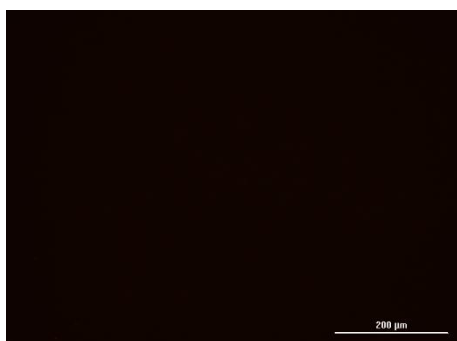


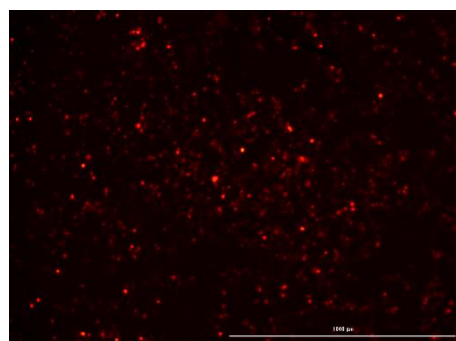
Supplementary information

Table S1. Primers used to clone the plasmids

Primers	Sequence 5' to 3'
SP6toBamHI-F	GCTCCTGGATCCAGGTGACAC
SP6toBamHI-R	GTGTCACCTGGATCCAGGAGC
DelNsP4-F	CATGGCCACCTTTGCAAG
DelNsP1-R	CTTCCTGTCCGACATCATC
DelCPE3-F	GCCATTATGGTGAGCGAG
DelCPE3-R	CTCCATTATGGCTGATTGG
DelFMtoE1-F	CTTGACAACCTAGGTACGAAGGTA
DelFMtoE1-R	TCTGTGCCCCAGTTTGCTA
ICD-F	AAAAAAAAAAAAAAAAAAGGGTCGGCATGGCATC
ICD-R	GTCTCACGCAGCCATACGGTTCACTAAACG
Chik-F	CGTTTAGTGAACCGTATGGCTGCGTGAGAC
Chik-R	ATGCCATGCCGACCCTTTTTTTTTTTTTTTTTTTTTTTT TTGAAATATT



(a)



(b)

Figure S1. Micrographs of HEK-293T cells transfected in fluorescent field (Texas Red filter) with 1 μ g of the plasmid: (a) pVax-Rep, bar 200 μ m; (b) pVax-Help, bar 1000 μ m.

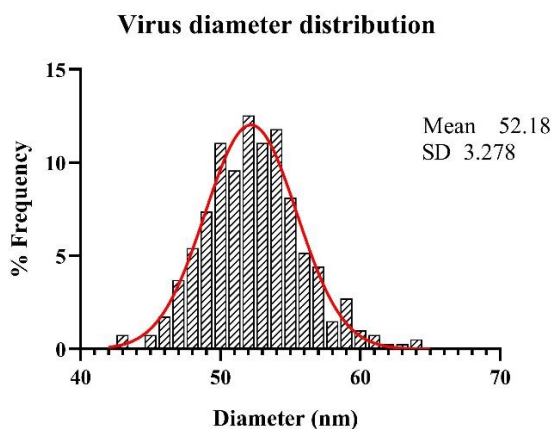


Figure S2. Histograms of CHIKV particles diameter distribution measure by transmission electron microscopy.

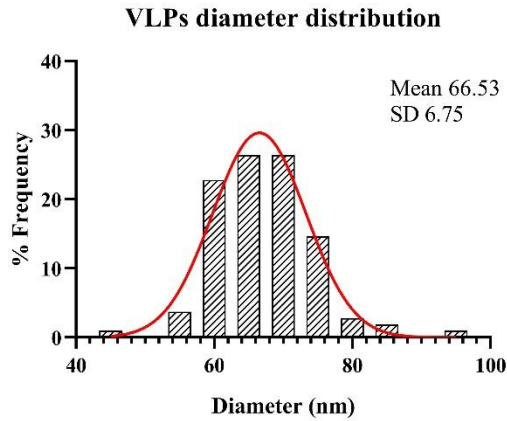


Figure S3. Histograms of VLPs diameter distribution measure by transmission electron microscopy.

pACNR-CHIKV plasmid sequence

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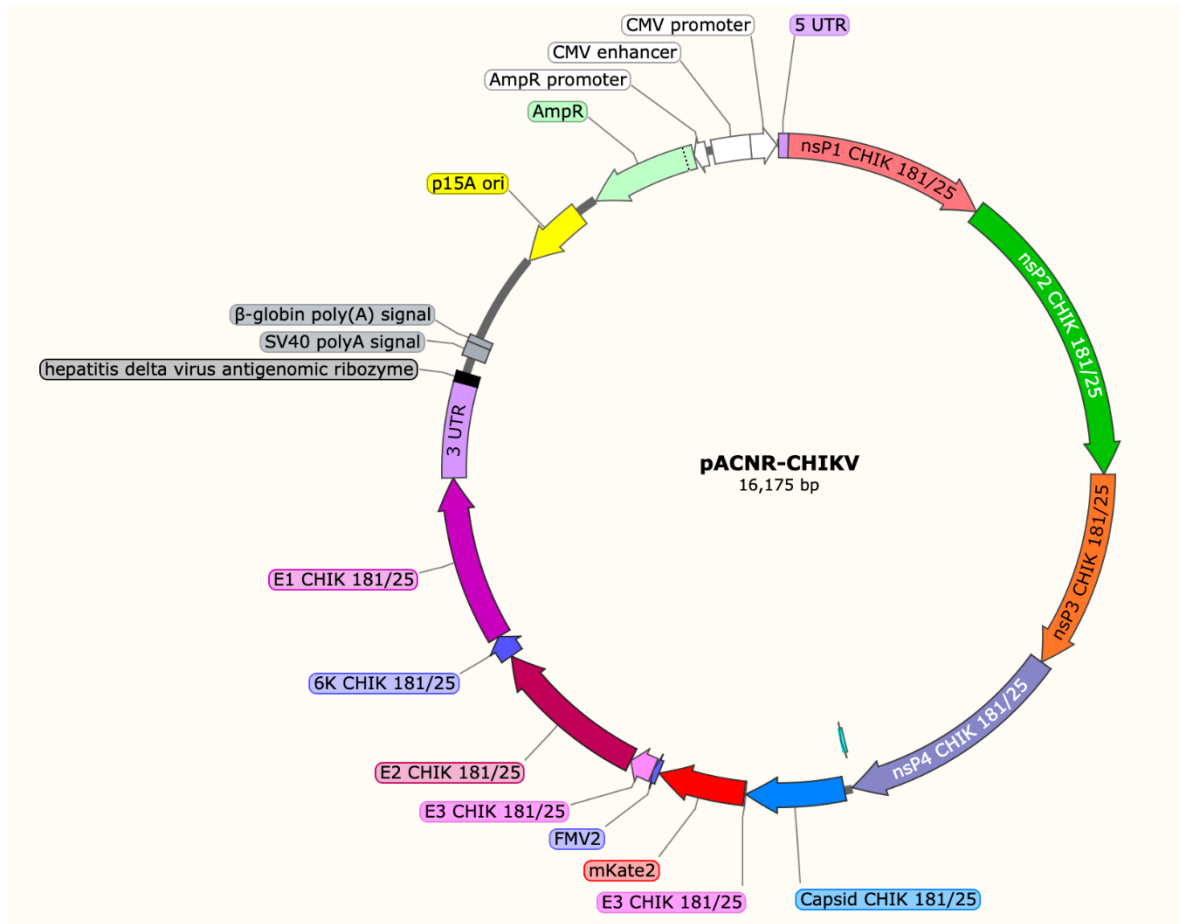
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pACNR-CHIKV plasmid sequence annotated

Feature	Location (nts)	Orientation with respect the CMV promoter	Type
5' UTR	1 to 76	Right	5' Untranslated region
nsp1	77 to 1,681	Right	CDS – nonstructural protein 1 – 59.5 kDa
nsp2	1,682 to 4,075	Right	CDS – nonstructural protein 2 – 89.6 kDa
nsp3	4,076 to 5,665	Right	CDS – nonstructural protein 3 – 57.5 kDa
nsp4	5,666 to 7,501	Right	CDS – nonstructural protein 3 – 68.2 kDa
SG promoter	7,472 to 7,504	Right	Promoter for the subgenomic RNA
Capsid	7,567 to 8,394	Right	CDS – capsid protein – 29.6 kDa
E3 (partial)	8,350 to 8,361	Right	CDS – partial sequence of the E3 chaperone – This allows the correct autoproteolytic cleavage between C and mKate2 – 402 Da
mKate2	8,362 to 9,057	Right	CDS – reporter gene mKate2 – 26.1 kDa
FMV2	9,058 to 9,108	Right	CDS – Foot and mouse disease virus cleave site to separate mKate 2 from E3.
E3	9,109 to 9,300	Right	CDS – E3 protein – 7.3 kDa
E2	9301 to 10,569	Right	CDS – E2 glycoprotein – 47.5 kDa
6K	10,570 to 10,752	Right	CDS – 6K protein – 6.8 kDa

E1	10,752 to 12,072	Right	CDS – E1glycoprotein – 47.5 kDa
3' UTR	12,073 to 12,822	Right	3' Untranslated region that includes the viral polyA
HDV	12,823 to 12,906	Right	Hepatitis delta virus ribozyme
SV40 polyA	13,032 to 13,147	Right	Simian vacuolating virus 40 polyadenylation signal and transcription terminator
β -globin polyA	13,148 to 13,203	Right	β -globin polyadenylation signal and transcription terminator
p15A ori	13,930 to 14,474	Left	Origin of replication for <i>E. coli</i>
AmpR	14,639 to 15,499	Left	Ampicillin resistant gene for selection in <i>E. coli</i> – 31.5 kDa
AmpR promoter	15,500 to 15,604	Left	<i>E. coli</i> promoter for AmpR
CMV enhancer	15,653 to 15,956	Right	Enhancer for the Cytomegalovirus promoter for mammalian cell lines.
CMV promoter	15,957 to 16,169	Right	Cytomegalovirus transcription promoter for mammalian cell lines.

pACNR-CHIKV plasmid map



pACNR-Rep plasmid sequence

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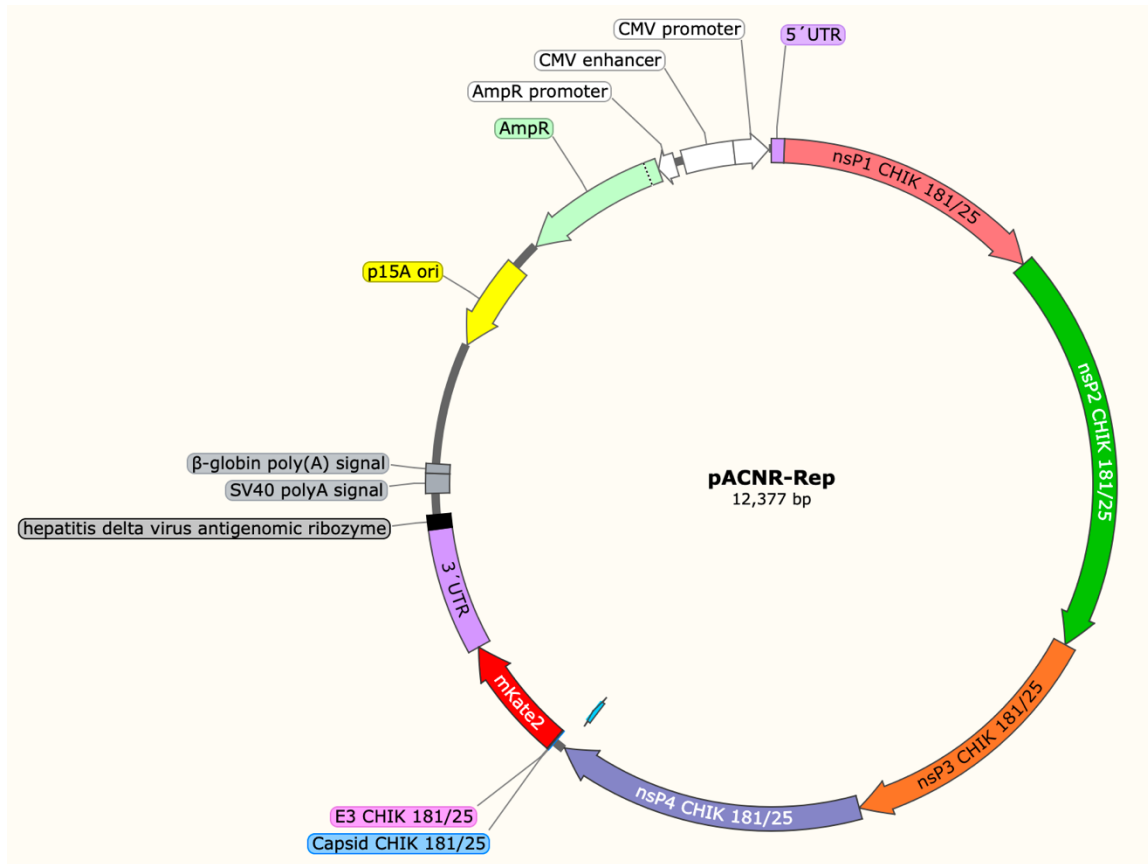
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pACNR-CHIKV replicon sequence annotated

Feature	Location (nts)	Orientation with respect the CMV promoter	Type
5' UTR	1 to 76	Right	5' Untranslated region
nsp1	77 to 1,681	Right	CDS – nonstructural protein 1 – 59.5 kDa
nsp2	1,682 to 4,075	Right	CDS – nonstructural protein 2 – 89.6 kDa
nsp3	4,076 to 5,665	Right	CDS – nonstructural protein 3 – 57.5 kDa
nsp4	5,666 to 7,501	Right	CDS – nonstructural protein 3 – 68.2 kDa
SG promoter	7,472 to 7,504	Right	Promoter for the subgenomic RNA
Capsid	7,567 to 7,572	Right	CDS – capsid protein – 2 amino acids – 278 Da
E3 (partial)	7,573 to 7,578	Right	CDS –E3 - 2 amino acids – 202 Da
mKate2	7,579 to 8,286	Right	CDS – reporter gene mKate2 – 26.4 kDa
3' UTR	8,287 to 9,024	Right	3' Untranslated region that includes the viral polyA
HDV	9,025 to 9,108	Right	Hepatitis delta virus ribozyme
SV40 polyA	9,234 to 9,349	Right	Simian vacuolating virus 40 polyadenylation signal and transcription terminator
β-globin polyA	9,350 to 9,405	Right	β-globin polyadenylation signal and transcription terminator
p15A ori	10,132 to 10,676	Left	Origin of replication for <i>E. coli</i>
AmpR	10,841 to 11,701	Left	Ampicillin resistant gene for selection in <i>E. coli</i> – 31.5 kDa
AmpR promoter	11,702 to 11,806	Left	<i>E. coli</i> promoter for AmpR

CMV enhancer	11,855 to 12,158	Right	Enhancer for the Cytomegalovirus promoter for mammalian cell lines.
CMV promoter	12,159 to 12,362	Right	Cytomegalovirus transcription promoter for mammalian cell lines.

pACNR-Rep plasmid map



pVax-Helper plasmid sequence

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pACNR-CHIKV replicon sequence annotated

Feature	Location (nts)	Orientation with respect the CMV promoter	Type
5' UTR	1 to 65	Right	5' Untranslated region
Capsid	66 to 848	Right	CDS – capsid protein – 29.6 kDa
E3 (partial)	849 to 860	Right	CDS – partial sequence of the E3 chaperone – This allows the correct autoproteolytic cleavage between C and mKate2 – 402 Da
mKate2	861 to 1,556	Right	CDS – reporter gene mKate2 – 26.1 kDa
FMV2	1,557 to 1,607	Right	CDS – Foot and mouse disease virus cleave site to separate mKate 2 from E3.
E3	1,608 to 1,799	Right	CDS – E3 protein – 7.3 kDa
E2	1,800 to 3,068	Right	CDS – E2 glycoprotein – 47.5 kDa
6K	3,069 to 3,251	Right	CDS – 6K protein – 6.8 kDa
E1	3,252 to 4,571	Right	CDS – E1 glycoprotein – 47.5 kDa
3' UTR	4,572 to 5,297	Right	3' Untranslated region that includes the viral polyA
β -globin polyA	5,298 to 5,522	Right	β -globin polyadenylation signal and transcription terminator
NeoR/KanR	5,695 to 6,489	Right	Aminoglycoside phosphotransferase confers resistance to neomycin, kanamycin, and G418 (Geneticin®) for selection in <i>E. coli</i> – 29.0 kDa
pUC ori	6,815 to 7,403	Right	Origin of replication for <i>E. coli</i>
CMV enhancer	7,504 to 7,883	Right	Enhancer for the Cytomegalovirus promoter for mammalian cell lines.
CMV promoter	7,884 to 8,087	Right	Cytomegalovirus transcription promoter for mammalian cell lines.

pACNR-Rep plasmid map

