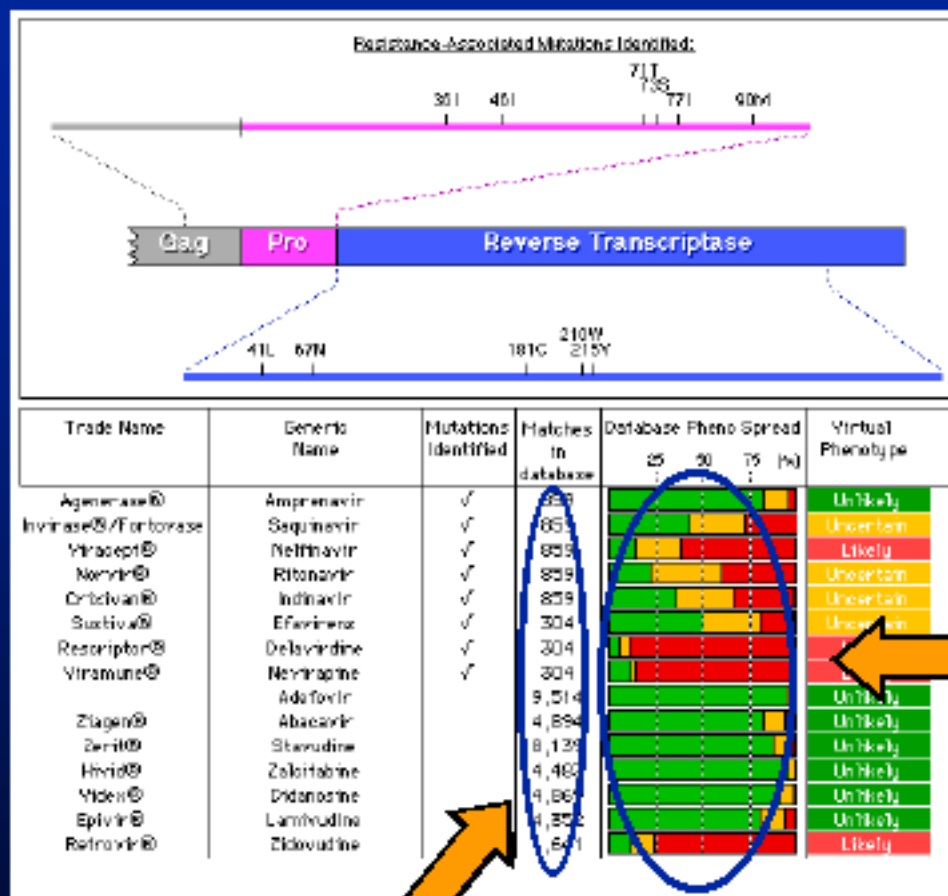
(e.g., nelfinavir)

VircoGEN II



Trade Name	Generic Name	Mutations Identified	Matches in database	Database Pheno Spread			Virtual Phenotype
				25	50	75 (%)	
Agenerase®	Amprenavir	✓	859				Unlikely
Invirase®/Fortovase	Saquinavir	✓	859				Uncertain
Viracept®	Nelfinavir	✓	859				Likely
Norvir®	Ritonavir	✓	859				Uncertain
Crixivan®	Indinavir	✓	859				Uncertain
Sustiva®	Efavirenz	✓	304				Uncertain
Rescriptor®	Delavirdine	✓	304				Likely
Viramune®	Nevirapine	✓	304				Likely
	Adefovir		9,514				Unlikely
Ziagen®	Abacavir		4,894				Unlikely
Zerit®	Stavudine		8,139				Unlikely
Hivid®	Zalcitabine		4,483				Unlikely
Videx®	Didanosine		4,869				Unlikely
Epivir®	Lamivudine		4,352				Unlikely
Retrovir®	Zidovudine	✓	1,641				Likely

VircoGEN II



Distribution of matching phenotypes

Number of matching samples in database

Technology Approval

- **Kit Based Assays**
 - *FDA Approval*
- **Service Based Assays**
 - *Lab accreditation*

Technology Approval

- **Virco's Accreditation's:**
 - Europe : EN 45001
 - US : CLIA, College American Pathologists,
New York State
- **LabCorp's Accreditation's:**
 - US : CLIA, College American Pathologists,
New York State

Recent Internal QA Data

- **Variation of wild-type reference IC₅₀ values**
 - **less than 3-fold over a period of >1 year**
(1700 to >2200 determinations per compound)

Wild-type reference IC₅₀ values (μM)

>1 year period of repeat testing

	n	Mean	Median	Low 95% CI	Up 95% CI
Zidovudine	2202	0.0494	0.0552	0.0481	0.0507
Lamivudine	2116	2.1154	2.0048	2.0578	2.1747
Didanosine	2087	5.1856	6.1319	5.0475	5.3276
Zalcitabine	2041	1.2882	1.4867	1.2576	1.3197
Stavudine	2054	2.0549	1.9192	1.9894	2.1226
Nevirapine	2103	0.0207	0.0211	0.0202	0.0212
Delavirdine	2213	0.0076	0.0080	0.0074	0.0078
Efavirenz	1720	0.0007	0.0006	0.0006	0.0007
Indinavir	2168	0.0186	0.0191	0.0182	0.0190
Ritonavir	2172	0.0283	0.0294	0.0277	0.0290
Nelfinavir	2236	0.0182	0.0175	0.0178	0.0185
Saquinavir	2261	0.0053	0.0062	0.0051	0.0054

Recent Internal QA Data

- **Inter-assay reproducibility (16 samples x10)**

Phenotype:

– variation in obtained IC₅₀ values: **1.2 to 2.5 fold**

Genotype:

– Nucleotide variation (1697bp/sample): **0.30 ± 0.19 %**
(99.7% accurate)

– Amino acid variation (565aa/sample) : **0.14 ± 0.11 %**
(about 99.9% accurate)

Recent Internal QA Data

- **HIV-1 Subtype Analysis:**

- Amplification & successful genotyping & phenotyping has been demonstrated for all Group M (A-H) subtypes of HIV-1 (De Bethune *et al.*, 1999)

- **Sensitivity:**

- About 95% of samples with viral load >1000 copies/ml successful

Summary

- **Antivirogram™ (phenotyping)**
 - High volume, accurate, miniaturised format
- **VircoGEN™ (genotyping)**
 - Use of database enhances interpretation
- **QA & accreditation is in place**
- **Virco's large relational phenotype-genotype database enables 'virtual phenotypes' to be derived from genotyping**