PURIFICATION OF MINICIRCLES BY COMBINED ENZYMATIC MODIFICATION OF MINIPLASMID TOPOLOGY AND MULTIMODAL CHROMATOGRAPHY

Duarte Miguel Prazeres, iBB- Institute for Bioengineering and Biosciences, Instituto Superior Técnico
miguelprazeres@tecnico.ulisboa.pt
Ana Rita Silva-Santos, iBB- Institute for Bioengineering and Biosciences, Instituto Superior Técnico
Cláudia Alves, iBB- Institute for Bioengineering and Biosciences, Instituto Superior Técnico
Michaela Šimčiková, iBB- Institute for Bioengineering and Biosciences, Instituto Superior Técnico
Gabriel Monteiro, iBB- Institute for Bioengineering and Biosciences, Instituto Superior Técnico
Ana Margarida Azevedo, iBB- Institute for Bioengineering and Biosciences, Instituto Superior Técnico

Key Words: gene therapy, multimodal chromatography, minicircle, nicking endonuclease, plasmid DNA.

Minicircle (MC) DNA vectors are able to generate a high-level transgene expression *in vivo*, which is superior to the one afforded by conventional plasmids. MC vectors are produced by replicating a parental plasmid (PP) and promoting its recombination in *Escherichia coli*. This generates a MC with the expression cassette, and a miniplasmid (MP) with the replication segment. Unfortunately, wider use of MC vectors is hampered by difficulties in isolating the target MCs from their MP counterpart. In this proof-of-concept study, a reproducible process is described to improve the purification of supercoiled (sc) MCs that combines an *in vitro* enzymatic relaxation of sc MP impurities with topoisomer separation and RNA clearance by multimodal chromatography.

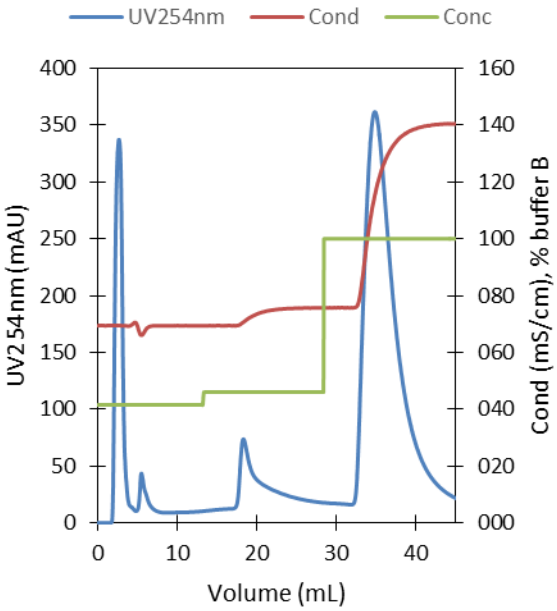

Figure 1 – Multimodal chromatography purification of sc MC DNA from a feed stream containing oc MP, oc MC and RNA

At the early stage of vector design, a site for the nicking endonuclease Nb.BbvCI was strategically placed in the MP part of the PP backbone. A process was then established that involves *E. coli* culture and recombination of PPs into target MC, cell harvesting and alkaline lysis, and tandem precipitation with isopropanol, ammonium acetate and PEG. Next, an *in vitro* digestion step was carried out with Nb.BbvCI to nick of one of the strands of the MPs and of non-recombined PPs by Nb.BbvCI. As a result, sc MPs and non-recombined PPs were converted into the corresponding open circular (oc) forms, whereas sc MCs remain unaffected. Finally, sc MC was isolated from oc DNA molecules (oc MPs, oc MC) and RNA by performing multimodal chromatography with a Capto-Adhere column using a series of elution steps with increasing NaCl concentrations (Figure 1).

On the basis of agarose gel electrophoresis, protein tests and quantitative PCR, the sc MC-containing fractions were determined to be virtually free from nucleic acid impurities and proteins. Furthermore, the process was reproducible and performed similarly with differently sized PP and MCs.