Supplementary information of "**Recovery of synthetic Zika virus based on Rio-U1 isolate using a genetically stable two plasmid system and cDNA amplification**"

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Supplemental Table 1. Set of primers employed in part or the entire ZIKV cDNA template amplification.

Target	Primer	Sequence 5' → 3'	Amplicon (bp)
pCC1-MCS-Z1Z2	1F	CCTAAGCTTAGGTACCTTGCAAGGTCCATC	8255
	1R	GCTTAGGATCCGAGCTCACGCGTCTGTACC	
pCC1-Z3Z4	2F	GGATGTGCTGCAAGGCGATTAAGTTGG	7456
	2R	CTCGTATGTTGTGTGGAATTGTGAGC	
IC.Rio-U1	3F	CCTTATGTATCATACACATACGATTTAGGTGACACTATAGAG	10847
	3R	AGACCCATGGATTTCCCCACACCGG	

Supplemental Table 2 – Primer set designed for RT-PCR and viral genome

sequencing.

Primer	Position	Sequence 5' → 3'
ZK1F	1 - 25	AGTTGTTGATCTGTGTGAATCAGAC
ZKGAP5'AR	166 - 182	GCTCACACGGGCTACTC
ZK1AR	623 – 644	ATCCAGCATAGGGCATTCATAG
ZK1AF	627 – 646	GAATGCCCTATGCTGGATGA
ZK1BF	982 – 1003	GGTGCATAGGAGTCAGCAATAG
ZK1BR	1007 – 1028	ACCTGACATACCTTCCACAAAG
ZK1CR	1202 – 1221	GGTAGGCTTCACCTTGTGTT
ZK1CF	1373 – 1395	AGAGAATCTGGAGTACCGGATAA
ZK2F	1732 – 1751	GGCAAACTGTCGTGGTTCTA
ZK1R	1872 – 1892	GTATGACACGCCCTTCAATCT
ZK2AF	2431 – 2450	CCCTTATGTGCTTGGCCTTA
ZK2AR	2434 – 2453	CCCTAAGGCCAAGCACATAA
ZK24L1F	2608 – 2626	GTAGATTGGCAGCAGCAGT
ZK2BR	2731 – 2751	CCGTCAGTTGAACTCCATTCT
ZK24L1R	2927 – 2948	AAAGCTGTTCCATGCTCTATGT
ZK3F	3085 – 3107	GCTACTGGATTGAGAGTGAGAAG
ZK2R	3364 – 3385	CTCAGAGATGGTCCTCTTGTTC
ZK3AR	3743 – 3764	ATCTCCTCCAGTGTTCATTTCC
ZK24L2F	3807 – 3827	GCGTTGCTGGTATCTTTCATC
ZK3AF	3972 – 3992	GTTGTTCCACGCACTGATAAC
ZK24L2R	4045 – 4061	TGCTCTCCACGCCACAA
ZK4F	4345 – 4366	GTTACGTGGTCTCAGGAAAGAG
ZK3R	4548 – 4567	GGGTATGGCTATTGGGTTCAT
ZK4BF	4715 – 4735	ACAAGTTGGAGTGGGAGTTAT
ZK4AR	5026 – 5047	CTCCCACACTTGTCTAGGATTG
ZK4AF	5034 – 5055	GACAAGTGTGGGAGAGTGATAG

ZK4BR	5358 – 5377	GTTCCAGAGTGGGTGACATT
ZK5F	5593 – 5613	CGGACTCCAACTCACCAATTA
ZK4R	5912 – 5933	GACTCTCTCGCCATCAAGTATG
ZK5AR	6277 – 6299	GCACCATCTTCTATCTGTGTAGG
ZK5AF	6282 – 6304	ACAGATAGAAGATGGTGCTTTGA
ZK5BR	6518 – 6537	TGGCTTCCTGGAATCTCTCT
ZK6F	6959 – 6980	TCTAATGGGAAGGAGAGAGGAG
ZK5R	7104 – 7125	CCATCGCCATTAAGGAGTAGTT
ZK6AF	7607 – 7628	CTTGGCTGGAGCTTCTCTAATC
ZK6AR	7607 – 7628	GATTAGAGAAGCTCCAGCCAAG
ZK6BF	8018 - 8039	AAGCTATGGGTGGAACATAGTC
ZK6BR	8062 - 8081	CTCAGCCGCCATATGAAAGA
ZK7F	8549 – 8569	CTTTGACGAGAACCACCCATA
ZK6R	8689 – 8708	GGTGTCGGTCATGGCTATTC
ZK7AF	9187 – 9206	GTGTTGAAGGGCTGGGATTA
ZK7AR	9228 – 9249	CTGGTATGCGACTCATCTCTTC
ZK7BF	9514 – 9534	ACCTAGTGGTGCAACTCATTC
ZK7BR	9537 – 9558	GAACTTCCTCAGCCTCCATATT
ZK8F	10034 – 10054	GGTTCCAACTGGGAGAACTAC
ZK7R	10205 – 10227	CTATGAGAGATCCACACCACAAG
ZKGAP3'F	10643 – 10664	TGGATCTCCAGAAGAGGGACTA
ZK8R	10788 – 10807	AGACCCATGGATTTCCCCAC
zkmelt65f	10034 - 10068	GGTTCCAACTGGGAGAACTACCTGGTCAATCCATG
zkmelt65r	10782 - 10807	AGACCCATGGATTTCCCCACACCGGC
zkmelt70f	10034 - 10090	GGTTCCAACTGGGAGAACTACCTGGTCAATCCATG GAAAGGGAGAATGGATGACCAC
zkmelt70r	10778-10807	AGACCCATGGATTTCCCCACACCGGCCGCC

Supplemental Table 3 - Clinical scores for monitoring signs of disease in AG129 mice

Observation	Score
Body weight	
Loss of 5-10%	1
Loss of 10-15%	2
Loss of 16-20%	3
Loss of 20% or more	6
Fur	
Slightly ruffled	2
Evidently ruffled	3
Respiration	
Accelerated breathing (tachypnea)	1
Difficult breathing (dyspnea)	3
Clinical complications	
Tension during handling	1
Tremors, breath noises, aggression, vocalizations	3
Motility	
Abnormal walking and posture (slightly hunched, reduced activity)	1
Massively abnormal walking and posture (evidently hunched, moderately reduced activity)	2
Motility only after stimulation, isolation, lethargy	3
No activity for more than 24 hours	6
Measures according to total score	
3	Animals are carefully observed, with monitoring 2 times a day
6	Implementation of humane endpoint, abort experiment (euthanasia)