HMP_MDG_454DEFAULTPROTOCOL.V4.2

1. PURPOSE

1.1. The current outline of the pilot production study is given below. This study is designed to meet a number of objectives including:

1.1.1. Test the use of the provisional 454 16S protocol thus providing additional feedback on the use of this protocol to generate 16S reads with actual samples

1.1.2. Further inform quality control procedures of 16S reads generated from 454 beyond what can be learned with the Mock Community

1.1.3. Further inform data analysis procedures of 16S reads generated from 454 beyond what can be learned with the Mock Community

1.1.4. Allow centers to test in house LIMs and workflows and use this information to craft efficient production procedures

1.1.5. Test procedures for data submission with the DACC and NCBI

1.1.6. Test screening procedures for human contamination

2. IMPORTANT PROTOCOL NOTES

2.1. This protocol describes the protocol for the clinical sample pilot study using barcoded primers for 16S variable regions V1-3 and V3-5.

2.1.1. DNA provided by Baylor for Pilot Experiment: clinical samples and HMP even mock minus Candida (positive control)

2.1.1.1. Centers will quantify the samples using a fluorescent based assay and record this information.

2.1.1.2. 1ul of each undiluted sample will be used per amplification reaction – samples may be diluted for accommodate robotic pipetting capacity, however the final concentration must be equivalent to the 1ul undiluted sample.

2.1.1.3. Two controls will be amplified:

2.1.1.3.1. The HMP even mock (1ul/amplification reaction) - positive control.

2.1.1.3.2. One barcoded primer pair will be tested with water as a negative control.

2.1.2. Each sequencing center will receive clinical samples from 12 individuals (12 individuals x 16.5 samples/individual = 198 samples).

2.1.2.1. Each sample will be amplified with 2, 16S variable regions: (198 samples x 2 variable regions = 396 amplicons)

2.1.2.2. Each sample will have a unique barcode (using either Broad or JCVI primers – see Appendix below).

2.1.2.3. The 396 PCR products that will be cleaned with AmPure bead (SPRI), quantified (Quant-iT, or SybrGreen), normalized and pooled:

2.1.2.3.1. 96 (up to 100) uniquely barcoded amplicons/pool totaling 4 pools (2 pools for V1-3 amplicons, and 2 pools for V3-5 amplicons).

2.1.2.3.2. Each 96 library pool will be sequenced on 1 PTP (~10,000 reads/amplicon) totaling 4 PTPs.

2.1.2.3.3. Alternative: 48 (up to 50) uniquely barcoded amplicons/pool totaling 8 pools (4 pools for V1-3 and 4 pools for V3-5 amplicons).

2.1.2.3.4. Each 48 library pool will be sequenced on ½ PTP (10,000reads/amplicon) totaling 8, ½ PTPs.

2.1.2.3.5. The HMP mock positive control should be included in each amplicon pool (96 or 48). For this study we are using V 1.1.

2.1.2.3.6. A negative control (water blank) should be included with each PCR core mix.

2.1.2.3.7. Note- multiplexed sample pools should consist of only one V region per pool (V1-V3 or V3-V5) and that pool should be run on a distinct half or full plate (in other words do not mix V regions in a pool or on the same plate 'real estate.')

2.1.2.3.8. If there are additional samples that cannot be accommodated in the pooling strategy desired by the center those samples (up to 8 samples) can be excluded **(Note- this is still under discussion by the centers)**

2.1.2.3.9. If the number of successfully amplified samples exceeds what a center can pool using one of the above strategies using four PTPs then the extra samples can be excluded from multiplexing.

2.1.2.3.10. **PCR failures**- (**Note- this is still under discussion by the centers**) if a PCR for a particular sample fails then the reaction should be repeated one time using the provisional protocol.

2.1.2.3.10.1. If the reaction fails a second time, it should be repeated with increased template (double the volume) if the sample was a low concentration sample (e.g., <50ng/ul) or diluted to one half the concentration if the concentration is high (e.g, >50ng/ul).'Plan B'

2.1.2.3.10.2. If the reaction fails again, the reaction can be considered a failure. However centers are free to try other adjustments to the PCR protocol at their discretion.

2.1.2.3.10.3. All deviations from the default PCR conditions should be noted in the metadata file.

3. REQUIREMENTS

Materials/Equipment	Vendor	Catalog Number
AccuPrime™ Taq DNA Polymerase High Fidelity	Invitrogen	12346-086
Forward and Reverse Primers premixed	Operon	custom order
96 well thermocyler plate		
clear adhesive plate seals		
DNAse/RNAse free water	-	-
Thermo Cycler	-	-
Vortex	-	-
Pipettes	-	-
Aerosol resistant pipette tips		
MinElute PCR Purification Kit	Qiagen	28004
Ampure (SPRI) Beads (60mL kit)	Agencourt	A29152
1x low TE, pH 8.0		
Quant-IT ds DNA Assay, high sensitivity	Invitrogen/Molecular Probes	Q33120

Documentation
Quant-iT ds DNA Assay protocol (manufacturer's specifications)
SybrGreen Assay protocol (manufacturer's specifications)
poolingCalculator.xls
MinElute PCR Purification Kit Manual

4. METHOD

The PCR will be carried out using AccuPrime Taq High Fidelity. It is not necessary to setup this reaction on ice, however it is recommended.

- **PCR Primer Setup** Set up of 10uM primer plates (combining barcoded A primer with non-barcoded B primer- see appendix below for primer & tag sequences):
    - 1:10 dilution of the 100uM stocks:
    - For each variable region, set up a working primer plate with 90ul of 1x low TE
    - Add 5ul of each barcoded primer A from 100uM plate to corresponding well position in 10uM dilution plate.
    - Add 5ul of the 100uM of corresponding B adapter to each well of the 96-well plate (final concentration 10uM primer pair).
    - Mix by pipetting up and down.
    - Working concentration of 4uM (2uM each primer)
        - Dilute the 10uM primers 1:2.5 in 1x low TE (add 150ul of TE to each well of the 10uM primer stock plate and mix

- Primers can be stamped out into multiple single use primer plates and store at -20oC until ready to use.

4.1. PCR Setup - Mastermix

4.1.1. MasterMix contains the following amounts per sample:

13.85uL	RNAse/DNAse free water
2uL	10X AccuPrime PCR Buffer II
0.15uL	Accuprime Taq Hifi
16uL	Total Volume of master mix

4.1.2. Multiply all volumes above by the amount of reactions needed plus 10%.

4.1.3. Combine reagents in a 2mL micro centrifuge tube and vortex to mix completely. If more than 100 reactions are needed a 15mL tube should be used.

4.1.4. Using an automated pipette transfer 16uL of master mix into individual wells in the 96 well reaction plate.

4.1.5. Cover plate and spin in a centrifuge at 2000rpm to collect sample at the bottom of the wells.

4.2. PCR Setup

4.2.1. For the initial reaction, transfer 2uL diluted (1:1 in water) DNA sample into the respective reaction wells. If the amplification fails repeat with 2uL of undiluted sample (See 2.1.1.2.)

4.2.2. Transfer 2uL of barcoded primers from primer plate to corresponding wells in 96 well PCR plate.

4.2.3. Securely seal with clear adhesive plate seal and vortex plate vigorously.

4.2.4. Spin briefly at 2000 rpm in a centrifuge.

4.2.5. Place in thermo cycler and cycle as follows:

95°C	2 min	
95°C	20 sec	} 30 cycles
50 or 56°C*	30 sec	
72°C	5min	
4°	forever	

*** 56°C for V3-1, 50°C for V5-3**

4.2.6. Clean PCR products using Agencourt AmPure Beads (use Agencourt protocol → 1.8x volume beads (36ul beads) – follow manufacturer's specifications.

4.2.7. Elute beads with 25ul 1x low TE, pH 8.0 and transfer to new 96 well plate.

4.3. PCR Gel Analysis – (E-gel alternative using 1ul of PCR product - faster) - we will know from the Quantification step below if we have product so this step is actually optional.

4.3.1. In a new reaction plate add 1uL PCR product to 1uL 6X loading dye
4.3.2. Cover, vortex to mix, briefly centrifuge to collect sample at the bottom of the well.
4.3.3. Prepare a 1% agarose 1X TAE gel with EtBr.
4.3.4. Load samples and run approximately 1 hour at 100V.
4.3.5. Capture gel image on gel-doc and retain for analysis.

4.4. PCR Product Quantification

4.4.1. Quantify PCR product using SYBR-Green Quantification or Quant-IT ds DNA high sensitivity assay according to the manufacturer's specifications.

4.5. PCR Pooling

4.5.1. Using values from the SYBR Green or Quant-IT quantification, calculate pooling amounts using the poolingCalculator.xls or according to the following formula:

Amount (uL) of each sample = ((vol/2)*(min)) / sampleconc
where:
Vol = total volume of each sample
Min = concentration in ng/ul of the sample with the lowest concentration
Sampleconc = concentration in ng/uL of target sample

4.5.2. Pool samples using a minimum transfer volume of 1uL. If less than 1uL is called for, a dilution must be made. If using the poolingCalculator.xls this will be accounted for.
4.5.3. Using a Qiagen minElute column, purify the pool according to the manufacturer's protocol.

(The Broad normalizes by converting all concentrations to molecules/ul. Determine which sample has the lowest concentration and then dilute all other samples to the same concentration. Pool equal volume of each (5-10ul) sample and then concentrate using a Qiagen MinElute column (elution with 30uL, 1x low TE, pH 8.0).

4.6. Sample Transfer for 454 Library Completion

4.6.1. Proceed directly to the qPCR library step.

Optional: Enter emPCR using ¼ the recommended primer concentration to avoid too many molecules amplified on bead. This results in high signal intensities during run, which leads to higher mixed reads and shorter read lengths.

5. POST SEQUENCING- Data Transfer to the DACC

5.1. After sequencing, the raw *.sff (e.g., no deconvoluting of samples or screening for human contamination) will be deposited at the DACC ftp site. Centers are of course free to work with this data in house in parallel to the DACC efforts.

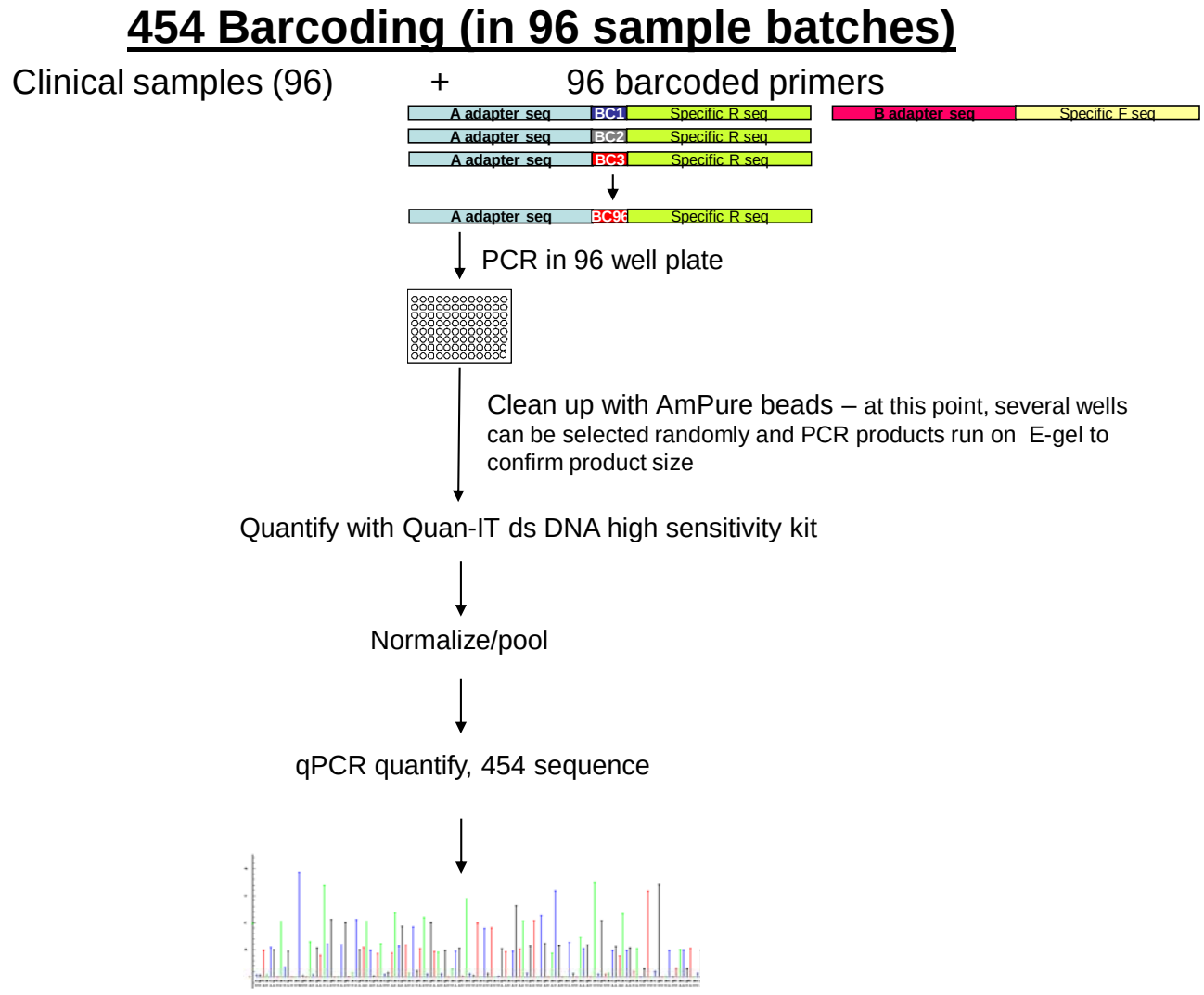
5.2. A sample spreadsheet containing metadata and library construction information as set up by the DACC will be completed by all centers and will accompany the submission of the *.sff files

5.3. The DACC will perform a screening for the presence of human sequence and report these results to the centers

APPENDIX:

BROAD INSTITUTE PRIMER SEQUENCES INCLUDING TAGS

Purpose: In this approach, we will 454 barcode sequences designed internally by the Broad (Pablo Alvarez and Will Brockman) between the A adapter and primer specific sequence (see picture below). Barcoded primer sets have been tested by the Broad Institute.



Broad Primer Sequences:

Primers were obtained from Operon reconstituted in TE for concentration of 100uM (100pmol/ul)

Variable regions V3 --> V1

"B" adapter for XLR + V1-3 27F
5' CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG

plate position	barcode name	barcode sequence	Barcode city name	primer name	A barcoded adapter for XLR system + barcode + V1-3 534R primer
A1	v2bBar8L	CACGC	Kabul	XLR_534R_v2bBar8L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCACGCATTACCGCGGCTGCTGG
A2	v2bBar23L	CGCAAC	Tirana	XLR_534R_v2bBar23L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCAACATTACCGCGGCTGCTGG
A3	v2bBar174L	TGAAGC	Algiers	XLR_534R_v2bBar174L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAAGCATTACCGCGGCTGCTGG
A4	v2bBar602L	ACTTGC	Canberra	XLR_534R_v2bBar602L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTTGCATTACCGCGGCTGCTGG
A5	v2bBar212L	TCACAC	Vienna	XLR_534R_v2bBar212L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACACATTACCGCGGCTGCTGG
A6	v2bBar25L	CGTGAC	Baku	XLR_534R_v2bBar25L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGACATTACCGCGGCTGCTGG
A7	v2bBar622L	ACGCGC	Nassau	XLR_534R_v2bBar622L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCGCATTACCGCGGCTGCTGG
A8	v2bBar72L	CCTCTC	Bridgetown	XLR_534R_v2bBar72L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTCTCATTACCGCGGCTGCTGG
A9	v2bBar600L	ACTCAC	Minsk	XLR_534R_v2bBar600L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCACATTACCGCGGCTGCTGG
A10	v2bBar559L	AGACAC	Brussels	XLR_534R_v2bBar559L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACACATTACCGCGGCTGCTGG
A11	v2bBar31L	CGACTC	Sarajevo	XLR_534R_v2bBar31L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGACTCATTACCGCGGCTGCTGG
A12	v2bBar551L	AGCTTC	Rio	XLR_534R_v2bBar551L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTTCATTACCGCGGCTGCTGG
B1	v2bBar1149L	AAGCCGC	Sofia	XLR_534R_v2bBar1149L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGCCGCATTACCGCGGCTGCTGG
B2	v2bBar15L	CAAGAAC	Ottawa	XLR_534R_v2bBar15L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAAGAACATTACCGCGGCTGCTGG
B3	v2bBar556L	AGTTGGC	Bangui	XLR_534R_v2bBar556L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTTGGCATTACCGCGGCTGCTGG
B4	v2bBar144L	TATCAAC	Santiago	XLR_534R_v2bBar144L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCAACATTACCGCGGCTGCTGG
B5	v2bBar575L	AGGCGGC	Beijing	XLR_534R_v2bBar575L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGCGGCATTACCGCGGCTGCTGG
B6	v2bBar48L	CGGTATC	Bogota	XLR_534R_v2bBar48L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTATCATTACCGCGGCTGCTGG
B7	v2bBar166L	TGACGAC	Kinshasa	XLR_534R_v2bBar166L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACGACATTACCGCGGCTGCTGG
B8	v2bBar613L	ACAAGGC	Brazzaville	XLR_534R_v2bBar613L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACAAGGCATTACCGCGGCTGCTGG
B9	v2bBar560L	AGACCTC	Zagreb	XLR_534R_v2bBar560L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACCTCATTACCGCGGCTGCTGG
B10	v2bBar741L	ATACCAC	Havana	XLR_534R_v2bBar741L	CCATCTCATCCCTGCGTGTCTCCGACTCAGATACCACATTACCGCGGCTGCTGG
B11	v2bBar228L	TCGCGGC	Nicosia	XLR_534R_v2bBar228L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCGGCATTACCGCGGCTGCTGG
B12	v2bBar807L	ATCTTAC	Prague	XLR_534R_v2bBar807L	CCATCTCATCCCTGCGTGTCTCCGACTCAGATCTTACATTACCGCGGCTGCTGG
C1	v2bBar1273L	AACCAGC	Copenhagen	XLR_534R_v2bBar1273L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCAGCATTACCGCGGCTGCTGG
C2	v2bBar441L	TTCGAGC	Djibouti	XLR_534R_v2bBar441L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGAGCATTACCGCGGCTGCTGG
C3	v2bBar1174L	AAGGTGC	Quito	XLR_534R_v2bBar1174L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGTGCATTACCGCGGCTGCTGG
C4	v2bBar209L	TCTTGGC	Cairo	XLR_534R_v2bBar209L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTGGCATTACCGCGGCTGCTGG
C5	v2bBar153L	TAATCTC	Suva	XLR_534R_v2bBar153L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAATCTCATTACCGCGGCTGCTGG
C6	v2bBar213L	TCACCTC	Helsinki	XLR_534R_v2bBar213L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACCTCATTACCGCGGCTGCTGG
C7	v2bBar298L	TCCGCTC	Paris	XLR_534R_v2bBar298L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCGCTCATTACCGCGGCTGCTGG
C8	v2bBar146L	TATTGAC	Berlin	XLR_534R_v2bBar146L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTGACATTACCGCGGCTGCTGG
C9	v2bBar554L	AGTCGAC	Accra	XLR_534R_v2bBar554L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCGACATTACCGCGGCTGCTGG
C10	v2bBar646L	ACGGCTC	Athens	XLR_534R_v2bBar646L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGGCTCATTACCGCGGCTGCTGG
C11	v2bBar158L	TGCGTTC	Guatemala	XLR_534R_v2bBar158L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGTTCATTACCGCGGCTGCTGG
C12	v2bBar207L	TCTCGAC	Conakry	XLR_534R_v2bBar207L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCGACATTACCGCGGCTGCTGG
D1	v2bBar77L	CCAGGAC	Bissau	XLR_534R_v2bBar77L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCAGGACATTACCGCGGCTGCTGG
D2	v2bBar601L	ACTCCTC	Budapest	XLR_534R_v2bBar601L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCCTCATTACCGCGGCTGCTGG
D3	v2bBar481L	TTCCTGC	Jakarta	XLR_534R_v2bBar481L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCATTACCGCGGCTGCTGG
D4	v2bBar419L	TTCATAC	Tehran	XLR_534R_v2bBar419L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCATACATTACCGCGGCTGCTGG
D5	v2bBar26L	CGTCGTC	Baghdad	XLR_534R_v2bBar26L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTCGTCATTACCGCGGCTGCTGG
D6	v2bBar1172L	AAGGCAC	Dublin	XLR_534R_v2bBar1172L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCACATTACCGCGGCTGCTGG
D7	v2bBar1210L	AACAACTC	Jerusalem	XLR_534R_v2bBar1210L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACAACTCATTACCGCGGCTGCTGG
D8	v2bBar606L	ACACGGAC	Rome	XLR_534R_v2bBar606L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACACGGACATTACCGCGGCTGCTGG
D9	v2bBar159L	TGCCGAAC	Kingston	XLR_534R_v2bBar159L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCCGAACATTACCGCGGCTGCTGG
D10	v2bBar147L	TATTCGTC	Tokyo	XLR_534R_v2bBar147L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTCGTCATTACCGCGGCTGCTGG
D11	v2bBar141L	TAGGAATC	Amman	XLR_534R_v2bBar141L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGGAATCATTACCGCGGCTGCTGG
D12	v2bBar119L	CCGGCCAC	Nairobi	XLR_534R_v2bBar119L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGCCACATTACCGCGGCTGCTGG
E1	v2bBar1379L	AATGGTAC	Tarawa	XLR_534R_v2bBar1379L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAATGGTACATTACCGCGGCTGCTGG
E2	v2bBar208L	TCTCCGTC	Pyongyang	XLR_534R_v2bBar208L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCCGTCATTACCGCGGCTGCTGG
E3	v2bBar1267L	AACCTGGC	Seoul	XLR_534R_v2bBar1267L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCTGGCATTACCGCGGCTGCTGG
E4	v2bBar637L	ACGAAGTC	Bishkek	XLR_534R_v2bBar637L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAAGTCATTACCGCGGCTGCTGG
E5	v2bBar435L	TTCGTGGC	Riga	XLR_534R_v2bBar435L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGGCATTACCGCGGCTGCTGG
E6	v2bBar1202L	AACACAAC	Beirut	XLR_534R_v2bBar1202L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACACAACATTACCGCGGCTGCTGG
E7	v2bBar413L	TTCTTGAC	Maseru	XLR_534R_v2bBar413L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTTGACATTACCGCGGCTGCTGG
E8	v2bBar289L	TCCAAGTC	Monrovia	XLR_534R_v2bBar289L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAAGTCATTACCGCGGCTGCTGG
E9	v2bBar433L	TTCGCGAC	Tripoli	XLR_534R_v2bBar433L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGCGACATTACCGCGGCTGCTGG
E10	v2bBar121L	CCGGTCGC	Vaduz	XLR_534R_v2bBar121L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGTCGCATTACCGCGGCTGCTGG
E11	v2bBar669L	ACCTGAAC	Vilnius	XLR_534R_v2bBar669L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTGAACATTACCGCGGCTGCTGG
E12	v2bBar1156L	AAGAGTTC	Luxembourg	XLR_534R_v2bBar1156L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGTTCATTACCGCGGCTGCTGG
F1	v2bBar370L	TTGACAAC	Bamako	XLR_534R_v2bBar370L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGACAACATTACCGCGGCTGCTGG
F2	v2bBar281L	TCCAGAAC	Valletta	XLR_534R_v2bBar281L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAGAACATTACCGCGGCTGCTGG
F3	v2bBar49L	CGGTCTTC	Kishinev	XLR_534R_v2bBar49L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTCTTCATTACCGCGGCTGCTGG
F4	v2bBar1173L	AAGGCCTC	Monaco	XLR_534R_v2bBar1173L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCCTCATTACCGCGGCTGCTGG
F5	v2bBar599L	ACTAATTC	Rabat	XLR_534R_v2bBar599L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTAATTCATTACCGCGGCTGCTGG
F6	v2bBar167L	TGACCGTC	Maputo	XLR_534R_v2bBar167L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACCGTCATTACCGCGGCTGCTGG
F7	v2bBar161L	TGTCGGAC	Kathmandu	XLR_534R_v2bBar161L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCGGACATTACCGCGGCTGCTGG
F8	v2bBar580L	AGGTTGTC	Amsterdam	XLR_534R_v2bBar580L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGTTGTCATTACCGCGGCTGCTGG
F9	v2bBar629L	ACGAGAAC	Wellington	XLR_534R_v2bBar629L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGAACATTACCGCGGCTGCTGG
F10	v2bBar184L	TGGTGAAC	Managua	XLR_534R_v2bBar184L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTGAACATTACCGCGGCTGCTGG
F11	v2bBar233L	TCGTTGTC	Abuja	XLR_534R_v2bBar233L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTTGTCATTACCGCGGCTGCTGG
F12	v2bBar364L	TTGTGTTC	Oslo	XLR_534R_v2bBar364L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGTGTTCATTACCGCGGCTGCTGG

G1	v2bBar78L	CCACGGTC	Muscat	XLR_534R_v2bBar78L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCACGGTCATTACCGCGGCTGCTGG
G2	v2bBar393L	TTGGAGGC	Islamabad	XLR_534R_v2bBar393L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCATTACCGCGGCTGCTGG
G3	v2bBar350L	TTATCGGC	Asuncion	XLR_534R_v2bBar350L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTATCGGCATTACCGCGGCTGCTGG
G4	v2bBar1164L	AAGAAGAC	Lima	XLR_534R_v2bBar1164L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAAGACATTACCGCGGCTGCTGG
G5	v2bBar1196L	AACTGTTC	Manila	XLR_534R_v2bBar1196L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACTGTTCATTACCGCGGCTGCTGG
G6	v2bBar411L	TTCTCAAC	Warsaw	XLR_534R_v2bBar411L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTCAACATTACCGCGGCTGCTGG
G7	v2bBar6L	CTTCCTTC	Lisbon	XLR_534R_v2bBar6L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTCCTTCATTACCGCGGCTGCTGG
G8	v2bBar1031L	ATTCGTAC	Doha	XLR_534R_v2bBar1031L	CCATCTCATCCCTGCGTGTCTCCGACTCAGATTCGTACATTACCGCGGCTGCTGG
G9	v2bBar76L	CCTTCCGC	Moscow	XLR_534R_v2bBar76L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTCCGCATTACCGCGGCTGCTGG
G10	v2bBar555L	AGTCCGTC	Kigali	XLR_534R_v2bBar555L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCCGTCATTACCGCGGCTGCTGG
G11	v2bBar378L	TTGAACTC	Riyadh	XLR_534R_v2bBar378L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAACTCATTACCGCGGCTGCTGG
G12	v2bBar1225L	AACGAGGC	Dakar	XLR_534R_v2bBar1225L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGAGGCATTACCGCGGCTGCTGG
H1	v2bBar99L	CCGTTCAC	Belgrade	XLR_534R_v2bBar99L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGTTCACATTACCGCGGCTGCTGG
H2	v2bBar236L	TCGAGGAAC	Singapore	XLR_534R_v2bBar236L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGAGGAACATTACCGCGGCTGCTGG
H3	v2bBar731L	ACCGGAAGC	Bratislava	XLR_534R_v2bBar731L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGGAAGCATTACCGCGGCTGCTGG
H4	v2bBar628L	ACGTTCCAC	Mogadishu	XLR_534R_v2bBar628L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGTTCCACATTACCGCGGCTGCTGG
H5	v2bBar1250L	AACGGAGTC	Pretoria	XLR_534R_v2bBar1250L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGGAGTCATTACCGCGGCTGCTGG
H6	v2bBar438L	TTCGTTATC	Madrid	XLR_534R_v2bBar438L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTTATCATTACCGCGGCTGCTGG
H7	v2bBar693L	ACCGTAATC	Colombo	XLR_534R_v2bBar693L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGTAATCATTACCGCGGCTGCTGG
H8	v2bBar672L	ACCTTGGTC	Khartoum	XLR_534R_v2bBar672L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTTGGTCATTACCGCGGCTGCTGG
H9	v2bBar355L	TTAAGATTC	Stockholm	XLR_534R_v2bBar355L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTAAGATTCATTACCGCGGCTGCTGG
H10	v2bBar187L	TGGTTGGTC	Bern	XLR_534R_v2bBar187L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTTGGTCATTACCGCGGCTGCTGG
H11	v2bBar162L	TGTCCGGTC	Damascus	XLR_534R_v2bBar162L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCCGGTCATTACCGCGGCTGCTGG
H12	v2bBar1292L	AACCGTGTC	Taipei	XLR_534R_v2bBar1292L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCGTGTCATTACCGCGGCTGCTGG

Variable regions V5 --> V3

"B" adapter for XLR + V3-5 357F
5' CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG

plate position	barcode name	barcode sequence	Barcode city name	primer name	"A" barcoded adapter for XLR system + barcode + V3-5 926R primer
A1	v2bBar8L	CACGC	Kabul	XLR_926R_v2bBar8L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCACGCCCGTCAATTCMTTTRAGT
A2	v2bBar23L	CGCAAC	Tirana	XLR_926R_v2bBar23L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCAACCCGTCAATTCMTTTRAGT
A3	v2bBar174L	TGAAGC	Algiers	XLR_926R_v2bBar174L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAAGCCCGTCAATTCMTTTRAGT
A4	v2bBar602L	ACTTGC	Canberra	XLR_926R_v2bBar602L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTTGCCCGTCAATTCMTTTRAGT
A5	v2bBar212L	TCACAC	Vienna	XLR_926R_v2bBar212L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACACCCGTCAATTCMTTTRAGT
A6	v2bBar25L	CGTGAC	Baku	XLR_926R_v2bBar25L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGACCCGTCAATTCMTTTRAGT
A7	v2bBar622L	ACGCGC	Nassau	XLR_926R_v2bBar622L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCGCCCGTCAATTCMTTTRAGT
A8	v2bBar72L	CCTCTC	Bridgetown	XLR_926R_v2bBar72L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTCTCCCGTCAATTCMTTTRAGT
A9	v2bBar600L	ACTCAC	Minsk	XLR_926R_v2bBar600L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCACCCGTCAATTCMTTTRAGT
A10	v2bBar559L	AGACAC	Brussels	XLR_926R_v2bBar559L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACACCCGTCAATTCMTTTRAGT
A11	v2bBar31L	CGACTC	Sarajevo	XLR_926R_v2bBar31L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGACTCCCGTCAATTCMTTTRAGT
A12	v2bBar551L	AGCTTC	Rio	XLR_926R_v2bBar551L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTTCCCGTCAATTCMTTTRAGT
B1	v2bBar1149L	AAGCCGC	Sofia	XLR_926R_v2bBar1149L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGCCGCCCGTCAATTCMTTTRAGT
B2	v2bBar15L	CAAGAAC	Ottawa	XLR_926R_v2bBar15L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCAAGAACCCGTCAATTCMTTTRAGT
B3	v2bBar556L	AGTTGGC	Bangui	XLR_926R_v2bBar556L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTTGGCCCGTCAATTCMTTTRAGT
B4	v2bBar144L	TATCAAC	Santiago	XLR_926R_v2bBar144L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCAACCCGTCAATTCMTTTRAGT
B5	v2bBar575L	AGGCGGC	Beijing	XLR_926R_v2bBar575L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGCGGCCCGTCAATTCMTTTRAGT
B6	v2bBar48L	CGGTATC	Bogota	XLR_926R_v2bBar48L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTATCCCGTCAATTCMTTTRAGT
B7	v2bBar166L	TGACGAC	Kinshasa	XLR_926R_v2bBar166L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACGACCCGTCAATTCMTTTRAGT
B8	v2bBar613L	ACAAGGC	Brazzaville	XLR_926R_v2bBar613L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACAAGGCCCGTCAATTCMTTTRAGT
B9	v2bBar560L	AGACCTC	Zagreb	XLR_926R_v2bBar560L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACCTCCCGTCAATTCMTTTRAGT
B10	v2bBar741L	ATACCAC	Havana	XLR_926R_v2bBar741L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGATACCACCCGTCAATTCMTTTRAGT
B11	v2bBar228L	TCGCGGC	Nicosia	XLR_926R_v2bBar228L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCGGCCCGTCAATTCMTTTRAGT
B12	v2bBar807L	ATCTTAC	Prague	XLR_926R_v2bBar807L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGATCTTACCCGTCAATTCMTTTRAGT
C1	v2bBar1273L	AACCAGC	Copenhagen	XLR_926R_v2bBar1273L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCAGCCCGTCAATTCMTTTRAGT
C2	v2bBar441L	TTCGAGC	Djibouti	XLR_926R_v2bBar441L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGAGCCCGTCAATTCMTTTRAGT
C3	v2bBar1174L	AAGGTGC	Quito	XLR_926R_v2bBar1174L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGTGCCCGTCAATTCMTTTRAGT
C4	v2bBar209L	TCTTGGC	Cairo	XLR_926R_v2bBar209L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTGGCCCGTCAATTCMTTTRAGT
C5	v2bBar153L	TAATCTC	Suva	XLR_926R_v2bBar153L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTAATCTCCCGTCAATTCMTTTRAGT
C6	v2bBar213L	TCACCTC	Helsinki	XLR_926R_v2bBar213L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACCTCCCGTCAATTCMTTTRAGT
C7	v2bBar298L	TCCGCTC	Paris	XLR_926R_v2bBar298L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCGCTCCCGTCAATTCMTTTRAGT
C8	v2bBar146L	TATTGAC	Berlin	XLR_926R_v2bBar146L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTGACCCGTCAATTCMTTTRAGT
C9	v2bBar554L	AGTCGAC	Accra	XLR_926R_v2bBar554L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCGACCCGTCAATTCMTTTRAGT
C10	v2bBar646L	ACGGCTC	Athens	XLR_926R_v2bBar646L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGGCTCCCGTCAATTCMTTTRAGT
C11	v2bBar158L	TGCGTTC	Guatemala	XLR_926R_v2bBar158L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGTTCCCGTCAATTCMTTTRAGT
C12	v2bBar207L	TCTCGAC	Conakry	XLR_926R_v2bBar207L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCGACCCGTCAATTCMTTTRAGT
D1	v2bBar77L	CCAGGAC	Bissau	XLR_926R_v2bBar77L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCAGGACCCGTCAATTCMTTTRAGT
D2	v2bBar601L	ACTCCTC	Budapest	XLR_926R_v2bBar601L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCCTCCCGTCAATTCMTTTRAGT
D3	v2bBar481L	TTCCTGC	Jakarta	XLR_926R_v2bBar481L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCCCGTCAATTCMTTTRAGT
D4	v2bBar419L	TTCATAC	Tehran	XLR_926R_v2bBar419L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCATACCCGTCAATTCMTTTRAGT
D5	v2bBar26L	CGTCGTC	Baghdad	XLR_926R_v2bBar26L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTCGTCCCGTCAATTCMTTTRAGT
D6	v2bBar1172L	AAGGCAC	Dublin	XLR_926R_v2bBar1172L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCACCCGTCAATTCMTTTRAGT
D7	v2bBar1210L	AACAACTC	Jerusalem	XLR_926R_v2bBar1210L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACAACTCCCGTCAATTCMTTTRAGT
D8	v2bBar606L	ACACGGAC	Rome	XLR_926R_v2bBar606L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACACGGAACCCGTCAATTCMTTTRAGT
D9	v2bBar159L	TGCCGAAC	Kingston	XLR_926R_v2bBar159L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCCGAACCCGTCAATTCMTTTRAGT
D10	v2bBar147L	TATTCGTC	Tokyo	XLR_926R_v2bBar147L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTCGTCCCGTCAATTCMTTTRAGT
D11	v2bBar141L	TAGGAATC	Amman	XLR_926R_v2bBar141L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGGAATCCCGTCAATTCMTTTRAGT
D12	v2bBar119L	CCGGCCAC	Nairobi	XLR_926R_v2bBar119L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGCCACCCGTCAATTCMTTTRAGT

E1	v2bBar1379L	AATGGTAC	Tarawa	XLR_926R_v2bBar1379L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAATGGTACCCGTCAATTCMTTTRAGT
E2	v2bBar208L	TCTCCGTC	Pyongyang	XLR_926R_v2bBar208L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCCGTCCCGTCAATTCMTTTRAGT
E3	v2bBar1267L	AACCTGGC	Seoul	XLR_926R_v2bBar1267L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCTGGCCCGTCAATTCMTTTRAGT
E4	v2bBar637L	ACGAAGTC	Bishkek	XLR_926R_v2bBar637L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAAGTCCCGTCAATTCMTTTRAGT
E5	v2bBar435L	TTCGTGGC	Riga	XLR_926R_v2bBar435L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGGCCCGTCAATTCMTTTRAGT
E6	v2bBar1202L	AACACAAC	Beirut	XLR_926R_v2bBar1202L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACACAACCCGTCAATTCMTTTRAGT
E7	v2bBar413L	TTCTTGAC	Maseru	XLR_926R_v2bBar413L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTTGACCCGTCAATTCMTTTRAGT
E8	v2bBar289L	TCCAAGTC	Monrovia	XLR_926R_v2bBar289L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAAGTCCCGTCAATTCMTTTRAGT
E9	v2bBar433L	TTCGCGAC	Tripoli	XLR_926R_v2bBar433L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGCGACCCGTCAATTCMTTTRAGT
E10	v2bBar121L	CCGGTCGC	Vaduz	XLR_926R_v2bBar121L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGTCGCCCGTCAATTCMTTTRAGT
E11	v2bBar669L	ACCTGAAC	Vilnius	XLR_926R_v2bBar669L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTGAACCCGTCAATTCMTTTRAGT
E12	v2bBar1156L	AAGAGTTC	Luxembourg	XLR_926R_v2bBar1156L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGTTCCCGTCAATTCMTTTRAGT
F1	v2bBar370L	TTGACAAC	Bamako	XLR_926R_v2bBar370L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGACAACCCGTCAATTCMTTTRAGT
F2	v2bBar281L	TCCAGAAC	Valletta	XLR_926R_v2bBar281L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAGAACCCGTCAATTCMTTTRAGT
F3	v2bBar49L	CGGTCTTC	Kishinev	XLR_926R_v2bBar49L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTCTTCCCGTCAATTCMTTTRAGT
F4	v2bBar1173L	AAGGCCTC	Monaco	XLR_926R_v2bBar1173L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCCTCCCGTCAATTCMTTTRAGT
F5	v2bBar599L	ACTAATTC	Rabat	XLR_926R_v2bBar599L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTAATTCCCGTCAATTCMTTTRAGT
F6	v2bBar167L	TGACCGTC	Maputo	XLR_926R_v2bBar167L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACCGTCCCGTCAATTCMTTTRAGT
F7	v2bBar161L	TGTCGGAC	Kathmandu	XLR_926R_v2bBar161L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCGGACCCGTCAATTCMTTTRAGT
F8	v2bBar580L	AGGTTGTC	Amsterdam	XLR_926R_v2bBar580L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGTTGTCCCGTCAATTCMTTTRAGT
F9	v2bBar629L	ACGAGAAC	Wellington	XLR_926R_v2bBar629L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGAACCCGTCAATTCMTTTRAGT
F10	v2bBar184L	TGGTGAAC	Managua	XLR_926R_v2bBar184L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTGAACCCGTCAATTCMTTTRAGT
F11	v2bBar233L	TCGTTGTC	Abuja	XLR_926R_v2bBar233L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTTGTCCCGTCAATTCMTTTRAGT
F12	v2bBar364L	TTGTGTTC	Oslo	XLR_926R_v2bBar364L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGTGTTCCCGTCAATTCMTTTRAGT
G1	v2bBar78L	CCACGGTC	Muscat	XLR_926R_v2bBar78L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCACGGTCCCGTCAATTCMTTTRAGT
G2	v2bBar393L	TTGGAGGC	Islamabad	XLR_926R_v2bBar393L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCCCGTCAATTCMTTTRAGT
G3	v2bBar350L	TTATCGGC	Asuncion	XLR_926R_v2bBar350L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTATCGGCCCGTCAATTCMTTTRAGT
G4	v2bBar1164L	AAGAAGAC	Lima	XLR_926R_v2bBar1164L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAAGACCCGTCAATTCMTTTRAGT
G5	v2bBar1196L	AACTGTTC	Manila	XLR_926R_v2bBar1196L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACTGTTCCCGTCAATTCMTTTRAGT
G6	v2bBar411L	TTCTCAAC	Warsaw	XLR_926R_v2bBar411L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTCAACCCGTCAATTCMTTTRAGT
G7	v2bBar6L	CTTCCTTC	Lisbon	XLR_926R_v2bBar6L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTCCTTCCCGTCAATTCMTTTRAGT
G8	v2bBar1031L	ATTCGTAC	Doha	XLR_926R_v2bBar1031L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGATTCGTACCCGTCAATTCMTTTRAGT
G9	v2bBar76L	CCTTCCGC	Moscow	XLR_926R_v2bBar76L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTCCGCCCGTCAATTCMTTTRAGT
G10	v2bBar555L	AGTCCGTC	Kigali	XLR_926R_v2bBar555L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCCGTCCCGTCAATTCMTTTRAGT
G11	v2bBar378L	TTGAACTC	Riyadh	XLR_926R_v2bBar378L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAACTCCCGTCAATTCMTTTRAGT
G12	v2bBar1225L	AACGAGGC	Dakar	XLR_926R_v2bBar1225L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGAGGCCCGTCAATTCMTTTRAGT
H1	v2bBar99L	CCGTTCAC	Belgrade	XLR_926R_v2bBar99L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGTTCACCCGTCAATTCMTTTRAGT
H2	v2bBar236L	TCGAGGAAC	Singapore	XLR_926R_v2bBar236L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGAGGAACCCGTCAATTCMTTTRAGT
H3	v2bBar731L	ACCGGAAGC	Bratislava	XLR_926R_v2bBar731L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGGAAGCCCGTCAATTCMTTTRAGT
H4	v2bBar628L	ACGTTCCAC	Mogadishu	XLR_926R_v2bBar628L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGTTCCACCCGTCAATTCMTTTRAGT
H5	v2bBar1250L	AACGGAGTC	Pretoria	XLR_926R_v2bBar1250L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGGAGTCCCGTCAATTCMTTTRAGT
H6	v2bBar438L	TTCGTTATC	Madrid	XLR_926R_v2bBar438L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTTATCCCGTCAATTCMTTTRAGT
H7	v2bBar693L	ACCGTAATC	Colombo	XLR_926R_v2bBar693L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGTAATCCCGTCAATTCMTTTRAGT
H8	v2bBar672L	ACCTTGGTC	Khartoum	XLR_926R_v2bBar672L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTTGGTCCCGTCAATTCMTTTRAGT
H9	v2bBar355L	TTAAGATTC	Stockholm	XLR_926R_v2bBar355L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTAAGATTCCCGTCAATTCMTTTRAGT
H10	v2bBar187L	TGGTTGGTC	Bern	XLR_926R_v2bBar187L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTTGGTCCCGTCAATTCMTTTRAGT
H11	v2bBar162L	TGTCCGGTC	Damascus	XLR_926R_v2bBar162L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCCGGTCCCGTCAATTCMTTTRAGT
H12	v2bBar1292L	AACCGTGTC	Taipei	XLR_926R_v2bBar1292L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCGTGTCCCGTCAATTCMTTTRAGT

JCVI primers:

Please note that the 27F (V1-> V3 region) and the 357F (V3->V5) primers consist of 'B' Adapter and the respective 16S primer. Therefore they are the same sequence for each respective V region (you only need to order one 'F' primer to pair with each individual 'R' primer). Each 'R' primer (534 R (V1->V3) and 926R (V3->V5) listed contains a unique barcodes. The barcodes have also been posted in a tab-delimited file on the DACC site (jcvi_barcodes_v1v3_v3v5_101409). Thanks to Kelvin Li for work on the barcode design.

Barcoded oligos for V3->V1 directional sequencing. The 534R primer includes the 454 'A' adapter, barcode and 16S primer. The 27F primer consists of the 'B' adapter and 16S primer.

10/14/09

oligo	27F	534R
27F/534R_000	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGTGACTGATTACCGCGGCTGCTGG
27F/534R_001	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGATACGACATTACCGCGGCTGCTGG
27F/534R_002	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTCGAGCGATTACCGCGGCTGCTGG
27F/534R_003	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTATCGAGAGATTACCGCGGCTGCTGG
27F/534R_004	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACTATCGATTACCGCGGCTGCTGG
27F/534R_005	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGATATATAGCGATTACCGCGGCTGCTGG
27F/534R_006	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGTACGATGATTACCGCGGCTGCTGG
27F/534R_007	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCGCTAGCATTACCGCGGCTGCTGG
27F/534R_008	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGTCTATCATTACCGCGGCTGCTGG
27F/534R_009	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGCACTACTATTACCGCGGCTGCTGG
27F/534R_010	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCGTACGTGATTACCGCGGCTGCTGG
27F/534R_011	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCGTGTACATTACCGCGGCTGCTGG
27F/534R_012	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTACAGTAGATTACCGCGGCTGCTGG
27F/534R_013	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTATACATCATTACCGCGGCTGCTGG
27F/534R_014	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGCGAGTGATTACCGCGGCTGCTGG
27F/534R_015	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCACACGTATTACCGCGGCTGCTGG
27F/534R_016	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTATATCGATTACCGCGGCTGCTGG
27F/534R_017	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGATCGTATATTACCGCGGCTGCTGG
27F/534R_018	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCATATACGATTACCGCGGCTGCTGG
27F/534R_019	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGAGACACTGATTACCGCGGCTGCTGG
27F/534R_020	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTGCGCTAGATTACCGCGGCTGCTGG
27F/534R_021	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTCACGCGATTACCGCGGCTGCTGG
27F/534R_022	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCACTCACTAGATTACCGCGGCTGCTGG
27F/534R_023	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTACAGCTCATTACCGCGGCTGCTGG
27F/534R_024	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGTCTGACGATTACCGCGGCTGCTGG
27F/534R_025	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCACACTCGCGATTACCGCGGCTGCTGG
27F/534R_026	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCTCGTCTGATTACCGCGGCTGCTGG
27F/534R_027	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCGACGTCTATTACCGCGGCTGCTGG
27F/534R_028	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTCACGACGCATTACCGCGGCTGCTGG
27F/534R_029	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGATGTCAGTCGATTACCGCGGCTGCTGG
27F/534R_030	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCATAGACACATTACCGCGGCTGCTGG
27F/534R_031	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGATGTACGTGATTACCGCGGCTGCTGG
27F/534R_032	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGATAGCGTGAGATTACCGCGGCTGCTGG
27F/534R_033	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGTAGCTCATTACCGCGGCTGCTGG
27F/534R_034	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGATATCGTCATTACCGCGGCTGCTGG
27F/534R_035	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTACATGATTACCGCGGCTGCTGG
27F/534R_036	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGATACGCAGATTACCGCGGCTGCTGG
27F/534R_037	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATGACTGAGATTACCGCGGCTGCTGG
27F/534R_038	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTGACTAGATTACCGCGGCTGCTGG
27F/534R_039	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCTACGCGCATTACCGCGGCTGCTGG
27F/534R_040	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTGAGTGAGATTACCGCGGCTGCTGG
27F/534R_041	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACGCTACTATTACCGCGGCTGCTGG
27F/534R_042	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCTAGACGATTACCGCGGCTGCTGG
27F/534R_043	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTACTATCATTACCGCGGCTGCTGG
27F/534R_044	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACAGTGAGCATTACCGCGGCTGCTGG
27F/534R_045	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGATCGATAGACATTACCGCGGCTGCTGG
27F/534R_046	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCAGAGACGATTACCGCGGCTGCTGG
27F/534R_047	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGACGTGCGCATTACCGCGGCTGCTGG
27F/534R_048	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCACTCTATCGATTACCGCGGCTGCTGG
27F/534R_049	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCTCAGACGATTACCGCGGCTGCTGG
27F/534R_050	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGATGCTCGATTACCGCGGCTGCTGG
27F/534R_051	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTAGCACGATTACCGCGGCTGCTGG
27F/534R_052	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCGCATCGATTACCGCGGCTGCTGG
27F/534R_053	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGACGCTCTATTACCGCGGCTGCTGG
27F/534R_054	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGACGCACAGATTACCGCGGCTGCTGG
27F/534R_055	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGTAGACTATTACCGCGGCTGCTGG
27F/534R_056	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTGTACTGCATTACCGCGGCTGCTGG
27F/534R_057	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAGACTCAGATTACCGCGGCTGCTGG
27F/534R_058	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCGCTGTAGATTACCGCGGCTGCTGG
27F/534R_059	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCGAGCAGATTACCGCGGCTGCTGG
27F/534R_060	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGAGTCGAGTATTACCGCGGCTGCTGG
27F/534R_061	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGCTAGTATATTACCGCGGCTGCTGG
27F/534R_062	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGAGTCGCGCATTACCGCGGCTGCTGG
27F/534R_063	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTCGTCAGTCATTACCGCGGCTGCTGG
27F/534R_064	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCTAGTCTATTACCGCGGCTGCTGG
27F/534R_065	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTACTCACTATTACCGCGGCTGCTGG
27F/534R_066	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTATGTACAGATTACCGCGGCTGCTGG
27F/534R_067	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTGATAGTATTACCGCGGCTGCTGG
27F/534R_068	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTGTACGAGATTACCGCGGCTGCTGG
27F/534R_069	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGATCTAGTCACATTACCGCGGCTGCTGG
27F/534R_070	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATGAGAGTGATTACCGCGGCTGCTGG
27F/534R_071	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACTGCTCAGATTACCGCGGCTGCTGG
27F/534R_072	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTATACTACTATTACCGCGGCTGCTGG
27F/534R_073	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACAGTGCTACATTACCGCGGCTGCTGG
27F/534R_074	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTATAGAGCATTACCGCGGCTGCTGG
27F/534R_075	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACATCGCGAGATTACCGCGGCTGCTGG
27F/534R_076	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGATGACGACTCATTACCGCGGCTGCTGG
27F/534R_077	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTATGTACTATTACCGCGGCTGCTGG
27F/534R_078	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCGAGATACATTACCGCGGCTGCTGG
27F/534R_079	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTACAGTGTGATTACCGCGGCTGCTGG
27F/534R_080	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCACGATGATTACCGCGGCTGCTGG
27F/534R_081	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCTACGTCGATTACCGCGGCTGCTGG
27F/534R_082	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTCACGTGCATTACCGCGGCTGCTGG
27F/534R_083	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCAGCACTCGATTACCGCGGCTGCTGG
27F/534R_084	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGACTGCGATTACCGCGGCTGCTGG
27F/534R_085	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTATAGTACATTACCGCGGCTGCTGG

27F/534R_085	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGACTATAGTACATTACCGCGGCTGCTGG
27F/534R_086	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGATATGTCGTGATTACCGCGGCTGCTGG
27F/534R_087	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTACTAGATGTATTACCGCGGCTGCTGG
27F/534R_088	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCTGTCTCATTACCGCGGCTGCTGG
27F/534R_089	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGACGATCATTACCGCGGCTGCTGG
27F/534R_090	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCGTGTCACATTACCGCGGCTGCTGG
27F/534R_091	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACGTATCTATTACCGCGGCTGCTGG
27F/534R_092	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGAGACTAGATTACCGCGGCTGCTGG
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27F/534R_094	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGCATATACACGATTACCGCGGCTGCTGG
27F/534R_095	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGACTCGATATTACCGCGGCTGCTGG
27F/534R_096	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGACACAGTCGTATTACCGCGGCTGCTGG
27F/534R_097	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTACACGTCATTACCGCGGCTGCTGG
27F/534R_098	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGCGATGACATTACCGCGGCTGCTGG
27F/534R_099	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGTATAGCATTACCGCGGCTGCTGG
27F/534R_100	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGCGACGCGATGATTACCGCGGCTGCTGG
27F/534R_101	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCACTGCGATTACCGCGGCTGCTGG
27F/534R_102	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGACTGTGACTCATTACCGCGGCTGCTGG
27F/534R_103	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTGATCGACAGATTACCGCGGCTGCTGG
27F/534R_104	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGTATCGATATTACCGCGGCTGCTGG
27F/534R_105	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGACGCATCATTACCGCGGCTGCTGG
27F/534R_106	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCGATCTCATTACCGCGGCTGCTGG
27F/534R_107	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCAGTCGTATTACCGCGGCTGCTGG
27F/534R_108	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGACAGCTATAGATTACCGCGGCTGCTGG
27F/534R_109	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGCACTCTCGACATTACCGCGGCTGCTGG
27F/534R_110	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTACTCTGATTACCGCGGCTGCTGG
27F/534R_111	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGATACGAGAGCATTACCGCGGCTGCTGG
27F/534R_112	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGACGTCGCAGTATTACCGCGGCTGCTGG
27F/534R_113	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGATGTCGTACTATTACCGCGGCTGCTGG
27F/534R_114	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGCATGTACGTCATTACCGCGGCTGCTGG
27F/534R_115	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGCACGCGTCTCATTACCGCGGCTGCTGG
27F/534R_116	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCTATCGAGATTACCGCGGCTGCTGG
27F/534R_117	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGACGACACGAGATTACCGCGGCTGCTGG
27F/534R_118	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGCGTCGCATTACCGCGGCTGCTGG
27F/534R_119	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTCAGCTCGTGATTACCGCGGCTGCTGG
27F/534R_120	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACGACTGTATTACCGCGGCTGCTGG
27F/534R_121	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGCACAGTATACATTACCGCGGCTGCTGG
27F/534R_122	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGACGTCATCTGATTACCGCGGCTGCTGG
27F/534R_123	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACTGTGAGATTACCGCGGCTGCTGG
27F/534R_124	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGTACACATCACATTACCGCGGCTGCTGG
27F/534R_125	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG CCATCTCATCCCTGCGTGTCTCCGACTCAGATAGCTCGACATTACCGCGGCTGCTGG

Barcoded oligos for V5->V3 directional sequencing. The 926R primer includes the 454 'A' adapter, barcode and 16S primer. The 357F primer consists of the 'B' adapter and 16S primer.

10/14/09

oligo	357F	926R
357F/926R_000	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCATAGACAGCCGTCAATTCMTTTRAGT
357F/926R_001	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCACTACGCCGTCAATTCMTTTRAGT
357F/926R_002	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCGTCAGTACCGTCAATTCMTTTRAGT
357F/926R_003	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGTACGTAGCCGTCAATTCMTTTRAGT
357F/926R_004	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCTCTAGACCGTCAATTCMTTTRAGT
357F/926R_005	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGATACACAGCCGTCAATTCMTTTRAGT
357F/926R_006	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCTAGTCTCCGTCAATTCMTTTRAGT
357F/926R_007	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCAGTGTACCGTCAATTCMTTTRAGT
357F/926R_008	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTACGTCTGTCCGTCAATTCMTTTRAGT
357F/926R_009	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGACTACGAGCCGTCAATTCMTTTRAGT
357F/926R_010	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGCACACTACCGTCAATTCMTTTRAGT
357F/926R_011	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACGAGTACACCGTCAATTCMTTTRAGT
357F/926R_012	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCTACTGAGCCGTCAATTCMTTTRAGT
357F/926R_013	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCACGATAGCGCCGTCAATTCMTTTRAGT
357F/926R_014	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATATCGACACCGTCAATTCMTTTRAGT
357F/926R_015	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTACTACATCCGTCAATTCMTTTRAGT
357F/926R_016	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGAGCGCGAGCCGTCAATTCMTTTRAGT
357F/926R_017	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTAGATCGACCGTCAATTCMTTTRAGT
357F/926R_018	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGATGACGCGCCGTCAATTCMTTTRAGT
357F/926R_019	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCTCGAGACCGTCAATTCMTTTRAGT
357F/926R_020	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGTGTAGCGCCGTCAATTCMTTTRAGT
357F/926R_021	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACGACGTACCGTCAATTCMTTTRAGT
357F/926R_022	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTAGAGTAGCCGTCAATTCMTTTRAGT
357F/926R_023	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGTACTCACCGTCAATTCMTTTRAGT
357F/926R_024	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCACACGTCCGTCAATTCMTTTRAGT
357F/926R_025	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGTATGAGCCGTCAATTCMTTTRAGT
357F/926R_026	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTATACGCTCCGTCAATTCMTTTRAGT
357F/926R_027	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGTGAGACGCCGTCAATTCMTTTRAGT
357F/926R_028	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAGTATGCGCCGTCAATTCMTTTRAGT
357F/926R_029	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTACAGCGTCCGTCAATTCMTTTRAGT
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357F/926R_035	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGAGTGCGCCGTCAATTCMTTTRAGT
357F/926R_036	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGAGCACGTCCGTCAATTCMTTTRAGT
357F/926R_037	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACATATCGCCGTCAATTCMTTTRAGT
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357F/926R_124	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCAGATGACGCCGTCAATTCMTTTRAGT
357F/926R_125	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTGTGAGTGCCGTCAATTCMTTTRAGT