Comparison of HIV Drug-Resistant Mutant Detection by NGS with and without Unique Molecular Identifiers (UMI)

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Introduction

Background

Sequencing error compromises the sensitivity of NGS for detection of HIV drug-resistant mutants.

Consensus building with UMIs can reduce sequencing artifacts and quantify the true sampling depth[1,2].

UMI-based consensus building has not been universally adopted for drug-resistant surveillance because it adds technical and bioinformatics challenges with uncertain gain.

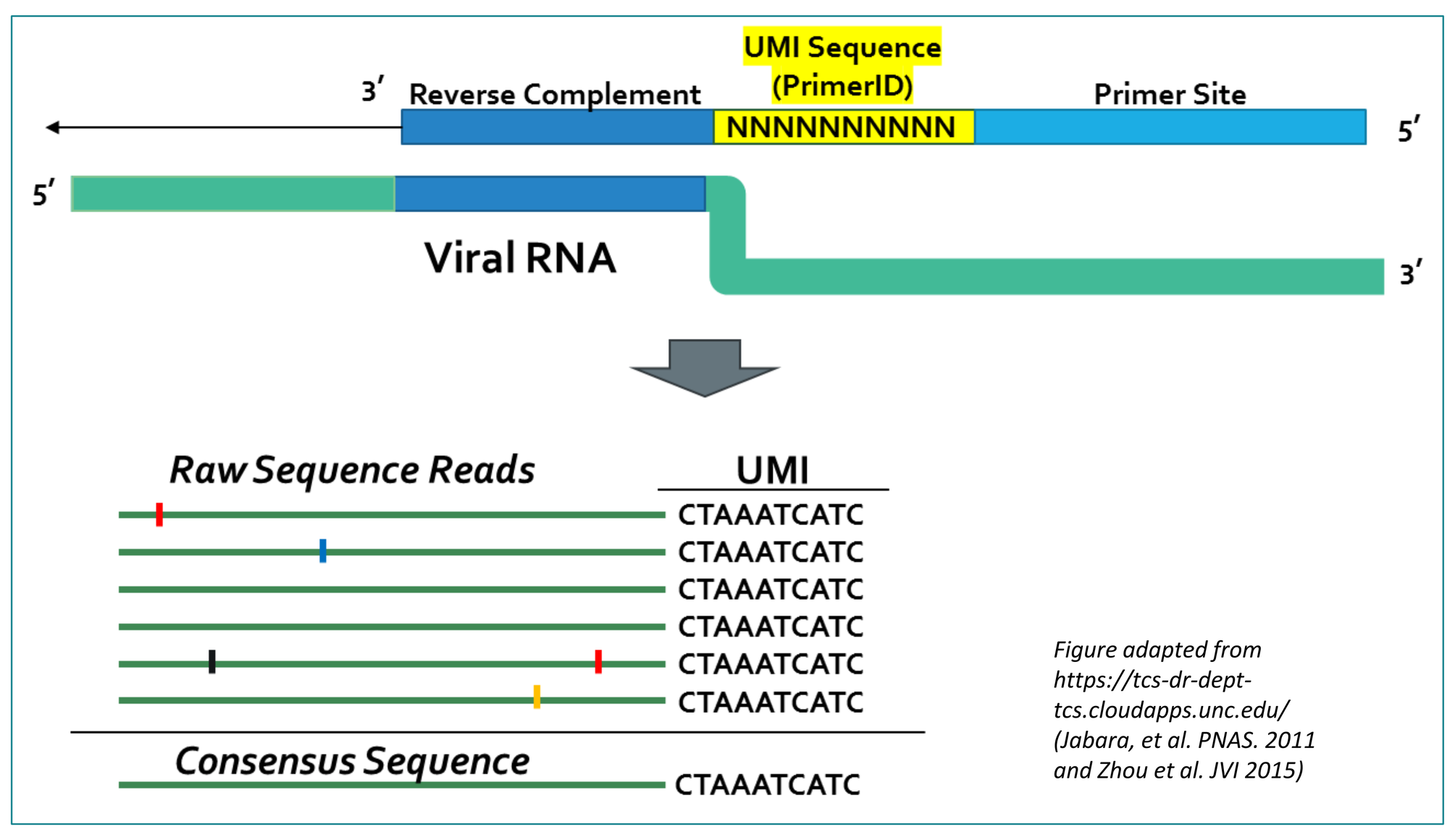

Figure 1: Consensus building from sequences derived from individual viral templates that are tagged with UMIs (N^{10} = 1 Million Combinations) during cDNA synthesis can be been used to remove PCR and sequencing artifacts and to quantify the depth of mutant and wild-type template sampling[1–3].

Objectives

Perform a comparative assessment of UMI- and non-UMI-based NGS assays for the accurate detection and confident analysis of low frequency HIV DR.

1)Determine the sensitivity and specificity

2)Determine the limit of detection

3)Determine the accuracy with clinical samples

Materials & Methods

Mixture Panel

We created a mixture panel of recombinant wild-type and mutant viruses that were spiked into HIV-negative blood.

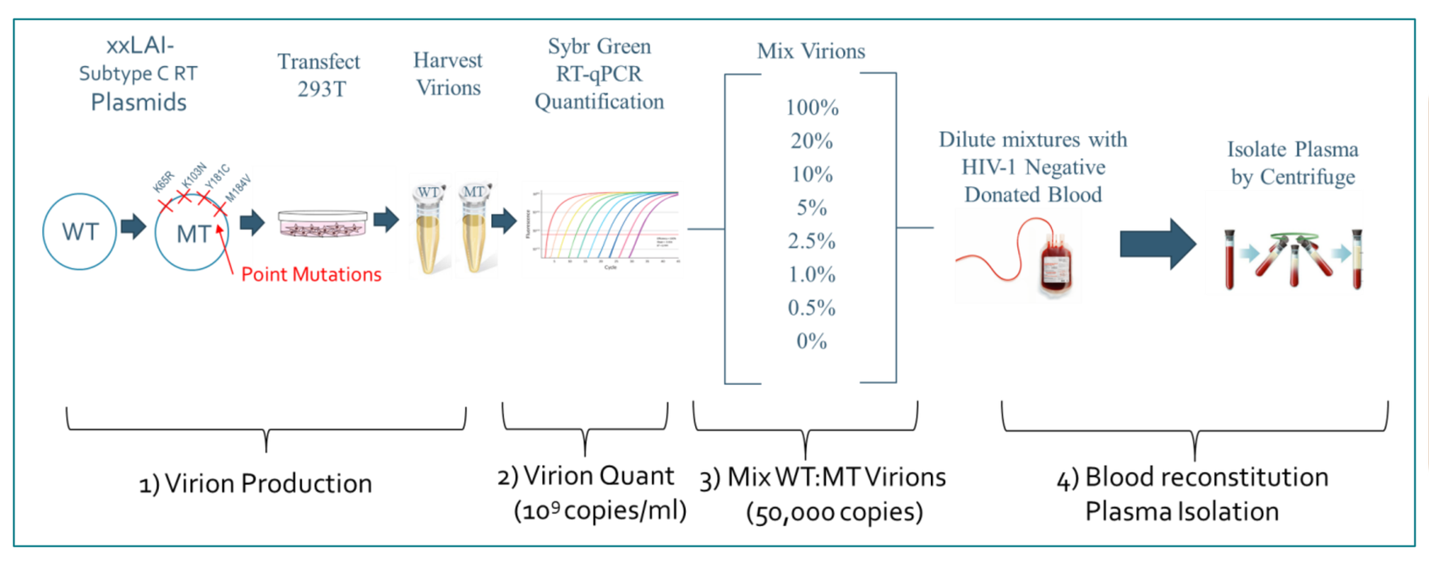

Figure 2: Patient-derived HIV-1 Subtype C RT was cloned into xxLAI and the HIV DR mutations K65R, K103N, Y181C and M184V were made by mutagenesis. Infectious clones of WT and MT plasmids were produced in 293T cells. Finally, mixtures of WT:MT virus were diluted in HIV negative blood.

We serial-diluted this plasma into cell culture media to determine the detection limits of each NGS assay.

To assess the effects of PCR bias, we performed UMI-NGS with PCR primers that contained mismatches for cDNA templates.

NGS Library Prep and Analysis

- NGS libraries were constructed using the ultrasensitive single-genome sequencing method as previously described3.
- The Zhou method was used for UMI consensus building and UMI bioinformatic analysis[4].
- PASeq v1.4 was used for non-UMI NGS analysis (https://www.paseq.org[5])

Clinical Data Set

A UMI-NGS dataset derived from plasma samples from viremic donors with HIV acute infection was re-analyzed without consensus building.

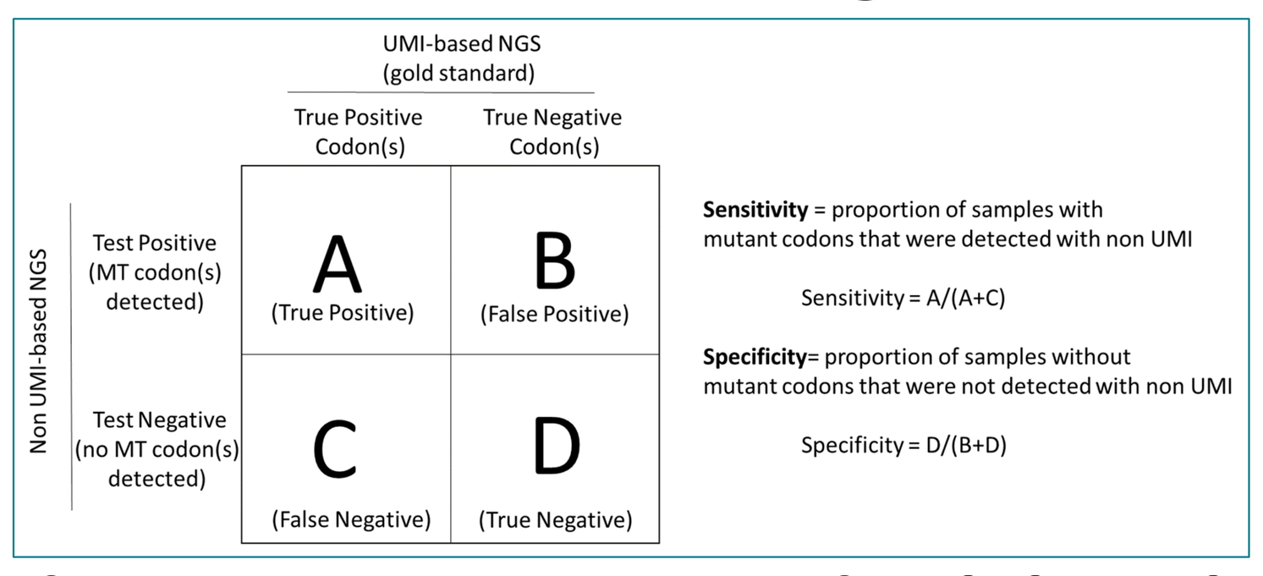

Figure 3: MiSeq libraries were prepared from the HIV-1 RT region (R1 codons 80-147; R2 codons 151-212) of virions isolated from 62 viremic donors with HIV acute infection. Sensitivity and Specificity for non-UMI analysis were calculated by the number of discordant samples relative to UMI-based NGS analysis.

To determine assay reproducibility, the final library preps were re-sequenced by running the final pooled samples in a fresh MiSeq flow cell.

Results

Mixture Panel

Table 1: Comparison of individual HIV-1 RT allele frequencies from known mixtures of WT and Mutant recombinant virus by UMI-based NGS and non-UMI-based NGS

Allele Frequency Expected (%)	non-UMI-based NGS Allele frequency detected (%)				UMI-based NGS Allele frequency detected (%)			
	K65R	K103N	Y181C	M184V	K65R	K103N	Y181C	M184V
100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
20.0	28.0	28.0	27.0	27.0	20.3	20.3	20.3	20.3
10.0	14.6	14.6	14.6	14.6	8.3	8.3	8.3	8.3
5.0	7.5	7.6	7.8	7.8	3.7	3.7	3.7	3.7
2.5	3.4	3.4	3.5	3.5	1.6	1.6	1.6	1.6
1.0	1.3	1.3	1.5	1.5	0.5	0.5	0.5	0.5
0.5	0.7	0.6	0.6	0.6	0.3	0.3	0.3	0.3
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2: Comparison of background mutation rates in mixtures of WT and Mutant recombinant virus by UMI-based NGS and non-UMI-based NGS

Spurious Mutation Rates	non-UMI-based NGS (n=8)	UMI-based NGS (n=17)
Ave Number	482	68
Mean	0.06%	0.08%
Median	0.02%	0.05%
Min	0.01%	0.01%
Max	0.46%	0.46%

Table 3: Comparison of the limit of detection for 1% and 5% HIV-1 RT allele frequencies in recombinant virus serial diluted by UMI-based NGS and non-UMI-based NGS

Expected	Viral Load (cp/ml)	non-UMI-based NGS % Detected			UMI-based NGS % Detected			# UMI
		K103N	Y181C	M184V	K103N	Y181C	M184V	
5%	1.00E+06	3.8	3.8	3.8	0.6	1.5	1.5	4308
	1.00E+05	4.0	4.0	4.0	1.3	3.2	3.2	24991
	1.00E+04	3.9	4.0	4.0	4.0	4.7	4.7	2149
	1.00E+03	4.8	4.8	4.8	3.1	3.1	3.1	261
	1.00E+02	0.8	0.9	0.9	0ᵃ	0ᵃ	0ᵃ	16
	1.00E+01	0ᵇ	0ᵇ	0ᵇ	0ᵃ	0ᵃ	0ᵃ	0
1%	1.00E+06	0.9	0.9	0.9	0ᵇ	0ᵇ	0ᵇ	5010
	1.00E+05	0.9	0.8	0.8	0ᵇ	0.7	0.7	20848
	1.00E+04	0.8	0.8	0.8	0.6	0.7	0.7	2325
	1.00E+03	1.6	1.6	1.6	0.8	0.8	0.8	266
	1.00E+02	0ᵇ	0ᵇ	0ᵇ	0ᵃ	0ᵃ	0ᵃ	19
	1.00E+01	0ᵇ	0ᵇ	0ᵇ	0ᵃ	0ᵃ	0ᵃ	3

0ᵃ false negative detection; 0ᵇ undetectable (insufficient UMI counts)

Summary

- We detected 0.5% drug-resistant associated mutations with and without UMI-based sequence consensus building, indicating that both methods are generally robust.
- False negative error rates are higher in non-UMI-based NGS in samples with limited template sampling.
- Non-UMI NGS had unreproducible background mutations (<5%) in clinical samples, which lowered the specificity relative to the UMI assay.

Table 4: Comparison of the effects from PCR primers that contained mismatches for the target cDNA templates with UMI- and non-UMI-based NGS (PCR Bias)

# of Reactions PCR 1	# of Reactions PCR 2	# of PCR1 mismatches	Primers with consensus	Allele Frequency Expected (%)	non-UMI-based NGS Allele frequency detected (%)	UMI-based NGS Allele frequency detected (%)
5	5	0	1447	1.0	1.5	0.9
			1213	5.0	7.0	4.0
		2	248	1.0	0.0ᵇ	0.8ᵃ
			215	5.0	7.7	10.7
5	1	0	1244	1.0	1.7	1.5
			1170	5.0	7.6	6.5
		2	120	1.0	1.9	3.3ᵃ
			178	5.0	8.3	11.2
3	3	0	1168	1.0	1.5	0.9
			1025	5.0	7.5	4.6
		2	246	1.0	1.2	1.2ᵃ
			183	5.0	11.4	8.2
3	1	0	1092	1.0	1.7	1.5
			989	5.0	8.0	7.3
		2	200	1.0	0.0ᵇ	1.0ᵃ
			184	5.0	8.5	6.0
1	1	0	1036	1.0	1.6	1.4
			911	5.0	9.5	8.5
		2	253	1.0	0.0ᵃ	0.0ᵃ
			211	5.0	10.3	8.5

0ᵃ false negative detection; 0ᵇ undetectable (insufficient UMI counts)

Clinical Data Set

Table 5: Comparison of HIV-1 RT allele frequency detection in clinical samples by UMI-based NGS and non-UMI-based NGS

	non-UMI-based NGS MiSeq Run 1	non-UMI-based NGS MiSeq Run 2	UMI-based NGS MiSeq Run 1	UMI-based NGS MiSeq Run 2
1	10.3% R83S, 0.5% E122K, 2.7% H198Q, 0.7% D192N, 0.51% E194K, 0.73% D204K,	0.53% E122K, 0.57% D192N, 0.71% D204K,	0.6% D192N, 0.5% E204K	0.6% D192N, 0.5% E204K
2	96.3 Q174S/2.4% Q174N/1.4% Q174R	96.8%Q174S/3.2% Q174N, 0.5% G196E	98.5 Q174S/1.5% Q174N	98.5 Q174S/1.5% Q174N
3	65.8% W88C, 12.7% K102R, 1.22% K104R, 1.1% V118I, 75.5% V118M, 66.8% S123N, 31.4% S123D 11.2% S123G, 10.0% S162C, 11.0% S162A, 22.4% S162F, 66.8% T165I 10% A173T, 22.0% E177D, 0.6% G196E, 76.0% I202V, 0.6% E203K, 1.0% R206K	67.1% W88C, 11.9% K102R, 1.1% K104R, 76.2% V118M, 58.5% S123N, 30.5% S123D 10.4% S123G, 10.1% S162C, 11.5% S162A, 23.1% S162F, 65.8% T165I 10.3% A173T, 22.9% E177D, 0.5% G196E, 76.0% I202V, 0.6% E203K, 0.9% R206K	65.7% W88C, 12.6% K102R, 1.2% K104R, 75.4% V118M, 65.6% S123N, 32% S123D, 10.1% S162C/23.6% S162F/2.7% S162A, 65.8% T165I, 10.2% A173T, 23.8% E177D, 76.0% I202V, 0.8% R206K,	65.3% W88C, 12.8% K102R, 1.3% K104R, 75.8% V118M, 2.1% D123E/65.3% S123N, 32% S123D, 10.1% S162C/23.6% S162F/2.7% S162A, 65.8% T165I, 10.2% A173T, 23.8% E177D, 76.0% I202V, 0.8% R206K,
4	1.5% K101N, 0.58% V118I, 0.8% R143K, 0.5% A173T, 0.53 I178V, 1.0% V179I, 1.9%V189I, 0.6% E194K, 0.58% D204K,	0.6% K101N, 0.8% R143K, 1% I178M, 2.3% I178V, 0.8% V179I, 1.7%V189I, 0.6% E194K,	0.5% R143K, 0.5% V179I, 1.9% V189I, 0.6% E194K	0.5% R143K, 0.5% V179I, 1.9% V189I, 0.6% E194K
5	4.1% Q91H, 1.34% K101N, 0.52% T165I, 0.58% E194K, 0.5% Q207A,	0.6% K101N, 0.65% E194K,	0.5% E194K	0.5% E194K
6	0.6% E122K, 1.27% E169K, 0.6% A173T, 0.6% E203K	0.7% E122K, 1.06% E169K, 0.5% E203K	0.9% E169K	0.9% E169K
7	31.8% A173T, 6.09% E177D, 6.15% I178L, 0.6% G190E, 0.9% D192N, 1.55% E194K, 1.05% E203K, 1.4% D204K, 0.6% Q207K	31.9% A173T, 6.3% E177D, 6.2% I178L, 0.5% D186N, 0.5% G190E, 0.9% D192N, 1.6% E194K, 0.9% E203K, 1.5% D204K, 0.5% Q207K	20% A173T, 2.5% E177D, 2.5% I178L, 0.7% D192N, 1.2% E194K, 0.9% E203K, 1.1% E204K	20% A173T, 2.5% E177D, 2.5% I178L, 0.7% D192N, 1.2% E194K, 0.9% E203K, 1.1% E204K
8	0.7% Q207E, 0.8% D204K, 1.6% K211R	1.5% K211R	1.5% K211R	1.5% K211R
9	-		-	-
10	47.5 K102R, 0.5% Q207E, 54.9% K211Q	52.0% K102R, 54.2% K211Q	52.7% K102R, 55.4% K211Q	52.7% K102R, 54.2% K211Q
11	8.7% F87L	23.8% F87L, 1.7% D123S, 1.9% R166K, 1.0% D204E	8.7% F87L, 0.6% E122K	8.7% F87L, 0.6% E122K
12	3.4% K103I	1.6% K174K, 0.6% I202I,	-	-
13	-	-	-	-
14	-	-	-	-
15	-	-	-	-
16	47.8% I178L, 47.9% V179I/50.4% V179T, 49.7% Q197P, 51.5% E207D/16.3% E207G, 31.92 K211R	48.5% I178L, 0.7% V179A, 48.7% V179I/50.4% V179T, 51% Q197P, 50.4% E207D/16.3% E207G, 31.2 K211R	43.4% I178L, 43.6% V179I/55.7% V179T, 54.4% Q197P, 56.4% E207D/8.4% E207G, 34.9% K211R	43.4% I178L, 43.6% V179I/55.7% V179T, 54.4% Q197P, 56.4% E207D/8.4% E207G, 34.9% K211R
17	-	0.7 R83K, 0.6% E122K, 6.6% S134N, 0.7% E169K, 2.2% A173K, 2.3% K174Q, 0.5% D192N, 0.8% E194K, 0.6% A200V, 0.6% D204K,	4.0% S134N	3.6% S134N
18	0.6% V118I, 1.0% S123D, 0.5% S162N, 0.9% D204K	0.5% V90I, 0.6% V118I, 1.0% S123D, 0.6% S162N, 0.6% A173T, 0.5% E194K, 0.7% D204K	-	0.6% E204K
19	0.5% A173D, 0.5% A173T, 4.0% A200T	0.5% A173Q, 4.0% A200T	3.3% A200T,	3.8% A200T,
20	2.83% K104I, 0.9% G112E, 0.6% D113N, 0.9% V189I, 1.0% E194K, 1.1% A200K, 0.6% R206K	1% K101K, 0.9% G112E, 0.5% I178I, 0.9% V189I, 1.0% E194K, 1.2% A200K, 0.6% R206K	0.9% A200K,	0.7% V189I, 0.9% A200K,
21	0.8% A114T, 0.7% E138K, 0.5% E194K,	0.5% K104N, 0.7% E138K, 0.6% E169K, 0.6% E194K,	0.6% E194K	0.6% E194K
22	0.6% S162N, 0.6% A200V	0.7% E169K	-	-
23	6.9% K102R	6.6% K102R,	2.1% K102R	1.9% K102R
24	1.3% E122K, 33.9% I178V, 0.9% D192N, 0.8% E194K, 0.7% R206K	1.2% E122K, 35% I178V, 1.0% D192N, 0.8% E194K, 0.7% R206K	0.6% E122K, 33.8% I178V, 0.6% D192N, 0.7% E194K, 0.5% R206K	0.6% E122K, 33.8% I178V, 0.6% D192N, 0.7% E194K, 0.5% R206K
25	2.2% P176L, 3.9% Q207K	2.4% P176L, 0.9% Q207K,	2.2% P176L	2.2% P176L
26	-	0.6% I178I	-	-
27	0.5% A173T, 1.0% Q207E	0.6% F160L, 2.9% Q207E	-	-
28	-	-	-	-
29	0.5 R143K, 0.5% E169K, 0.7% D192N, 0.9% D194K,0.7% E196K, 0.6% E203K, 1.5% E206K	0.5 R143K, 0.6% E169K, 0.9% D192N, 0.9% D194K, 0.9% E203K, 1.1% D204K, 1.5% E206K	0.5% D194N, 0.7% E196K, 0.8% E206K, 1.3% R208K	0.5% D192N, 0.5% D194K, 0.8% E204K, 0.8% E206K,
30	-		-	-
31	2.16% L193I, 0.57% Q207E, 1.51% K211R	0.65% L193I	-	-
32	5.7% E207D	0.6%I178I, 5.7% E207D	5.2% E207D	5.2% E207D
33	0.8% R199K, 1.3% A200R, 0.9% E204K	0.8% F160L, 0.7% R199K, 4.1% A200R, 0.9% E204K	0.6% E122K, 0.7% E204K	0.8% R199K, 0.7% E204K
34	0.6% E89K, 0.7% E122K, 0.5% G141E, 25% T165I, 0.81% A173E, 0.64% G190E, 0.51% A200T, 0.6% D204K,	0.6% E89K, 0.7% E122K, 25% T165I, 0.81% A173E, 0.64% G190E, 0.6% D204K,	0.7% E122K, 25% T165I, 0.81% A173E, 0.6% D204K,	0.7% E122K, 25% T165I, 0.7% A173E, 0.6% D204K,
35	0.8% A200K, 0.5% E203K	0.5% T139M, 0.9% A200K, 0.5% D204K	0.7% A200K	0.7% A200K
36	0.5% S162T	-	-	-
37	0.9% G93E, 0.5% I167F, 0.6% I178R,	1.0% G93E, 0.7% I167F, 0.7% I178R,	0.9% I167F, 0.9% I178R	0.9% I167F, 0.9% I178R,
38	0.7% A173T, 2.7% R206K	0.6% S162S, 0.7% A173T, 2.7% R206K	2.1% R206K	2.1% R206K
39	0.8% G93E, 0.5% H96Y, 0.8% K104N, 0.6% I178T, 0.8% D186N, 0.9% E177K, 1.6% I202V	0.8% G93E. 0.7% K104N, 1.1% E177K, 0.6% I178T, 0.9% D186N, 1.6% I202V	0.9% I202V	0.9% I202V
40	6.9% N175C, 55.2% N175Y, 37.3% N175H , 55.3% I187L	6.8% N175C, 55.2% N175Y, 37.3% N175H , 55.6% I187L	4.1% N175C, 59.8% N175Y, 34% N175H , 58.8% I187L	5.2% N175C, 58.3% N175Y, 34.2% N175H , 57.2% I187L
41	-	0.6% E194K	-	-
42	85% I142V, 1.7% A173T, 9.9% A173G, 11% A173K, 76%A173E, 0.8% K174T, 4.4 K174R,	85% I142V, 0.8% K174T, 3.9 K174R,	83.6% I142V	83.8% I142V
43	14% V90L, 3.1% Q91H, 1.5% P176S	4% V90L, 5.8% Q91H, 1.5% P176S	-	-
44	-	1.0% E177E	-	-
45	-	-	-	-
46	81% G123S, 2.7% I132V, 1% A173I, 73.1% Q174K, 0.5% A200K, 22.3% A200E, 18.8% A200S	81% G123S, 2.7% I132V, 0.9% A173I, 72.4% Q174K, 22.3% A200E, 18.8% A200S	84% G123S, 2% I132V, 1% A173I, 72.8% Q174K, 22.6% A200E, 16.1% A200S	84% G123S, 2% I132V, 0.6% A173I, 73.4% Q174K, 21.6% A200E, 16.9% A200S
47	12% A173T, 87% Q174R, 2.5% I178V, 85% K211R	12% A173T, 88% Q174R, 0.7% I178V, 85% K211R	65% A173T, 34% Q174R, 34.5% K211R	64% A173T, 34% Q174R, 34.5% K211R
48	1% R83K, 0.6% D86N, 0.9% E89K, 1.4% G93E, 1.1% D110N, 0.6% V111M, 0.7 D113N, 0.7% R125K, 1.3% D121N, 2.0% E122K, 0.7% R143K, 0.5 M164I, 1.5% E169K, 0.5% R172K, 0.6% P176S, 0.8% V179G, 1.0% V179I, 1.2% D185N, 0.9% D186N, 0.5% G190E, 3.1% D192N, 2.5% E194K, 0.7% G196R, 1.0% I202V, 1.8% E203K, 1.6% E204K, 0.8% R206K	1.2% R83K, 0.6% D86N, 1.06% E89K, 1.4% G93E, 1.1% D110N, 0.7% V111M, 0.5 D113N, 0.7% R125K, 1.3% D121N, 2.4% E122K, 0.7% R143K, 1.2% S162S, 0.5 M164I, 1.6% E169K, 0.7% R172K, 1%K174K, 0.6% P176S, 0.6% E177K, 1.3% I178I, 0.8% V179G, 1.1% V179I, 1.2% D185N, 1.0% D186N, 3.1% D192N, 2.7% E194K, 0.7% G196R, 0.9% I202V, 1.9% E203K, 1.6% E204K, 0.8% R206K, 0.8% Q207D	0.7% R83K, 0.9% G93E, 0.6% D121N, 1.2%E122K, 1% V179G, 0.5% D186N, 1.6% D192N, 1.4% E194K, 1% E203K, 0.7% E204K	0.7% R83K, 0.9% G93E, 0.7% D121N, 1.3%E122K, 0.5% R125K, 0.5% E169K, 1% V179G, 0.5% D186N, 1.8% D192N, 1.5% E194K, 0.9% E203K, 0.7% E204K
49	15% G123N, 85% G123D, 35% Q174R, 65% I178M, 68% E207N, 31% E207D, 35% K211R	16% G123N, 85% G123D, 35% Q174R, 65% I178M, 68% E207N, 31% E207D, 0.8% E207K, 35% K211R	15% G123N, 85% G123D, 35% Q174R, 65% I178M, 70% E207N, 30% E207D, 34% K211R	15% G123N, 85% G123D, 35% Q174R, 65% I178M, 70% E207N, 30% E207D, 34% K211R
50	1.4% G123N, 0.54 G123D 1.1% Q174K, 2.5% G196E, 1% I202V, 0.9% E204N, 1.1% E207D, 1.2 L210I	1.1% G123N, 1.1% Q174K, 1.2% P176P, 3.1% G196E, 1% I202V, 0.9% E204N, 1.1% E207D, 1.3% K211K	1.1% G123N, 1.1% Q174K, 2.5% G196E, 1% I202V, 1.1% E204N, 1.1% E207D,	1.1% G123N, 1.1% Q174K, 2.5% G196E, 1% I202V, 1.1% E204N, 1.1% E207D, 1.1% R211K
51	1.0% E122K, 20% G123N, 20% T142I, 20% 165T, 20% L178I, 0.5% P176L, 0.7% G196E, 19% I202V, 2.6% D204K, 17% D204N, 20% Q207D,	1.0% E122K, 19.2% G123N, 20% T142I, 18.7% 165T, 18.8% L178I, 0.7% G196E, 19% I202V, 1.8% D204K, 17% D204N, 18.2% Q207D,	1% E122K, 15% G123N, 15% T142I, 15% I165T 15% L178I, 15% I202V, 0.7% D204K 15% D204N, 15% Q207D,	1% E122K, 15% G123N, 15% T142I, 15% I165T 15% L178I, 15% I202V, 0.7% D204K 15% D204N, 15% Q207D,
52	13% K102Q, 5% G123N, 95% G123S, 95% I135V, 0.5% I142V, 95% S162C, 5% Q174K, 81% I178M, 0.6% A200V, 0.6% K201R, 5% I202V, 5% D204N, 0.7% D204K, 5% E207D	13% K102Q, 6% G123N, 95% G123S, 95% I135V, 0.6% I142V, 95% S162C, 5% Q174K, 81% I178M, 0.6% V189I, 0.6% A200V, 0.7% K201R, 5% I202V, 5% D204N, 0.7% D204K, 5% E207D	13% K102Q, 5% G123N, 95% G123S, 95% I135V, 5% I142V, 95% S162C, 5% Q174K, 81% I178M, 5% I202V, 5% D204N, 5% E207D	13% K102Q, 5% G123N, 95% G123S, 95% I135V, 5% I142V, 95% S162C, 5% Q174K, 81% I178M, 5% I202V, 5% D204N, 5% E207D
53	0.6% D192N, 0.7% A173T, 9.4% D204K	0.5% D192N, 0.8% A173, 0.5% E194K, 0.7% I202I, 5.7% D204K, 3.54% Q207E	-	-
54	1.1% K174K, 0.6% I178I, 0.9% L193I, 2% H208Y	0.7%K166K 0.6% K174K, 0.6% I178I, 0.9% L193I, 1.8% H208Y	1.5% H208Y	1.5% H208Y
55	0.6% R83K, 0.5% D121N, 0.6% R143K, 0.8% A173I, 5.3% D192N, 0.9% E194K, 0.6% E203K, 3% L205I, 0.6% Q207K,	0.9% S123N, 0.8% I135K, 0.6% R143K, 0.8% A173I, 0.6% G190E, 5.7% D192N, 0.8% E194K, 0.9% L205I, 0.7% Q207K,	3.8% I118V, 0.5% A173I, 4.3% D192N, 0.6% E194K	4.7% D192N, 0.6% E194K
56	47% Q174K, 3% L193L, 1% D204K	47% Q174K, 3% L193L, 0.5% E194K, 1% D204K	44% Q174k, 0.8% D204K	45% Q174k, 0.8% D204K
57	0.6% A173T	0.6% A173T	-	-
58	3.6% Q91H, 2.1% A200R	6.1% Q91H, 0.5% Q207K	-	-
59	1.1% N81T, 9.4% Q85H, 0.9%R83K, 1.1% H96Y, 0.9% S134N, 0.6% F171L, 1.4% A173T, 1.1% I178L, 0.6% I195R	1% N81T, 0.7% R83K, 2.6% Q85H, 1.0% H96Y, 0.9% S134N, 1.4% A173T, 1.1% I178L, 2.0% Q207D, 0.5% L210W, 3% K211R	0.9% N81T, 0.9%R83K, 0.9% H96Y, 0.9% S134N, 0.9% A173T, 0.9% I178L,	0.9% N81T, 0.9%R83K, 0.9% H96Y, 0.9% S134N, 0.9% A173T, 0.9% I178L
60	2% Q91H, 0.6% R125K, 33% G196E	4% Q91H, 0.8% R125K, 33% G196E, 4%Q207D, 5%K211R	0.6% E194K, 19.2% G196E	0.6% E194K, 19.5% G196E
61	0.9% K211R	0.8% L193I, 0.9% K211R	0.7% K211R	0.7% K211R
62	2.0% G99R, 0.6% S123D, 4.6% P140Q	1.7% G99R, 0.5% S123D, 4.7% P140Q	3.7% G99R, 3.7% P140Q	3.7% G99R, 3.7% P140Q

Bold = Discordant mutations between UMI and non-UMI-based analysis; Red = Discordant mutations of each analysis in 2 subsequent MiSeq runs

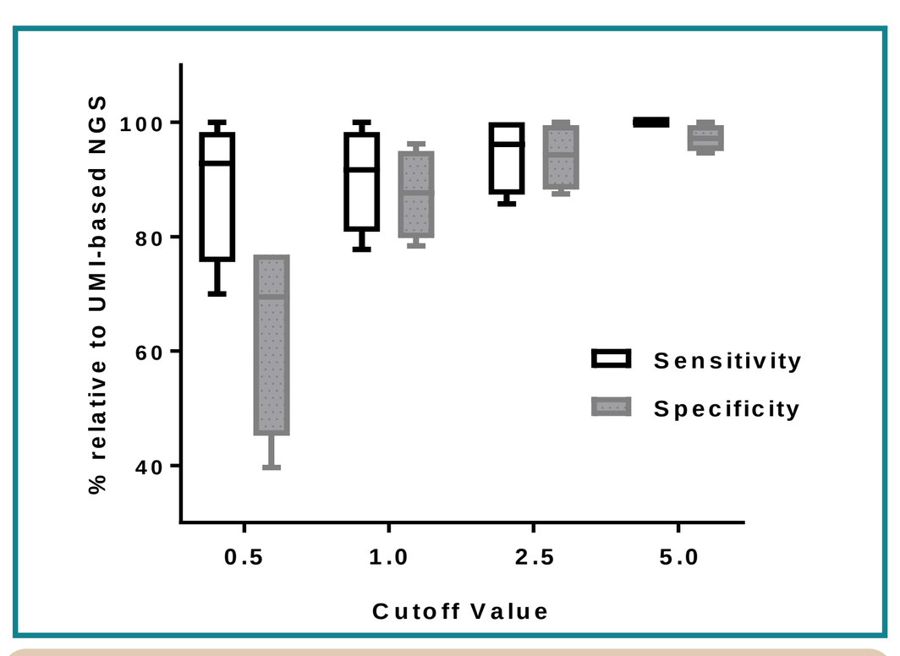

Figure 4: Sensitivity and Specificity from non-UMI-based NGS reanalysis of a clinical dataset.

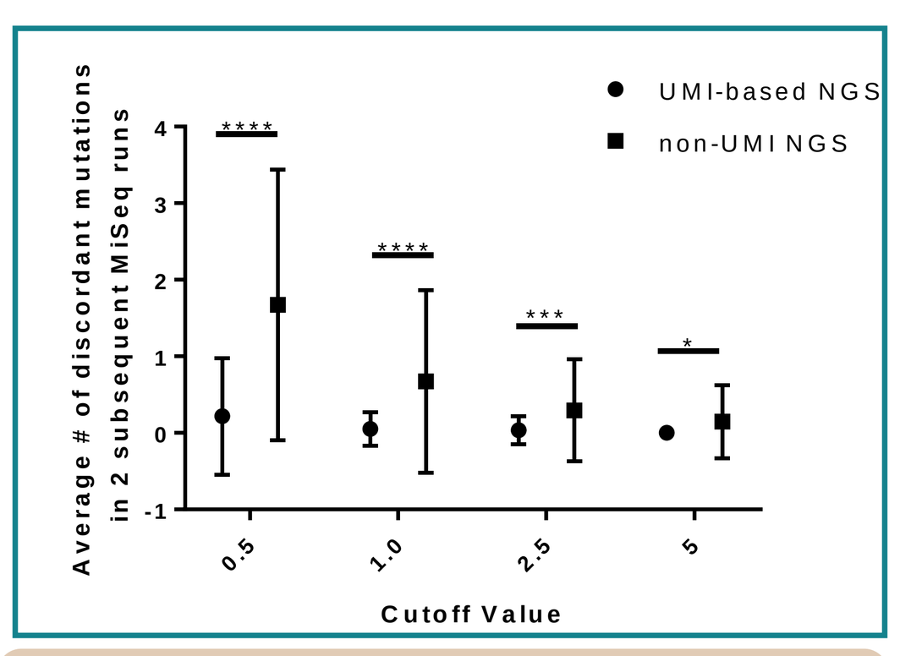

Figure 5: Reproducibility of UMI- and non-UMI-based NGS in 2 subsequent MiSeq runs.

Conclusions

- UMI-based NGS should be used when calling mutations at frequencies below 5%
- This is predominantly true for samples that are likely to have limited sampling depth from low viral inputs (e.g. from DBS) or with diverse samples (polymorphic primer binding sites).

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MTN microbicide trials network