
 **Department of Health** | Wadsworth Center


Whole Genome Next Generation Sequencing of TB in a Public Health Laboratory: A New Diagnostic Era

March 31, 2016

Kimberlee Musser, PhD
Chief, Bacterial Diseases
Wadsworth Center

 **Department of Health** | Wadsworth Center

- 900,000 sq. ft. state-of-the-art-facilities- 5 locations
- ~700 staff, >150 doctoral level scientists
- \$25 million in external grant funding
- Laboratories in four scientific divisions:
 - Environmental Health, Infectious Disease, Genetics, Translational Medicine

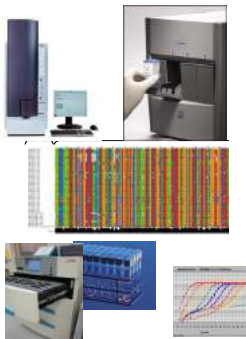


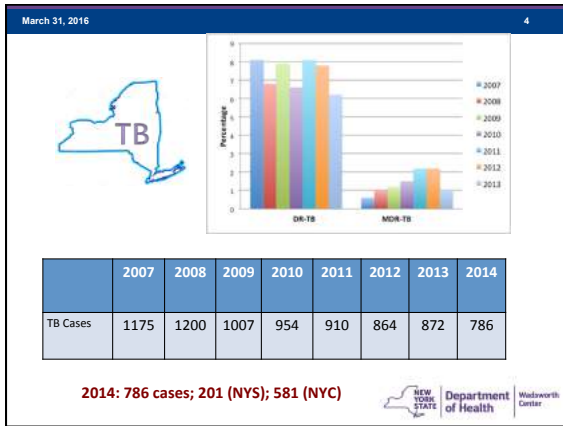
Division of Infectious Diseases

Viral Diseases	Bacterial Diseases	Bloodborne Diseases	Mycotic & Parasitic Diseases	Viral Replication and Vector Biology
Virology	Bacteriology	Bloodborne Virus	Parasitology	Arbivirology
Viral Encephalitis	Mycobacteriology	Diagnostic Immunology	Mycology	Rabies
Enteric Virus	Biodefense			

Roles of the Wadsworth Center Bacteriology and Mycobacteriology Laboratories

- Reference services
- Outbreak and hospital investigations, surveillance
- Specialized testing
- Applied research (NIH, CDC, contracts)
 - Development of diagnostics, algorithms
 - Evaluation of FDA approved tests
 - Retrospective analysis
 - Shared Services





Focus on continual improvement of TB Diagnostics

NAAT (isolates and specimens):

MTBC	real-time PCR	since 2007
MTBC differentiation	real-time PCR	since 2010

TB positive- resistance assessment:

<i>rpoB</i> gene	pyrosequencing	since 2009
<i>katG</i> gene	pyrosequencing	since 2010
<i>inhA</i> promoter	pyrosequencing	since 2012
<i>gyrA, gyrB</i> genes	pyrosequencing	since 2013

Non-tuberculous mycobacteria (NTM):

MAC	real-time PCR	since 2011
<i>M. abscessus</i>	real-time PCR	since 2014
NTM ID		
	<i>rpoB, hsp65, 16S</i> gene sequence analysis	since 2012
	MALDI-TOF MS	since 2013

Whole-genome sequencing for TB?

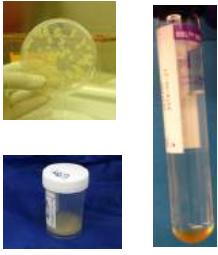
2013- Wadsworth Center Public Health Genomics Center (PHGC) funding announcement
 2014- PHGC funding to test 60 TB isolates by WGS

- **Goals for TB WGS:**
 - Utilize as soon as possible in testing algorithm to impact patient treatment
 - Expand molecular resistance prediction
 - Provide more comprehensive results
 - mixed infections, heteroresistance, typing
 - Assess costs and staff time


March 31, 2016 7

Where to start?

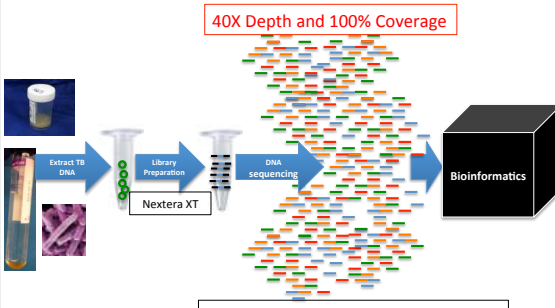
- Isolates
 - Solid
 - MGITs
- Primary specimens
 - sputum
 - other



Worst case scenario- Day 0 Flag Positive MGIT Culture



Whole Genome Sequencing TB- Assay Development



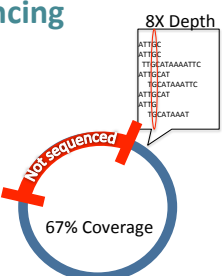
40X Depth and 100% Coverage

Sequencing on MiSeq, 2 x 250bp PE reads

March 31, 2016 9

Sequencing

- **Depth:** Essentially the number of times the base was read; measure of confidence in correct call
 - Can be given as a genome average
 - We are aiming for 40X
- **Coverage:** A percentage that describes how much of the genome was sequenced
 - Best 100%




8X Depth

```

ATTTTC
ATTTTC
TTTATAAAATTC
ATTTGAT
TGCATAAATTC
ATTTGAT
ATTTTC
TGCATAAAT
    
```


67% Coverage



March 31, 2016 10

TB DNA Preparation for WGS

- **InstaGene matrix (Chelex resin)**
 - The Chelex matrix binds to PCR inhibitors rather than DNA, preventing DNA loss due to irreversible DNA binding.
- **Fastprep tissue homogenizer**
 - Good enough yield to provide reliable WGS data even with 0 day MGIT



Success!

NEW YORK STATE Department of Health Wadsworth Center

March 31, 2016 11

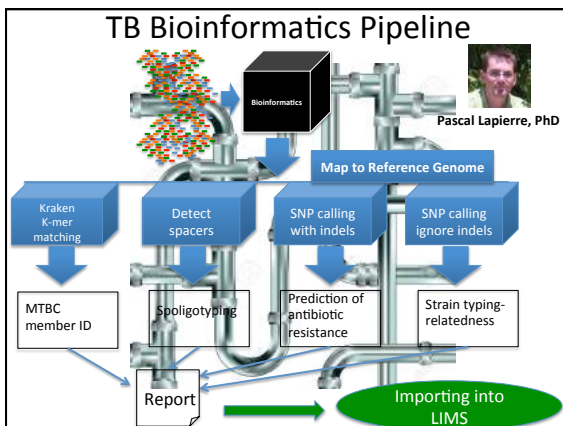
Library Preparation is Another Key Factor

- **Votintseva et al. suggested using 15 cycle library preparation**
 - 2015 paper about WGS of early positive MGIT

Sample	Method	stock ng/ul	12 cycle library prep		15 cycle library prep	
			Avg depth	coverage %	Avg depth	coverage %
<i>M. bovis</i> BCG (0day)	InstaGene	0.268	FAIL	FAIL	27.66	97.23
	InstaGene	0.344	FAIL	FAIL	19.4	97.07
	InstaGene	0.346	FAIL	FAIL	14.22	96.78

Success!

NEW YORK STATE Department of Health Wadsworth Center



Retrospective study: Fluoroquinolone comparison

6. Fluoroquinolone Resistance:

Table 21. Summary of *gyrA/gyrB* mutations identified used to predict fluoroquinolone resistance

High confidence mutations in <i>gyrA</i>	Number of isolates found to harbor mutation by WGS during retrospective study	Number of isolates with mutation confirmed by pyrosequencing or Sanger sequencing
AbsP9Val	4	4
Ser51Pro	1	1
Asp84Asn	1	1
Arg86Gly	7	7
Total	13	13 (100%)

No high confidence *gyrB* mutations were identified in this retrospective study.

Table 22. DST Phenotype results compared to WGS Genotype results for fluoroquinolone resistance (target_{gyrA})

WGS Genotype	Fluoroquinolone DST Phenotype	
	Resistant	Susceptible
Resistant	13 ¹	0
Susceptible	0	60

Resistant Predictive Value=100%
Susceptible Predictive value=100%

Validating against other molecular tests

Validating against DST


March 31, 2016 17

Retrospective Study: Isoniazid comparison

		DST Phenotype	
		R	S
WGS Genotype	R	55	1 ¹
	S	6 ²	32

¹This SNP is known to be a good but not perfect predictor of INH resistance (14/15 resistant)
² Each of the 6 has a different mutation that could potentially account for the missed resistance

Resistance Predictive Value= 98%
Susceptible Predictive value= 84%



March 31, 2016 18


Drug Resistance Prediction

WGS genotype prediction vs. DST phenotype *

Current	EMB	FLQ	INH	PZA	RIF	SM	KAN	ETA
Sensitivity	0.77	1.00	0.88	0.92	1.00	0.71	1.00	0.65
Specificity	0.98	1.00	0.99	0.97	0.96	1.00	1.00	0.95
PPV (Res)	0.87	1.00	0.99	0.88	0.85	1.00	1.00	0.89
NPV (Susc)	0.96	1.00	0.94	0.98	1.00	0.89	1.00	0.80
Concordance	0.94	1.00	0.95	0.96	0.97	0.91	1.00	0.83


- 7/8 Drugs have >90% concordance
- Predictions will be improved as we gather more data
- Increased support for new genes or new mutations to be included as 'high confidence'

*phenotype based on the critical concentrations used at WC



March 31, 2016 19

Why we don't think we can live without TB WGS...




March 31, 2016 20

Prospective specimens (received 7/3-7/13/15)

Results as of 8/3/15:

Sample	Current method results				WGS results				
	DST	Pyro results	spoligo	Genome coverage	Ave Depth	ID	High confidence mutations detected	Frameshift and/or large deletions detected	spoligo
IDR15-51087	Invalid; PZA S	No mutations	ND	98.36	91.05	Mtb	None	none	S00034
IDR15-52024	pending; PZA S	ND	ND	98.46	75.32	Mtb	gyrA Ala90Val; gyrA Asp94Gly (FLC)	none	Unknown (new)
IDR15-52248	pending	ND	ND	98.82	79.08	Mtb	emhB Met305Val (EMH); gyrA Ser91Pro (FIC); katG Ser315Thr (INH)	rpoB (+TTC) in-frame insertion (RIF)	S00034

No Results
WGS complete




March 31, 2016 21

Results as of 8/18/15:

Sample	Current method results				WGS results				
	DST	Pyro results	spoligo	Genome coverage	Ave Depth	ID	High confidence mutations detected	Frameshift and/or large deletions detected	spoligo
IDR15-51087	Pan-Susceptible	No mutations	ND	98.36	91.05	Mtb	None	none	S00034
IDR15-52024	pending; PZA S	gyrA Asp94Gly	pending	98.46	75.32	Mtb	gyrA Ala90Val; gyrA Asp94Gly (FLC)	none	Unknown (new)
IDR15-52248	pending	rpoB insertion of 3 bases at codon 534; katG; Ser315Thr; gyrA; Ser91Pro	ND	98.82	79.08	Mtb	emhB Met305Val (EMH); gyrA Ser91Pro (FIC); katG Ser315Thr (INH)	rpoB (+TTC) in-frame insertion (RIF)	S00034

Some Results
WGS complete



March 31, 2016 22

Results as of 10/8/15:

Sample	Current method results			WGS results					
	DST	Pyro results	spoligo	Genome coverage	Ave Depth	ID	High confidence mutations detected	Frameshift and/or large deletions detected	spoligo
IDR15-51087	Pan-Susceptible	No mutations	ND	98.36	91.05	Mtb	None	none	S00034
IDR15-52024	FLQ	grrA: Asp94Gly	S02467	98.46	75.32	Mtb	grrA: Ala90Val; grrA: Asp94Gly (FLQ)	none	Unknown (new)
IDR15-52248	1 st line invalid; 2 nd line pending	rpoB: insertion of 3 bases at codon 516: katG Ser315Thr; grrA: Ser31Pro	S00034	98.82	79.08	Mtb	embB: Mnt500val (EMB); grrA: Ser31Pro (FLQ); katG Ser315Thr (NH)	rpoB (-TTC) in-frame insertion (RF)	S00034

More Results
WGS complete

March 31, 2016 23

Heteroresistance

AS Applied Biosystems 4058_C08_15-52024-grrAF_006

S/N Q:270 A:151 T:145 C:418 15-52024-grrAF

KB bcp KB_3130_PCPYF_501V3.mcb

KB 1.4.1.8 Cap:6 Ph: 1720 to 7333 Pk1 Loc:1697

Version 6.0 HISGV Bases: 429 4068-59 C8

Wadsworth Center

March 31, 2016 24

Turn-around Time


Sample	Date Received	Date processed and sent to core	Date of DST results	Date of WGS report	DST TAT from receipt of specimen to DST results (days)	WGS TAT from receipt of specimen to report (days)
IDR15-51087	7/3/15	7/17/15	8/13/15	7/30/15	41	27
IDR15-52024	7/10/15	7/17/15	8/24/15 (1 st line); 9/1/15 (2 nd line)	7/30/15	53	20
IDR15-52248	7/13/15	7/17/15	1 st line invalid; 2 nd line still pending	7/30/15	>60	17

Imagine this is 4-5 days!

March 31, 2016 25

We have found that TB WGS provides:

- 1- MDR identification in ~ 2 weeks or less on TB cases (some that are not even known as cases) weeks-months before DST available
- 2- Resolution/ early identification of mixed samples (NTM/TB)
- 3- Resolves inconclusive identifications MTB complex due to missing RD regions
- 4- Resolves issues where pyrosequencing or Sanger sequencing will FAIL due to deletion in target genes
- 5- Finds mutations outside of pyrosequencing region of target genes
- 6- Finds mutations in 2nd line drugs which would never have been found when 1st line drugs are susceptible
- 8- Resolving spoligotyping issues
- 9- Can identify heteroresistance
- 10- Predicts resistance when DST is invalid. WGS is even more valuable because the normal time to susceptibility results is pushed back. In some cases these specimens turn out to have contamination so that DST can never be completed and is canceled.



March 31, 2016 26

Ongoing TB WGS Activities


- Received conditional approval to offer this test on 2/8/16!
- Finalizing LIMs import, reporting
- Performing TB WGS 1x/ week- TAT 7 days
- Created guidance document CLEP
- NIH R03- Evaluate TB WGS directly on sputum specimens
- APHL/CDC RFA to perform TB WGS on every case of TB in NYS and NYC- completed first 34

March 31, 2016 27

Whole Genome Sequencing of TB: A "One Stop Shop"

WGS	COST
Single assay	Estimated around \$100-\$200 per sample
Species identification	
Genotyping (more accurate)	TURN-AROUND TIME
Drug resistance mutations (more comprehensive)	DNA preparation (1 days) WGS result (4-5 days)

Reality for TB Cultures!



March 31, 2016 Acknowledgements 28

Wadsworth Center

MYCOBACTERIOLOGY LAB
Vincent Escuyer
Donna Kohlerschmidt
Michelle Isabelle
Susan Wolfe
Dennis Biggins

BACTERIOLOGY LAB
Joe Shea
Tanya Halse
Tammy Quinlan
Justine Edwards
Linda Gebhardt

APPLIED GENOMIC TECHNOLOGIES CORE
Matt Schudt
Patrick VanRoey
Pascal Lapierre
Mike Palumbo

APHL
Establishment of Mycobacterium tuberculosis complex WGS Reference Centers

CDC
James Posey
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention

NIH National Institutes of Health
Office of Extramural Research
R03 NIH- Use of whole genome sequencing for tuberculosis diagnostics

Wadsworth Center, NYSDOH
Public Health Genomics Initiative

NEW YORK STATE Department of Health Wadsworth Center

Questions?

http://biocomicals.blogspot.com/2011_05_01_archive.html

March 31, 2016 30

NEW YORK STATE Department of Health Wadsworth Center

March 31, 2016 31

Rapid Diagnosis of *Mycobacterium tuberculosis* with WGS

- **Faster turn-around time**
- **More comprehensive results**
 - Detect mixed infections
 - Many predictors of drug resistance
 - Emerging resistance
- **Cost effective**
 - Replace existing assays (real-time PCR, pyrosequencing, spoligotyping)
 - Staff time savings




Table 1: List of high confidence mutations associated with 8 anti-tuberculous drug drug classes

Antituberculous (Abbreviation)	Gene	SNP/indel position	Transition
Rifampin (RIF)	<i>rpoB</i>	251, 511, 513, 516, 522, 526, 531, 534, 572*	Coding
Isoniazid (INH)	<i>katG</i>	279, 115, 525	Coding
Isoniazid (INH)	<i>inhA</i> / <i>inhA</i> / <i>inhA</i>	-81	Non-coding
Isoniazid (INH)	<i>inhA</i> / <i>inhA</i> / <i>inhA</i>	-17, -15, -8	Non-coding
Isoniazid (INH)	<i>inhA</i>	203	Coding
Pyrazinamide (PZA)	<i>pyrA</i>	Any change	Coding
Pyrazinamide (PZA)	<i>pyrA</i> / <i>pyrA</i>	Any change	Non-coding
Ethambutol (EMB)	<i>embB</i>	206, 406, 497	Coding
Streptomycin (SM)	<i>rps</i>	512, 513, 516, 506	Non-coding
Streptomycin (SM)	<i>rpsL</i>	41, 88	Coding
Fluoroquinolones (FLQ)	<i>gyrA</i>	74, 90, 93, 94	Coding
Fluoroquinolones (FLQ)	<i>gyrB</i>	310	Coding
Ethambutol (EMB)	<i>inhA</i> / <i>inhA</i> / <i>inhA</i>	-17, -15, -8	Non-coding
Ethambutol (EMB)	<i>inhA</i>	203	Coding
Kanamycin (KAN)	<i>rps</i>	1400	Non-coding
Kanamycin (KAN)	<i>rns</i>	-27, -20	Non-coding
Kanamycin (KAN)	<i>rnsA</i>	1000	Coding


*A branchlet deletion/insertion in *rpoB*, *pyrA*, and *katG* may be considered a high confidence mutation.
 †Z-coil embedding system is utilized, which is uncommonly found in literature.

March 31, 2016 33

Sequence Confirmation- Using another molecular method (used to predict resistance)

Drug	Gene	# Mutations found	# Confirmed	# Not Confirmed
Rifampin	<i>rpoB</i>	46	44	2 ¹
Isoniazid	<i>katG</i>	40	36	4 ¹
Isoniazid & Ethionamide	<i>inhA</i> / <i>mabA</i>	22	17	5 ¹
Fluoroquinolones	<i>gyrA</i>	13	12	1 ²
Streptomycin	<i>rps</i>	11	11	0
	<i>rpsL</i>	28	26	2 ^{2,3}
Pyrazinamide	<i>pyrA</i>	31	19	12 ^{1,2,3}
Ethambutol	<i>embB</i>	28	26	2 ^{1,3}
Kanamycin	<i>rps</i>	8	8	0
	<i>eis</i>	1	0	1 ¹

¹No assay/ Mutation found outside range of confirmatory assay/ deletion caused assay failure
²pending
³No remaining DNA stock



NEW YORK STATE DEPARTMENT OF HEALTH WADSWORTH CENTER																																																																																																																																																																																																									
FINAL LABORATORY REPORT																																																																																																																																																																																																									
<p>Clinical Microbiology Laboratory Department of Clinical Microbiology 139 New Scotland Avenue Albany, NY 12242-1502 Phone: 518.402.1222 ext. 3100, 402.6204 Fax: 518.402.1222 ext. 3100, 402.6204 Personnel: Type Specimen (Initials)</p>																																																																																																																																																																																																									
<p>Specimen Identifier: Real-time PCR Identification: Genotype Date/Time: 02/24/16 Requested by: Dr. [Name] Requested for: Real-time PCR Method: PCR</p>	<p>139 New Scotland Avenue Albany, NY 12242-1502 Phone: 518.402.1222 ext. 3100, 402.6204 Fax: 518.402.1222 ext. 3100, 402.6204 Personnel: Type Specimen (Initials)</p>																																																																																																																																																																																																								
<p>Reference Information and Drug Susceptibility Profiles: Whole Genome Sequencing</p>																																																																																																																																																																																																									
<p>High resolution reference sequence</p> <table border="0"> <tr> <td>gag (ORF1)</td> <td>Multiple protein, 36213 bp</td> </tr> <tr> <td>pol (ORF2)</td> <td>Multiple protein, 80417 bp</td> </tr> <tr> <td>env (ORF3)</td> <td>A 100% to 100% similarity to reference for full gene</td> </tr> <tr> <td>gag-pol (ORF4)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF5)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF6)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF7)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF8)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF9)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF10)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF11)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF12)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF13)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF14)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF15)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF16)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF17)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF18)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF19)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF20)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF21)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF22)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF23)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF24)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF25)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF26)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF27)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF28)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF29)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF30)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF31)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF32)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF33)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF34)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF35)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF36)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF37)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF38)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF39)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF40)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF41)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF42)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF43)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF44)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF45)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF46)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF47)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF48)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF49)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF50)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF51)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF52)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF53)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF54)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF55)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF56)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF57)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF58)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF59)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF60)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF61)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF62)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF63)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF64)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF65)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF66)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF67)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF68)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF69)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF70)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF71)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF72)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF73)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF74)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF75)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF76)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF77)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF78)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF79)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF80)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF81)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF82)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF83)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF84)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF85)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF86)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF87)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF88)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF89)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF90)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF91)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF92)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF93)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF94)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF95)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF96)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF97)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF98)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF99)</td> <td>No reaction</td> </tr> <tr> <td>gag-pol-env (ORF100)</td> <td>No reaction</td> </tr> </table>		gag (ORF1)	Multiple protein, 36213 bp	pol (ORF2)	Multiple protein, 80417 bp	env (ORF3)	A 100% to 100% similarity to reference for full gene	gag-pol (ORF4)	No reaction	gag-pol-env (ORF5)	No reaction	gag-pol-env (ORF6)	No reaction	gag-pol-env (ORF7)	No reaction	gag-pol-env (ORF8)	No reaction	gag-pol-env (ORF9)	No reaction	gag-pol-env (ORF10)	No reaction	gag-pol-env (ORF11)	No reaction	gag-pol-env (ORF12)	No reaction	gag-pol-env (ORF13)	No reaction	gag-pol-env (ORF14)	No reaction	gag-pol-env (ORF15)	No reaction	gag-pol-env (ORF16)	No reaction	gag-pol-env (ORF17)	No reaction	gag-pol-env (ORF18)	No reaction	gag-pol-env (ORF19)	No reaction	gag-pol-env (ORF20)	No reaction	gag-pol-env (ORF21)	No reaction	gag-pol-env (ORF22)	No reaction	gag-pol-env (ORF23)	No reaction	gag-pol-env (ORF24)	No reaction	gag-pol-env (ORF25)	No reaction	gag-pol-env (ORF26)	No reaction	gag-pol-env (ORF27)	No reaction	gag-pol-env (ORF28)	No reaction	gag-pol-env (ORF29)	No reaction	gag-pol-env (ORF30)	No reaction	gag-pol-env (ORF31)	No reaction	gag-pol-env (ORF32)	No reaction	gag-pol-env (ORF33)	No reaction	gag-pol-env (ORF34)	No reaction	gag-pol-env (ORF35)	No reaction	gag-pol-env (ORF36)	No reaction	gag-pol-env (ORF37)	No reaction	gag-pol-env (ORF38)	No reaction	gag-pol-env (ORF39)	No reaction	gag-pol-env (ORF40)	No reaction	gag-pol-env (ORF41)	No reaction	gag-pol-env (ORF42)	No reaction	gag-pol-env (ORF43)	No reaction	gag-pol-env (ORF44)	No reaction	gag-pol-env (ORF45)	No reaction	gag-pol-env (ORF46)	No reaction	gag-pol-env (ORF47)	No reaction	gag-pol-env (ORF48)	No reaction	gag-pol-env (ORF49)	No reaction	gag-pol-env (ORF50)	No reaction	gag-pol-env (ORF51)	No reaction	gag-pol-env (ORF52)	No reaction	gag-pol-env (ORF53)	No reaction	gag-pol-env (ORF54)	No reaction	gag-pol-env (ORF55)	No reaction	gag-pol-env (ORF56)	No reaction	gag-pol-env (ORF57)	No reaction	gag-pol-env (ORF58)	No reaction	gag-pol-env (ORF59)	No reaction	gag-pol-env (ORF60)	No reaction	gag-pol-env (ORF61)	No reaction	gag-pol-env (ORF62)	No reaction	gag-pol-env (ORF63)	No reaction	gag-pol-env (ORF64)	No reaction	gag-pol-env (ORF65)	No reaction	gag-pol-env (ORF66)	No reaction	gag-pol-env (ORF67)	No reaction	gag-pol-env (ORF68)	No reaction	gag-pol-env (ORF69)	No reaction	gag-pol-env (ORF70)	No reaction	gag-pol-env (ORF71)	No reaction	gag-pol-env (ORF72)	No reaction	gag-pol-env (ORF73)	No reaction	gag-pol-env (ORF74)	No reaction	gag-pol-env (ORF75)	No reaction	gag-pol-env (ORF76)	No reaction	gag-pol-env (ORF77)	No reaction	gag-pol-env (ORF78)	No reaction	gag-pol-env (ORF79)	No reaction	gag-pol-env (ORF80)	No reaction	gag-pol-env (ORF81)	No reaction	gag-pol-env (ORF82)	No reaction	gag-pol-env (ORF83)	No reaction	gag-pol-env (ORF84)	No reaction	gag-pol-env (ORF85)	No reaction	gag-pol-env (ORF86)	No reaction	gag-pol-env (ORF87)	No reaction	gag-pol-env (ORF88)	No reaction	gag-pol-env (ORF89)	No reaction	gag-pol-env (ORF90)	No reaction	gag-pol-env (ORF91)	No reaction	gag-pol-env (ORF92)	No reaction	gag-pol-env (ORF93)	No reaction	gag-pol-env (ORF94)	No reaction	gag-pol-env (ORF95)	No reaction	gag-pol-env (ORF96)	No reaction	gag-pol-env (ORF97)	No reaction	gag-pol-env (ORF98)	No reaction	gag-pol-env (ORF99)	No reaction	gag-pol-env (ORF100)	No reaction
gag (ORF1)	Multiple protein, 36213 bp																																																																																																																																																																																																								
pol (ORF2)	Multiple protein, 80417 bp																																																																																																																																																																																																								
env (ORF3)	A 100% to 100% similarity to reference for full gene																																																																																																																																																																																																								
gag-pol (ORF4)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF5)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF6)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF7)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF8)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF9)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF10)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF11)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF12)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF13)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF14)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF15)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF16)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF17)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF18)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF19)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF20)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF21)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF22)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF23)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF24)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF25)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF26)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF27)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF28)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF29)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF30)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF31)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF32)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF33)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF34)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF35)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF36)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF37)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF38)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF39)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF40)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF41)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF42)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF43)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF44)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF45)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF46)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF47)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF48)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF49)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF50)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF51)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF52)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF53)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF54)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF55)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF56)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF57)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF58)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF59)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF60)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF61)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF62)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF63)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF64)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF65)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF66)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF67)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF68)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF69)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF70)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF71)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF72)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF73)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF74)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF75)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF76)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF77)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF78)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF79)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF80)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF81)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF82)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF83)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF84)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF85)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF86)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF87)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF88)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF89)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF90)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF91)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF92)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF93)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF94)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF95)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF96)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF97)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF98)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF99)	No reaction																																																																																																																																																																																																								
gag-pol-env (ORF100)	No reaction																																																																																																																																																																																																								
<p>Final Report</p>																																																																																																																																																																																																									
