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ABSTRACT WITHDRAWN

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Comparison of genomic and complementary hGH gene sequences utilized for electrotransfer in dwarf mice

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Poor gene expression is the main limitation for the use of naked DNA in vivo gene therapy approaches. However, this obstacle has been overcome by employing techniques as electroporation to improve the DNA delivery. The choice of the best gene sequence to be used is also essential for its success. A gene sequence used in an assay may be genomic, complementary or optimized. In this work a comparison between a plasmid containing the CMV promoter and encoding the genomic DNA (gDNA) or the complementary DNA (cDNA) sequence of the human growth hormone (hGH) was carried out. Lit/scid or lit/lit mice were injected with 50 μg of each plasmid into quadriceps muscle, followed by electrotransfer, using eight 50-V pulses of 20 ms at a 0.5-s interval. The lit/scid group that received the cDNA-hGH presented higher levels of hGH in the circulation throughout a 45 day experiment, with a peak of $\sim$20 ng hGH/ml on day six. In the immunocompetent mice (lit/lit), hGH serum level were also higher by using the cDNA-hGH vector. As expected, the use of a viral promoter in immunocompetent animals resulted in lower levels of circulating hGH, at least 2–3 fold in our case, when compared with immunodeficient mice. The bioactivity of hGH expressed by each vector and the mIGF-I levels will be also determined. These results demonstrate the importance of selecting the elements to be used in gene therapy vectors, such as the most efficient gene sequence.

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Analytical tools in minicircle production.

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Here, we give an overview on our analytical tools in the production of minicircle DNA, a safe and efficient vector system for gene and cell therapy and genetic vaccination approaches.

This minicircle technology is removing needless sequences like marker genes, the bacterial origin of replication etc., only used for stable maintenance and amplification of plasmids in bacteria. The resulting minicircle DNA consists almost only of the gene of interest, leading to significant size reduction and improved performance. Furthermore, our minicircle is a homogenous monomer and not a cocktail of various multimeric derivatives as in other minicircle systems published so far.

Throughout the DNA manufacturing process, certain analytical samples are taken in order to ensure the quality. For minicircle production it is especially important that the recombination does not start before this is required. A technique first time used for the analysis of DNA from a minicircle system is shown here in detail: the atomic force microscopy (AFM) is a versatile tool that shows and measures surface structures with unprecedented resolution and accuracy at the nm-scale. Additionally, we show results obtained by capillary gel electrophoresis (CGE) - a tool to identify and quantify minicircle topologies. Finally, the reliability of the non-existence of recombination product during cultivation is shown by use of a novel microfluidic channel. The continuous separation of biomolecules in microfluidic channels enables the implementation of measurements of small analytic volumes along the minicircle production. As a tool for product quality control we can detect the existence of unwanted multimers.

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Generation of neutralizing antibodies against botulinum neurotoxin B serotype by DNA electroporation

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Botulinum neurotoxin B (BoNTB) is a subtype of a family of seven (A-G) distinct proteins produced by *Clostridium botulinum*, a gram-positive, spore forming anaerobic bacillus. BoNTs inhibit the release of acetylcholine at the synapse of motor neurons, leading to flaccid paralysis. They are among the most potent poisons known, and might present a potential threat as a biological weapon. BoNTs type A, B and E are commonly linked to human disease.

Current therapies for botulism consist mainly of supportive care, prophylactic vaccine and passive antibody administration. Trivalent equine-based antitoxins (A, B and E) antibodies are available and used clinically to neutralize and clear BoNT from the circulatory system.

Production of high titer antisera against BoNTA by genetic immunization mediated by intramuscular DNA electrotropration has previously been reported. However, this technique failed to produce such neutralizing antibodies against BoNTB.

In this work we investigated the reasons of the low immunogenicity of the nontoxic C-terminal half of the heavy chain