Production of High-purity Minicircle-DNA for Use in Gene Transfer

The present invention relates to a highly efficient method for the production and purification of minicircle-DNA. Based on the coordinated and consecutively conducted in vivo processes of recombination, restriction and subsequent affinity purification, the technology provides highly pure minicircle-DNA with an optimized safety profile for direct application in gene therapy and vaccination. The technology was developed by Vienna based researchers.

Background

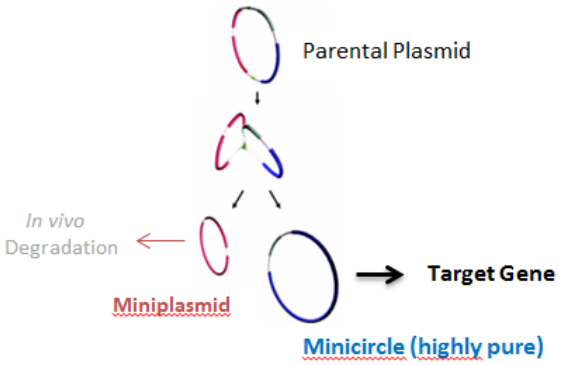
Among non-viral technologies, gene transfer using minicircle-DNA is regarded as a promising emerging technology that has already been shown to be superior to conventional plasmid-DNA in a variety of studies. Minicircles are derived from conventional plasmids via a site specific recombination process removing bacterial backbone sequences. The resulting minicircle-DNA constitutes a supercoiled, minimal expression cassette with an optimized safety and efficiency profile.
However, large scale production of these optimized DNA-molecules is not trivial and was so far hampered by inefficient recombination and/or purification processes.

Technology

To overcome the shortcomings in conventional mini-circle production systems a new high-efficiency process has been developed by researchers from Vienna.
The novel technology is based on coordinated consecutive steps of in vivo recombination, in vivo restriction and an in vitro purification step for the removal of residual impurities.
The recombination process is an inducible, sequence specific process, starting from a parental plasmid and leading to minicircles containing the desired DNA for gene transfer applications and miniplasmids carrying the unwanted bacterial backbone sequences. The elements on the parental plasmid are arranged in a way that endonuclease expression is activated by the removal of the minicircle sequence via the recombination process. The corresponding endonuclease restriction sites are integrated in the parental plasmid and – after recombination - on the miniplasmid. Upon expression of the endonuclease, plasmids carrying the corresponding sites are linearised and subsequently further degraded by host exonucleases. After recombination and in vivo restriction/degradation the host cell harbours almost exclusively minicircles. Remaining trace amounts of residual parental plasmids and mini
plasmids can subsequently be removed specifically via an affinity chromatography technology based on protein-DNA interaction.

Application

- Non-viral gene therapy and vaccination
- Minicircle or plasmid purification



Benefits

- Combination of minicircle production and purification work flow
- Consecutive complementary processing steps
- Optimized safety profile of minicircles
- High product purity (99% and higher)

Patent family status

EP1620559B1, US8647863B2, AU2004236352B2, EP2213744A1, US20140065705A1, GB1307075.0
Additional patent application claiming even better coordination of production and purification processes filed in 2013.

Development status

Technology is ready for upscaling

Cooperation options

License agreement, collaboration

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