

Supplementary Materials: Synthesis and Characterization of Mannosylated Formulations to Deliver a Minicircle DNA Vaccine

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E7 protein

atgcatggagatacaccttacattgcatgaatatatgttagatttgcaaccagagacaact
M H G D T P T L H E Y M L D L Q P E T T
gatctctactggttatgagcaattaaatgacagctcagaggaggaggatgaaatagatggt
D L Y C Y E Q L N D S S E E E D E I D G
ccagctggacaagcagaaccggacagagcccattacaatattgtaaccttttggtgcaag
P A G Q A E P D R A H Y N I V T F C C K
tgtgactctacgcttcggttggtgcgtacaaagcacacacgtagacatttcgtactttggaa
C D S T L R L C V Q S T H V D I R T L E
gacctgttaatgggcacactaggaattgtgtgcccatctgttctcagaaccataa
D L L M G T L G I V C P I C S Q K P -

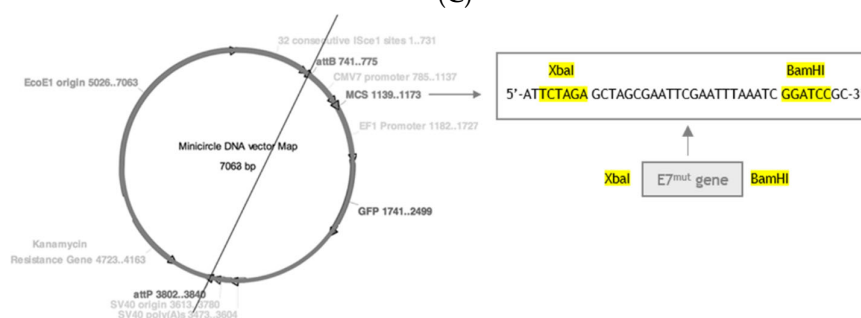
(A)

E7 protein - H2P (CAT → CCT)
 - C24G (TGT → GGT)
 - E46A (GAA → GCA)

(B)

E7wt MHGDIPTLHEYMLDLQPETTDLYCYEQNLDSSEEEDEIDGPAQGAEPDRAHYNIVTFCK 60
E7mutant MFGDPTLHEYMLDLQPETTDLYGYEQNLDSSEEEDEIDGPAQGAAPDRAHYNIVTFCK 60
* * * * *
E7wt CDSTLRCLVQSTHVDIRTLEDLLMGTLGIVCPICSKQP- 98
E7mutant CDSTLRCLVQSTHVDIRTLEDLLMGTLGIVCPICSKQP- 98
* * * * *

(C)



(D)

Figure S1. Schematic representation of three mutations in the HPV E7 gene and cloning of mutated gene in the parental plasmid vector. (A) E7 gene sequence from HPV wildtype; (B) Identification of three mutations to perform in the HPV E7 wildtype gene; (C) Sequencing of E7 mutant gene; (D) cloning of E7 mutant gene in the parental plasmid vector.

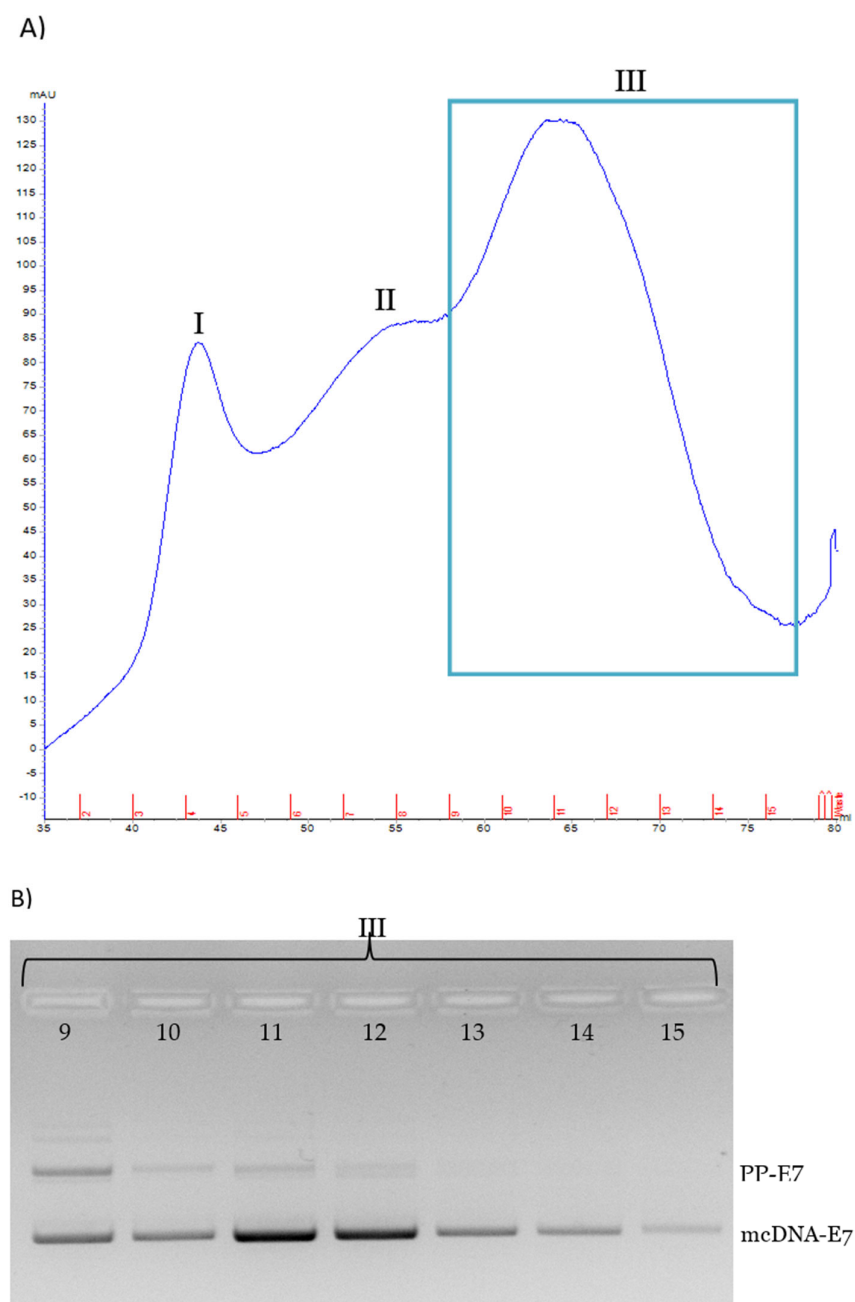


Figure S2. Chromatographic profile of mcDNA isolated by size exclusion chromatography in the Sephacryl SF-1000 column (A), using the following conditions, flow-rate of 0.3 mL/min, sample loading of 2 mL and fractionation of 3 mL; and agarose gel electrophoresis of fractions from peak III (B).

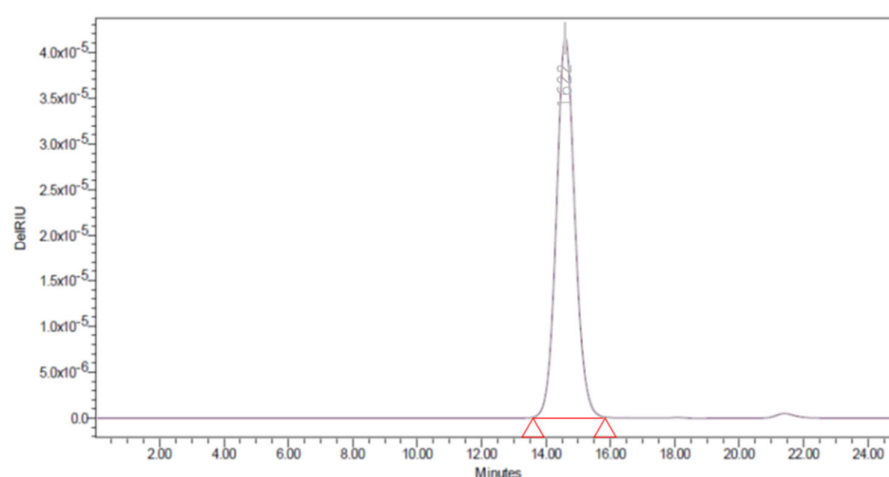


Figure S3. SEC chromatogram of MPITC-R8.

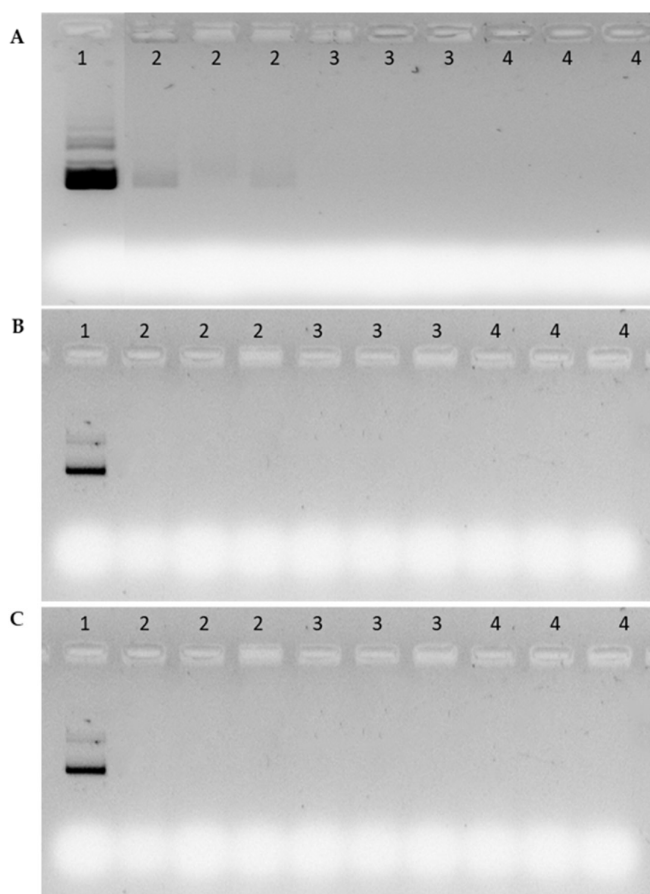


Figure S4. Electrophoretic mobility of supernatants from several formulations studied at various N/P ratios. (A) R8-mannose/mcDNA, (B) R8-mannose/PEI/mcDNA maintaining PEI N/P ratio at 5 and changing R8 N/P ratios and (C) R8-mannose/PEI/mcDNA maintaining PEI N/P ratio at 10 and changing R8 N/P ratios. Image A: lane 1-R8-mannose/mcDNA N/P ratio of 1:1; lane 2-R8-mannose/mcDNA N/P ratio of 1.5:1; lane 3-R8-mannose/mcDNA N/P ratio of 2:1. Image B: lane 1-R8-mannose/PEI/mcDNA N/P ratio of 1:5:1; lane 2-R8-mannose/PEI/mcDNA N/P ratio of 1.5:5:1; lane 3-R8-mannose/PEI/mcDNA N/P ratio of 2:5:1. Image C: lane 1-R8-mannose/PEI/mcDNA N/P ratio of 1:10:1; lane 2-R8-mannose/PEI/mcDNA N/P ratio of 1.5:10:1; lane 3-R8-mannose/PEI/mcDNA N/P ratio of 2:10:1.

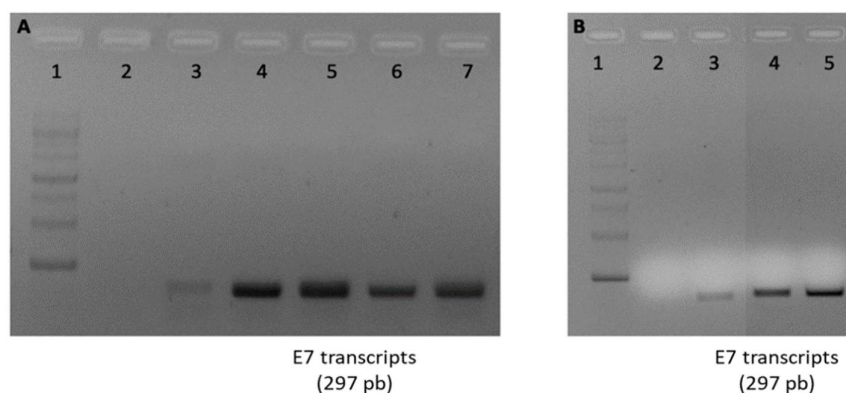


Figure S5. Analysis of RT-PCR products by agarose gel electrophoresis. Evaluation of E7 transcripts in Raw cells (**A**) and Fibro cells (**B**). Lane 1 - DNA molecular weight marker; lane 2- control without cDNA sample; lane 3-non-transfected cells; lane 4-cells transfected by PEI/mcDNA N/P ratio 5:1; lane 5-cells transfected by R8-mannose/PEI/mcDNA N/P ratio 2:5:1; lane 6-cells transfected by PEI/mcDNA N/P ratio 10:1; lane 7-cells transfected by R8-mannose/PEI/mcDNA N/P ratio 2:10:1.

Table S1. The molecular weight of each MPITC-R8 conjugate by SEC analysis.

Conjugates	Observed Relative Molecular Weight (Mw) (Da)	Theoretical Molecular Weight (Da)
MPITC-R8	1622	1643