GENERATION OF TILED VIRUS AMPLICONS FOR RSV B SEQUENCING

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Adapted from: Quick, J. *et al*. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nature Protocols 12 (6), 1261-1276.* https://www.nature.com/nprot/journal/v12/n6/abs/nprot.2017.066.html

Notes: The panel has been optimized using RSV B positive samples circulating in Europe in 2025-2026. Processing samples from previous years or from regions outside of Europe might require further adjustments.

Reference sequence is B/Australia/VIC-RCH056/2019 which is available at OP975389 and also deposited under accession number EPI_ISL_1653999 in GISAID

MATERIAL

- Nucleospin 8 Virus Core Kit (Macherey Nagel)
- Lunascript RT Supermix 5X kit (NEB)
- Q5 High-Fidelity PCR Kit (NEB) and dNTPs 10 mM
- Primers purchased at 200 µM from Sigma-Aldrich
- AMPure XP Bead-Based Reagent Protocol for PCR Purification (Beckman coulter)

Equivalent kit and reagents can be used.

PROTOCOL

1.1. GENERATION OF TILED AMPLICONS

Reverse Transcription :

Composition	Reaction	Final Concentration
Lunascript Supermix 5X	2 µL	1X
RNA*	8 µL	-

*RNA was extracted from 100 µL of respiratory sample and eluted in 100 µL.

LUNA RT	
T°	**Time**
25°C	2 min
55°C	10 min
95°C	1 min
4°C	∞

Amplicon generation by PCR in 2 independent reactions:

Primer pools.
Primers were purchased at 200 µM from Sigma-Aldrich
Prepare two 50X pre-mix (primers pools) with concentrations indicated in Annex 1

Composition	Reaction RSVB_pool 1	Reaction RSVB_pool 2	Final Concentration in 25 µL
5X Q5 reaction buffer	5 µL	5 µL	1X
10mM dNTP	0,5 µL	0,5 µL	200 µM
50X Primer pool 1 or primer pool 2 (see concentration in Annex 1)	0,5 µL	0,5 µL	See annex 1
cDNA	2,5 µL	2,5 µL	-
Q5 polymerase (2000 U/mL)	0,25 µL	0,25 µL	20 U
H2O RNase DNase free	For final volume 25 µL	For final volume 25 µL	

PCR (Q5)		
T°	**Time**	**Nb Cycle**
98°C	30 s	1
98°C	15 s	35
65°C	5 min	
4°C	∞	1

Check amplification on agarose gel:
Run 5 µL of each reaction on a 1% agarose gel. Each should produce a visible ~400 bp band and the negative control (no cDNA) should be clean.

1.2. PURIFICATION OF AMPLICONS USING MAGNETIC BEADS

Two purification techniques are described, one manual, and one automated using the King Fisher (Thermo Scientific).
Alternative purification techniques can be used but should be validated by the user.

1.2.A. MANUAL PURIFICATION OF AMPLICONS

1. Vortex Agencourt AMPureXP beads bottle (placed at room temperature) to resuspend magnetic particles
2. For each sample, combine the PCR products from pool 1 and pool 2 PCRs (~50 µL final volume)
3. Add 72 µL (1,44X) of RNAclean XP beads to PCR; mix by pipetting 10 times
4. Incubate samples 10 minutes at room temperature.

5. Place the tubes on an appropriate magnetic rack to separate beads from the supernatant.
6. When the solution is clear (about 5-10 minutes), discard the supernatant without disturbing the beads.
7. Add 200 µL of freshly prepared 70% ethanol to the tube while in the magnetic rack. Incubate at room temperature for 1 min, and then carefully remove and discard the supernatant
8. Repeat Step 7 once for a total of 2 washes.
9. Completely remove the residual ethanol, and air-dry beads on the magnetic rack (5-10 minutes).
10. Add 40 µL of RNase Dnase free H20 on the beads and remove the plate from the magnetic rack.
11. Mix well by pipetting up and down and put the tube in the magnetic rack until the solution is clear.
12. Transfer the 40 µL to a clean plate.
13. Quantify the DNA concentration using the Qubit High Sensitivity DNA kit (or equivalent) from 1 µL of each product. Expected range > 80 ng/µL DNA.
14. Proceed with NGS library construction or other downstream application. Alternatively, the samples can be stored at –20°C.

1.2.B. PURIFICATION OF AMPLICONS USING KING FISHER

1. Vortex Beckman Coulter AMPureXP beads bottle (placed at room temperature) to resuspend magnetic particles
2. For each sample, combine the PCR products from pool 1 and pool 2 PCRs (~50 µL final volume) and transfer the whole PCR products to a DeepWell plate
3. Add 108 µL of AMPureXP beads to the PCR products in a DeepWell plate
4. Fill a U-bottom plate with 150 µL of 70% ethanol for washing
5. Fill a U-bottom plate with 50 µL of Ambion water for elution
6. Start the protocol on the robot Kingfisher and follow the instructions.

Purification Protocol Steps for KingFisher Automation
Step 1 Pick up tips from position 1.
Step 2 – Binding (Position 2 - DW sample) • Pre-collect beads: ON • Release beads: ON • Mix for 6 minutes at medium speed • Collect beads: 10 cycles, 5 seconds each
Step 3 – Wash (Position 3 - 96-well plate with 150 µL of 70% ethanol) • Mix for 15 seconds at medium speed *(no bead release / pre-collect / collect)*
Step 4 – Dry (Position 3 - outside well) Dry outside the well for 2 minutes
Step 5 – Elution (Position 4 - 96-well plate with 50 µL NF-water) • Release beads: ON • Mix for 4 minutes at medium speed

Step 6 – Bead collection (Position 4) • Pre-collect beads: ON • Mix for 2 minutes at slow speed • Collect beads: 3 cycles, 30 seconds each
Step 7 Leave tips in wash plate at position 3
Step 8 - **Final step** Hold at **4°C indefinitely (∞)**

The panel is compatible with Oxford Nanopore Technologies and Illumina sequencing. If using a combined paired read length shorter than the amplicons size (~400nt) with DNA fragmentation or tagmentation, adapt the bioinformatic pipeline for primer trimming. 150 nt long reads are the minimum recommended.

2. ANNEX 1

Primers are ordered dry and resuspended at a concentration of 200 μM.
Two 50X concentrated primer pools are prepared (Pool 1 and Pool 2), aliquoted and frozen at -20°C until use (« pre-Mix » in tables below). Aliquots are freeze/thawed a maximum of 5 times to prepare PCR-Mixes.

Primer Version 1.5	Sequence	Pool	Conc. Final in Mix (nM)	Conc. Final PRE-MIX (50X) (µM)
RSVB_1_LEFT	CAGAAATGGGGTGCAATTCACTG	1	15	0,75
RSVB_1_RIGHT	TCATGAATGGAGATCAAGCCCAA	1	15	0,75
RSVB_3_LEFT	ACC ATG AGC ACT ACA AAM GAC AA	1	30	1,50
RSVB_3_RIGHT	GTT GGA AGT TAC GGG TTG AGG T	1	30	1,50
RSVB_5_LEFT	TGA CAC TCC CAA TTA TGA TGT GCA	1	30	1,50
RSVB_5_RIGHT	TTA CAA GGG CAG CTA TAC ACA GT	1	30	1,50
RSVB_7_LEFT	AGC ACA ATC ATC CAC AAG AGG G	1	224	11,22
RSVB_7_RIGHT	GAG TTG CTC TGC ATA TGC TTT GG	1	224	11,22
RSVB_9_LEFT	TCC AAA GAT CCT AAG AAG AAA GAT AGC A	1	11	0,56
RSVB_9_RIGHT	TAT TCC GTC ACG AGC CGA AGT R	1	11	0,56
RSVB_11_LEFT	TTG GAA GAC AAC GAT AGC GAC AA	1	15	0,75
RSVB_11_RIGHT	GCT TTA CTA GTA TGT TGA TGC TTG CA	1	15	0,75
RSVB_13_LEFT	TGC AGT TTA ACA TGC TTR AAA GTA AAA AG	1	30	1,50
RSVB_13_RIGHT	GTG GTT TGA TTG AAA AAC GTG TAG C	1	30	1,50
RSVB_15_LEFT	CCT AGA GTG CGA ATA GGT AAA TAA AAC AAG	1	150	7,48
RSVB_15_RIGHT	TGT GAT GCC ATG ACT CTG TGA G	1	150	7,48
RSVB_17_LEFT	GAA AAG ACC TGG GAT ACT CTT AAT CAT CT	1	75	3,74
RSVB_17_RIGHT	GTG CTT GGC TTG TTG TTC TGT G	1	75	3,74
RSVB_19_LEFT	AGA AAC CAC CAT TAA CCC AAC AAA AA	1	15	0,75
RSVB_19_RIGHT	GCA AGA GTT AGG AAG ATT GCA CTT G	1	15	0,75
RSVB_21_LEFT	CAG AGG TTA CTT GAG TGC TTT AAG AAC	1	150	7,48
RSVB_21_RIGHT	CTT TGT TCG TAA GCT GCA AAG CA	1	150	7,48
RSVB_23_LEFT	AGC ACT TAC ATG TTA ACA AAC AGT GAG	1	30	1,50
RSVB_23_RIGHT	ACA AAG GCT GAC TTC ACT TGG T	1	30	1,50
RSVB_25_LEFT	GCT GTG ATT ATG TGT CAA ACA AAG GA	1	30	1,50
RSVB_25_RIGHT	TGC AAT ATT ATT GAT TCC ACT TAG TTG GTC	1	30	1,50
RSVB_27_LEFT	TAC ATC AAC ATC ACA DCA CAG GC	1	150	7,48
RSVB_27_RIGHT	CTC TCT AGC ACT CCA ACT ATA CCA AG	1	150	7,48
RSVB_29_LEFT	CTT AAA CGA TTA CCA GCA GAC GTR	1	15	0,75
RSVB_29_RIGHT	GGT TGG ATG ATA TAG AAT CGT TAT GAT CAA G	1	15	0,75
RSVB_31_LEFT	ACG GCC CTT ATC TTA AAA ATG ATT ATA CC	1	15	0,75
RSVB_31_RIGHT	ACC AGT GTA TTA ACC ATG ATG GAG G	1	15	0,75
RSVB_33_LEFT	AGA TAA TCA AAC TTT GAG TGG TTT TCA GT	1	15	0,75
RSVB_33_RIGHT	ACT CTT GAT AGT AGA TCC TTT TGA GCC	1	15	0,75
RSVB_35_LEFT	ACT TGA GTG AGC TAT ATT TTC TCT TCA GA	1	15	0,75
RSVB_35_RIGHT	ACT AGT CCA TAT TAG ATC TTT TGG AGG AG	1	15	0,75
RSVB_37_LEFT	AGA AAG AGA GCT CAG TGT GGG T	1	15	0,75
RSVB_37_RIGHT	TCC TTT ATA AAA GGA GGT GCA TGT CT	1	15	0,75
RSVB_39_LEFT	CAC AGC TCT GAT AAA TGG TGA TAA TCA G	1	15	0,75
RSVB_39_RIGHT	TTG CAG AGC AAT TTG ATT GTA TAA CCA	1	15	0,75
RSVB_41_LEFT	GAG AAC TCC AGA CTT CCT TAC AGA R	1	75	3,74
RSVB_41_RIGHT	GCG CAC TTT TAG AAA ATA TTT TGT TTG GA	1	75	3,74
RSVB_43_LEFT	TGT AAG AGA AAG ATC TTG GTC ATT ATC CA	1	15	0,75
RSVB_43_RIGHT	ACT GTT AAC CGG TGT AAA TAA TTK ACA C	1	15	0,75
RSVB_45_LEFT	CTT AGC TTG ATG TCA GTW GTG GAA C	1	15	0,75
RSVB_45_RIGHT	TGA TCA GTT ATA TAC CCY TCT CCC C	1	15	0,75
RSVB_47_LEFT	GCT ACT GGA AAT CTA TGT CTA AAG TTT TCC	1	37	1,87
RSVB_47_RIGHT	GGG GTT GGG TGA TAT AGT TTG TTR TAA TT	1	37	1,87
RSVB_49_LEFT	GTG ATA GAC AGG ATT ATA GAT CAT TCA GGT	1	15	0,75
RSVB_49_RIGHT	GTT GCA TCT GTA GCA GGA ATG GT	1	15	0,75
RSVB_51_LEFT	ACA GCC AAT TGG AGT AAA ATY ATA ATT GA	1	15	0,75
RSVB_51_RIGHT	TCT CCA TTA ACT ACA CTC TTC AAT TTT GAC	1	15	0,75
RSVB_53_LEFT	AAC AAG CTT ATA AAC CAC AAG CAT ATG A	1	150	7,48
RSVB_53_RIGHT	TGT CTC GTT GTG TTG TAA ATG CAC	1	150	7,48

RSVB_2_LEFT	AGT GAA ATC TAA CTT TAC AAC AAT GCC A	2	72	3,61	10,83
RSVB_2_RIGHT	GGT GAG AGA TGT TAT TAT TGA ATC CAT CG	2	72	3,61	10,83
RSVB_4_LEFT	TGG CGG GTT TCT AGA ATG TAT TGG	2	29	1,44	4,33
RSVB_4_RIGHT	TGA TTT GCA TCT TCA GTG ATT AAT AGC A	2	29	1,44	4,33
RSVB_6_LEFT	AAG CTT GAC ATC AGA AAT ACA AGT CAA	2	14	0,72	2,17
RSVB_6_RIGHT	CTT GTC CTG AAC CAT AGG CAT TCA	2	14	0,72	2,17
RSVB_8_LEFT	AGC AGG TCT AGG CAT AAT GGG A	2	14	0,72	2,17
RSVB_8_RIGHT	TGT TGG TGC CAG ATG TTA TCG G	2	14	0,72	2,17
RSVB_10_LEFT	TGA CCA AAC AAA TGA TAA CAT TAC AGC A	2	14	0,72	2,17
RSVB_10_RIGHT	GGT GTC TGT TTT GTT GAT GYT GTT	2	14	0,72	2,17
RSVB_12_LEFT	CAG CAG CTG TCC AGT ACA ATG T	2	14	0,72	2,17
RSVB_12_RIGHT	TTC ACA TAG AGC AAT GAT CTC ATG AGT	2	14	0,72	2,17
RSVB_14_LEFT	GGA GCA TTC AAG TAT ATC AAG CCA C	2	29	1,44	4,33
RSVB_14_RIGHT	GCT TGT GAA TTC TAT TGT RAT GGA TGT	2	29	1,44	4,33
RSVB_16_LEFT	ACA GAT GTA TCA AAT CGA CAC ATA GTG	2	14	0,72	2,17
RSVB_16_RIGHT	GCA GAG ATG ATG AAT ATT ATG GCT GC	2	14	0,72	2,17
RSVB_18_LEFT	ACC CAA TAC AAA ATC AGA AAC ACA CC	2	14	0,72	2,17
RSVB_18_RIGHT	ACA GTG GAT TGT GGG GTG CT	2	14	0,72	2,17
RSVB_20_LEFT	GTG ATC CCT CAA GCA AGA ACK A	2	72	3,61	10,83
RSVB_20_RIGHT	TGA TTG TAT AGT TCA TAT ACT GTG GTG CT	2	72	3,61	10,83
RSVB_22_LEFT	AGC AAG TGG TAT AGC TGT ATC CAA AG	2	144	7,22	21,67
RSVB_22_RIGHT	GCC TTA CTA TCT GAA CAT TGC TTG AC	2	144	7,22	21,67
RSVB_24_LEFT	TCC TTC TTT CCA CAA GCT GAC AC	2	14	0,72	2,17
RSVB_24_RIGHT	GAA GGA AAC ACT AGA GGG TCA TAG T	2	14	0,72	2,17
RSVB_26_LEFT	CAA AGT TTA GCT TTT ATT CGT AGA TCY GA	2	14	0,72	2,17
RSVB_26_RIGHT	GTG TTG ATT GCG ATA TTT GAG GCT T	2	14	0,72	2,17
RSVB_28_LEFT	TGT TAA ACA AGA TAC TCA AGT CAA TGG AC	2	14	0,72	2,17
RSVB_28_RIGHT	TGG TTG ACT CTT TTG GGT TGC T	2	14	0,72	2,17
RSVB_30_LEFT	GCC ACT CAA CAA TTT CTC CAA CA	2	14	0,72	2,17
RSVB_30_RIGHT	AGT AAT GAC TGG AAA TAA GTT GGT TCT TC	2	14	0,72	2,17
RSVB_32_LEFT	GTG ATG AAA ACT CAG TAC TTA CAA CCA	2	29	1,44	4,33
RSVB_32_RIGHT	GCT GAT GTC TTT CCA TGT CAA AAA TTG	2	29	1,44	4,33
RSVB_34_LEFT	CTA TCA CAA TTA TTC CTT TAC GGA GAT TGT	2	14	0,72	2,17
RSVB_34_RIGHT	TCA TTG CAG TTA ATT CTT ACA GCA TCC	2	14	0,72	2,17
RSVB_36_LEFT	TTG CGG TTC TAT CGT GAG TTT CA	2	29	1,44	4,33
RSVB_36_RIGHT	CAG GGA AGA ATT GTA AAA TAT TTT CGG C	2	22	1,08	3,25
RSVB_36_RIGHT_bis	CAGGGAAGAATTGTAAAATATTTTCAGC	2	7	0,36	1,08
RSVB_38_LEFT	ACG AAC TGC ATG GRG TAC AAT C	2	14	0,72	2,17
RSVB_38_RIGHT	TCC CTT AAG CTT ATG GCC TAT GC	2	14	0,72	2,17
RSVB_40_LEFT	AGG TAG CTT AAC ACA GGA RTT AGA ATA C	2	108	5,42	16,25
RSVB_40_RIGHT	GAC ACA AGT CAA GAA TTT GTT CAG TCT	2	108	5,42	16,25
RSVB_42_LEFT	ATC CAC AGG CTT TAG GGT CTG A	2	14	0,72	2,17
RSVB_42_RIGHT	GGC TAT AGT GCT AGT TGT ATA TTT AAT RTC C	2	14	0,72	2,17
RSVB_44_LEFT	CAA AGA TGA ATT CAT GGA AGA ACT GAG T	2	14	0,72	2,17
RSVB_44_RIGHT	GCT TCA ACT TGA TGA TAT CRA CAT CTC C	2	14	0,72	2,17
RSVB_46_alt_LEFT	TTAGCTGGACATTGGATTCTGA	2	72	3,61	10,83
RSVB_46_RIGHT	CAACCCAAGGGCATACGGT	2	54	2,71	8,13
RSVB_46_RIGHT_bis	CAACCCAAGGGCATACAGT	2	18	0,90	2,71
RSVB_48_LEFT	CTTTTCAGACAACACTCATCTGCT	2	72	3,61	10,83
RSVB_48_RIGHT	CTGATCTTGCATCCTGTGGAACT	2	72	3,61	10,83
RSVB_50_LEFT	CCC AGT TGT ATA GCA TTC ATA GGT GA	2	29	1,44	4,33
RSVB_50_RIGHT	TTT GAA ATC AAT ATC ATC TTG AGC ATG GT	2	29	1,44	4,33
RSVB_52_LEFT	GAT GTT GTG CAA AAT GCT AAA TTG ATT CT	2	14	0,72	2,17
RSVB_52_RIGHT	CAC TAC CTG TTA TTT TAA TCA GCT TCT TGA	2	14	0,72	2,17

Sequence	Pool	Pool	Conc. Final in Mix (nM)	Conc. Final PRE-MIX (50X) (µM)
RSVB_2_LEFT	AGT GAA ATC TAA CTT TAC AAC AAT GCC A	2	72	3,61
RSVB_2_RIGHT	GGT GAG AGA TGT TAT TAT TGA ATC CAT CG	2	72	3,61
RSVB_4_LEFT	TGG CGG GTT TCT AGA ATG TAT TGG	2	29	1,44
RSVB_4_RIGHT	TGA TTT GCA TCT TCA GTG ATT AAT AGC A	2	29	1,44
RSVB_6_LEFT	AAG CTT GAC ATC AGA AAT ACA AGT CAA	2	14	0,72
RSVB_6_RIGHT	CTT GTC CTG AAC CAT AGG CAT TCA	2	14	0,72
RSVB_8_LEFT	AGC AGG TCT AGG CAT AAT GGG A	2	14	0,72
RSVB_8_RIGHT	TGT TGG TGC CAG ATG TTA TCG G	2	14	0,72
RSVB_10_LEFT	TGA CCA AAC AAA TGA TAA CAT TAC AGC A	2	14	0,72
RSVB_10_RIGHT	GGT GTC TGT TTT GTT GAT GYT GTT	2	14	0,72
RSVB_12_LEFT	CAG CAG CTG TCC AGT ACA ATG T	2	14	0,72
RSVB_12_RIGHT	TTC ACA TAG AGC AAT GAT CTC ATG AGT	2	14	0,72
RSVB_14_LEFT	GGA GCA TTC AAG TAT ATC AAG CCA C	2	29	1,44
RSVB_14_RIGHT	GCT TGT GAA TTC TAT TGT RAT GGA TGT	2	29	1,44
RSVB_16_LEFT	ACA GAT GTA TCA AAT CGA CAC ATA GTG	2	14	0,72
RSVB_16_RIGHT	GCA GAG ATG ATG AAT ATT ATG GCT GC	2	14	0,72
RSVB_18_LEFT	ACC CAA TAC AAA ATC AGA AAC ACA CC	2	14	0,72
RSVB_18_RIGHT	ACA GTG GAT TGT GGG GTG CT	2	14	0,72
RSVB_20_LEFT	GTG ATC CCT CAA GCA AGA ACK A	2	72	3,61
RSVB_20_RIGHT	TGA TTG TAT AGT TCA TAT ACT GTG GTG CT	2	72	3,61
RSVB_22_LEFT	AGC AAG TGG TAT AGC TGT ATC CAA AG	2	144	7,22
RSVB_22_RIGHT	GCC TTA CTA TCT GAA CAT TGC TTG AC	2	144	7,22
RSVB_24_LEFT	TCC TTC TTT CCA CAA GCT GAC AC	2	14	0,72
RSVB_24_RIGHT	GAA GGA AAC ACT AGA GGG TCA TAG T	2	14	0,72
RSVB_26_LEFT	CAA AGT TTA GCT TTT ATT CGT AGA TCY GA	2	14	0,72
RSVB_26_RIGHT	GTG TTG ATT GCG ATA TTT GAG GCT T	2	14	0,72
RSVB_28_LEFT	TGT TAA ACA AGA TAC TCA AGT CAA TGG AC	2	14	0,72
RSVB_28_RIGHT	TGG TTG ACT CTT TTG GGT TGC T	2	14	0,72
RSVB_30_LEFT	GCC ACT CAA CAA TTT CTC CAA CA	2	14	0,72
RSVB_30_RIGHT	AGT AAT GAC TGG AAA TAA GTT GGT TCT TC	2	14	0,72
RSVB_32_LEFT	GTG ATG AAA ACT CAG TAC TTA CAA CCA	2	29	1,44
RSVB_32_RIGHT	GCT GAT GTC TTT CCA TGT CAA AAA TTG	2	29	1,44
RSVB_34_LEFT	CTA TCA CAA TTA TTC CTT TAC GGA GAT TGT	2	14	0,72
RSVB_34_RIGHT	TCA TTG CAG TTA ATT CTT ACA GCA TCC	2	14	0,72
RSVB_36_LEFT	TTG CGG TTC TAT CGT GAG TTT CA	2	29	1,44
RSVB_36_RIGHT	CAG GGA AGA ATT GTA AAA TAT TTT CGG C	2	22	1,08
RSVB_36_RIGHT_bis	CAGGGAAGAATTGTAAAATATTTTCAGC	2	7	0,36
RSVB_38_LEFT	ACG AAC TGC ATG GRG TAC AAT C	2	14	0,72
RSVB_38_RIGHT	TCC CTT AAG CTT ATG GCC TAT GC	2	14	0,72
RSVB_40_LEFT	AGG TAG CTT AAC ACA GGA RTT AGA ATA C	2	108	5,42
RSVB_40_RIGHT	GAC ACA AGT CAA GAA TTT GTT CAG TCT	2	108	5,42
RSVB_42_LEFT	ATC CAC AGG CTT TAG GGT CTG A	2	14	0,72
RSVB_42_RIGHT	GGC TAT AGT GCT AGT TGT ATA TTT AAT RTC C	2	14	0,72
RSVB_44_LEFT	CAA AGA TGA ATT CAT GGA AGA ACT GAG T	2	14	0,72
RSVB_44_RIGHT	GCT TCA ACT TGA TGA TAT CRA CAT CTC C	2	14	0,72
RSVB_46_alt_LEFT	TTAGCTGGACATTGGATTCTGA	2	72	3,61
RSVB_46_RIGHT	CAACCCAAGGGCATACGGT	2	54	2,71
RSVB_46_RIGHT_bis	CAACCCAAGGGCATACAGT	2	18	0,90
RSVB_48_LEFT	CTTTTCAGACAACACTCATCTGCT	2	72	3,61
RSVB_48_RIGHT	CTGATCTTGCATCCTGTGGAACT	2	72	3,61
RSVB_50_LEFT	CCC AGT TGT ATA GCA TTC ATA GGT GA	2	29	1,44
RSVB_50_RIGHT	TTT GAA ATC AAT ATC ATC TTG AGC ATG GT	2	29	1,44
RSVB_52_LEFT	GAT GTT GTG CAA AAT GCT AAA TTG ATT CT	2	14	0,72
RSVB_52_RIGHT	CAC TAC CTG TTA TTT TAA TCA GCT TCT TGA	2	14	0,72