



HIV Drug Resistance Testing Using the Stanford University Drug Resistance Database

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MTN Virology Core

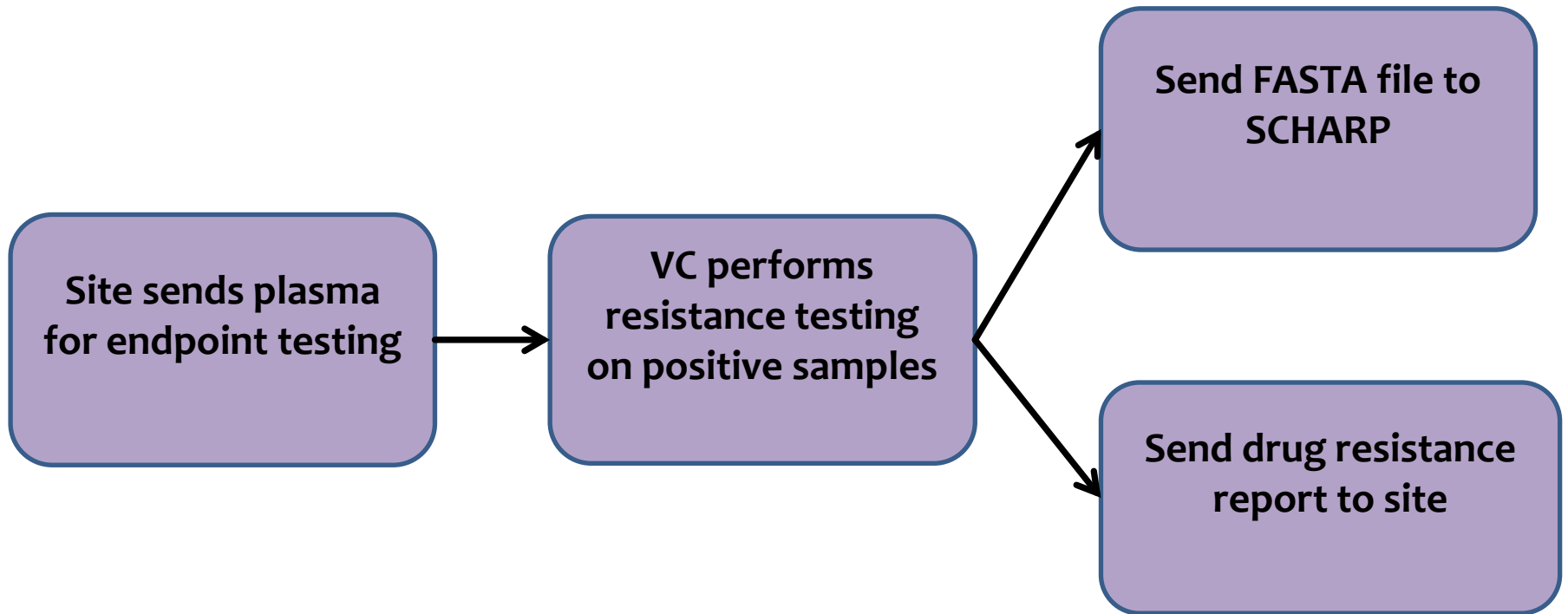
10/07/2015

Overview



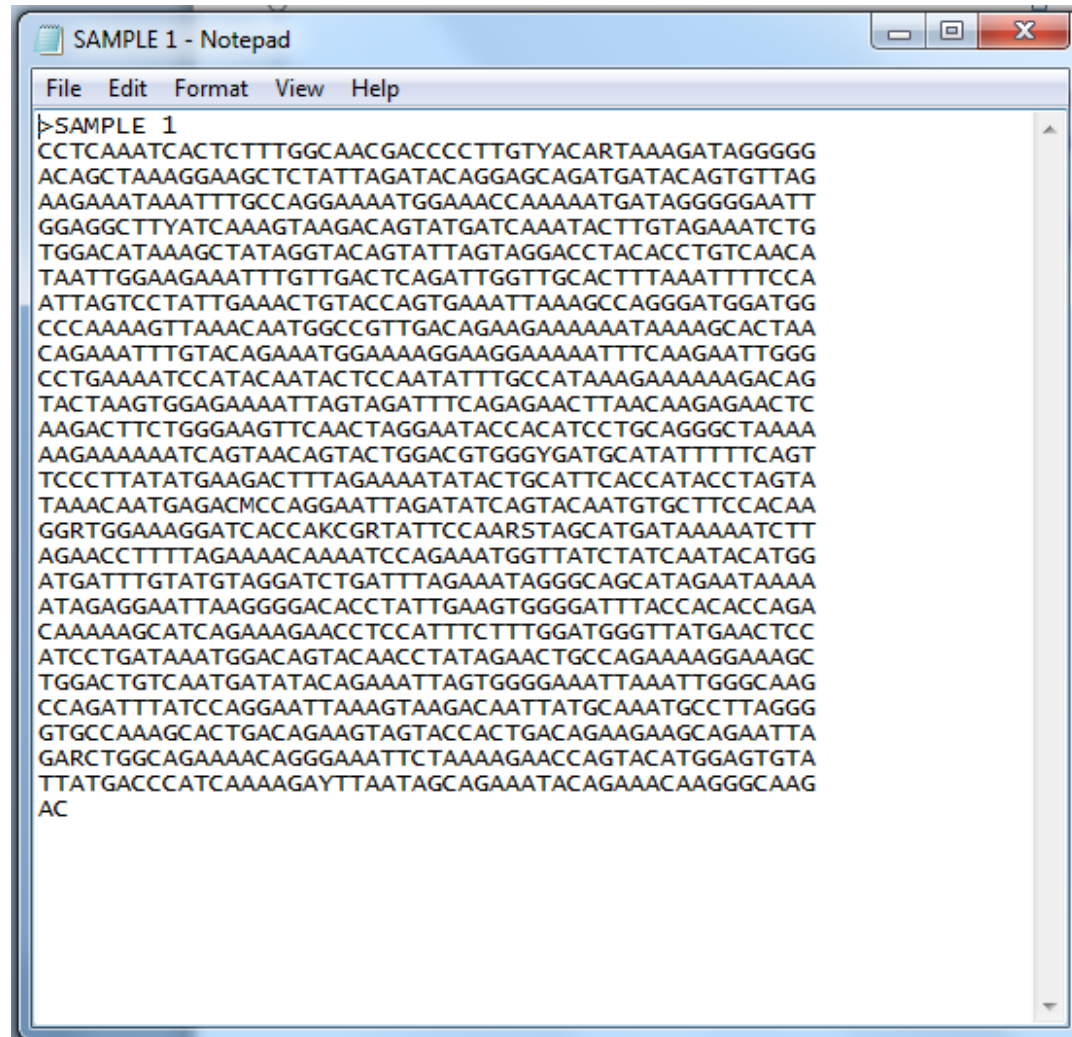
- How do we generate sequences at the VC?
- What is the Stanford Resistance Database?
- How do we generate the resistance report that is sent to sites?

Virology Core Resistance Testing



Final Result – FASTA file

- FASTA file



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SAMPLE 1
>SAMPLE 1
CCTCAAATCACTCTTTGGCAACGACCCCTTGTACARTAAAGATAGGGGG
ACAGCTAAAGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTGTTAG
AAGAAATAAATTTGCCAGGAAAATGGAAACCAAAAATGATAGGGGGAATT
GGAGGCTTYATCAAAGTAAGACAGTATGATCAAATACTTTGTAGAAAATCTG
TGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACA
TAATTGGAAGAAAATTTGTTGACTCAGATTGGTTGCACTTTAAATTTTCCA
ATTAGTCTATTGAACTGTACCAGTGAAATTAAGCCAGGGATGGATGG
CCAAAAGTTAAACAATGGCCGTTGACAGAAGAAAAAATAAAGCACTAA
CAGAAAATTTGTACAGAAATGGAAAAGGAAGAAAAATTTCAAGAATTGGG
CCTGAAAATCCATACAATACTCCAATATTTGCCATAAAGAAAAAAGACAG
TACTAAGTGGAGAAAATTAGTAGATTTTCAGAGAACTTAACAAGAGAACTC
AAGACTTCTGGGAAGTTCAACTAGGAATACCACATCCGTCAGGGCTAAAA
AAGAAAAAATCAGTAACAGTACTGGACGTGGGYGATGCATATTTTTCAGT
TCCCTTATATGAAGACTTTAGAAAATATACTGCATTCACCATACCTAGTA
TAAACAATGAGACMCCAGGAATTAGATATCAGTACAATGTGCTTCCACAA
GGRTGGAAAAGGATCACCAKCGRTATTCCAARSTAGCATGATAAAAAATCTT
AGAACC TTTTAGAAAACAAAATCCAGAAATGGTTATCTATCAATACATGG
ATGATTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAATAAAA
ATAGAGGAATTAAGGGGACACCTATTGAAGTGGGGATTTACCACACCAGA
CAAAAAGCATCAGAAAAGAACCTCCATTTCTTTGGATGGGTTATGAACTCC
ATCCTGATAAATGGACAGTACAACCTATAGAATGCCAGAAAAGGAAAAGC
TGGACTGTCAATGATATACAGAAATTAGTGGGGAAATTAATTTGGGCAAG
CCAGATTTATCCAGGAATTAAGTAAGACAATTAATGCAAAATGCCTTAGGG
GTGCCAAAGCACTGACAGAAGTAGTACCCTGACAGAAGAAGCAGAATTA
GARCTGGCAGAAAAACAGGGAAATTTAAAAGAACCAGTACATGGAGTGTA
TTATGACCCATCAAAAAGAYTTAATAGCAGAAATACAGAAAACAGGGCAAG
AC
```

Stanford University HIV Drug Resistance Database Website

- An online database created from published HIV sequences.
- Three main types of content:
 1. Database queries and references
 - Info relating to genotype-treatment correlations, genotype-clinical outcome correlations, references, new submissions to database
 2. Interactive programs
 - HIVdb program, HIValg program, HIVseq program, ART-AiDE, Rega HIV-1 subtyping tool
 3. Educational resources.
 - Drug resistance summaries, surveillance drug resistance mutation list

Interactive Programs

- HIVdb Program
 1. May enter a list of mutations
 2. May enter a complete sequence containing protease, RT and/or integrase
- VC uses option 2 to generate drug resistance report which contains:
 1. **Summary Data**
 2. **Sequence Quality Assessment**
 3. **Drug resistance Interpretation**
 4. **Mutation scoring table**

Drug Resistance Report

- Stanford HIV Drug Resistance Database Output
 1. **Summary Data** → Defines sample subtype, region of HIV, QC summary

Summary Data

Sequence includes PR: codons: 1 - 99

Sequence includes RT: codons: 1 - 334

There are no insertions or deletions

Subtype and % similarity to closest reference isolate:

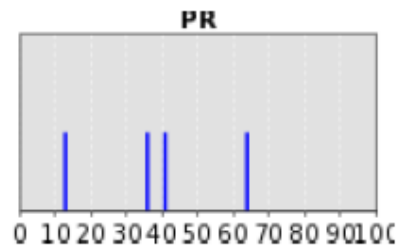
1. *PR: D (97.0%)*
2. *RT: D (95.9%)*

Drug Resistance Report

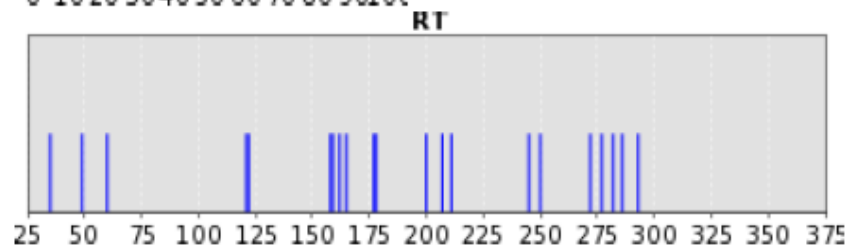
- Stanford HIV Drug Resistance Database Output
 2. **Sequence Quality Assessment** → a quality assessment sequence analysis (insertions, deletions, unusual bases)

Sequence Quality Assessment

Gene	QA Problem	Codons
PR	Stop Codons, Frame Shifts:	None
PR	Ambiguous Positions:	None
PR	Unusual Residues:	None



Gene	QA Problem	Codons
RT	Stop Codons, Frame Shifts:	None
RT	Ambiguous Positions:	None
RT	Unusual Residues:	None



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

Drug Resistance Report

- Stanford HIV Drug Resistance Database Output

3. Drug resistance Interpretation → Scans sequence for any mutations that are associated with 19 commonly used protease, nucleoside reverse transcriptase and non-nucleoside reverse transcriptase inhibitors.

Drug Resistance Interpretation: PR

PI Major Resistance Mutations: None
PI Minor Resistance Mutations: None
Other Mutations: I131V, M36I, R41K, I64V

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

Drug Resistance Interpretation: RT

NRTI Resistance Mutations: None
NNRTI Resistance Mutations: None
Other Mutations: V35T, K49R, V60I, D121Y, K122E, A158AS, I159IV, S162AGST, T165I, D177E, I178M, T200I, Q207G, R211K, V245E, D250E, A272P, K277R, L282C, T286A, I293V

Nucleoside RTI

lamivudine (3TC)	Susceptible
abacavir (ABC)	Susceptible
zidovudine (AZT)	Susceptible
stavudine (D4T)	Susceptible
didanosine (DDI)	Susceptible
emtricitabine (FTC)	Susceptible
tenofovir (TDF)	Susceptible

Non-Nucleoside RTI

efavirenz (EFV)	Susceptible
etravirine (ETR)	Susceptible
nevirapine (NVP)	Susceptible
rilpivirine (RPV)	Susceptible

Drug Resistance Report

- Stanford HIV Drug Resistance Database Output

4. Mutation scoring → each mutation is given a score based on Stanford's internal algorithm. The score determines the level of resistance.

(higher score = greater resistance)

Genotypic Score	
0 – 9	Susceptible
10 – 14	Potential Low-Level Resistance
15 – 29	Low-Level Resistance
30 – 59	Intermediate Resistance
≥ 60	High-Level Resistance

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

RT	3TC	ABC	AZT	D4T	DDI	FTC	TDF	EFV	ETR	NVP	RPV
K103N	-	-	-	-	-	-	-	<u>60</u>	<u>0</u>	<u>60</u>	<u>0</u>
Y188L	-	-	-	-	-	-	-	<u>60</u>	<u>15</u>	<u>60</u>	<u>60</u>
Total:	0	0	0	0	0	0	0	120	15	120	60

Examples



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.

<http://hivdb.stanford.edu/>