Docket #: S08-108

Minicircle vector for non-insertional transgene expression in quiescent cells and tissues

The minicircle is a non-viral DNA vector for non-insertional transgene expression. A typical minicircle contains a transgene expression cassette, and is free of all other plasmid DNA elements, including an antibiotic resistance gene and a plasmid DNA replication origin. This reduces the potential for silencing and inflammatory effects associated with these sequences.

The minicircle produced with this method provides a stable and persistent expression of the transgene expression cassette in quiescent cells and tissues, and reduces CpG inflammatory responses observed in some plasmid transfection schemes.

This invention improves the production of minicircle DNA by making a versatile bacterial strain. This bacterium is genetically engineered to express (1) multiple copies of an inducible phiC31 gene, the recombinase which mediates the formation of a minicircle expression cassette from the parental plasmid; (2) multiple copies of an inducible ISce1 gene, the restriction enzyme, which when expressed destroys the unwanted plasmid bacterial backbone DNA, and (3) two different genes encoding transporters of L-arabinose, which is the inducer of the araC.BAD system controlling the expression of both the recombinase phiC31 and the restriction enzyme ISce1.

Consequently, the new system has the following advantages:

(1) Reduces contamination of parental plasmid and its derivatives from 5 -15% to 1%.
(2) Reduces the time of minicircle preparation to that of routine plasmid preparations
(3) Reduces the amount of arabinose required by over 100-fold.
(4) Reduces the risk of phiC31/ISceI genes by removing them from the parental

plasmid to the bacterial genome making the final process GMP compatible.

Applications

- To produce more effective and pure minicircle DNA vectors than current methods for therapeutic (including gene therapy) in vitro and in vivo use, diagnostic, prophylactic or research applications.

Advantages

- The previous method of minicircle production gives 5 to 15% contamination with the parental plasmid. This requires additional purification that is timely and costly. This new invention has many improvements, including:
- 1. Minicircles (MC) are ready for GMP production of clinical grade MC: both øC31 and ISce1 genes have been relocated from parental plasmid to bacterial genome; contamination of these two genes, if any, will be in linear DNA and easily eliminated;
- 2. Significantly reduced impurity DNAs--which are hardly visible--in the MC prep.
- 3. Greatly simplified procedure: just adding some fresh broth with L-arabinose and incubating for a few more hours before MC isolation;
- 4. Cost reduction: only 1/100 to 1/1000 of the concentration of L-arabinose in original system is needed;
- 5. A smaller and more easily manipulated parental plasmid.
- 6. A friendlier bacterial strain.

Publications

- "A robust system for production of minicircle DNA vectors." Kay MA, He CY, Chen ZY. Nat Biotechnol. 2010 Dec;28(12):1287-9. Epub 2010 Nov 21.
- "A nonviral minicircle vector for deriving human iPS cells." Jia F, Wilson KD, Sun N, Gupta DM, Huang M, Li Z, Panetta NJ, Chen ZY, Robbins RC, Kay MA, Longaker MT, Wu JC. Nat Methods. 2010 Mar;7(3):197-9. Epub 2010 Feb 7.
- Hum Gene Ther. 2005 Jan;16(1):126-31. Improved production and purification of minicircle DNA vector free of plasmid bacterial sequences and capable of

persistent transgene expression in vivo. Chen ZY, He CY, Kay MA.

- Zhi-Ying Chen, Cheng-Yi He, Anja Ehrhardt, Mark A. Kay. Minicircle DNA Vectors Devoid of Bacterial DNA Result in Persistent and High-Level Transgene Expression in-Vivo. Molecular Therapy. Vol. 8, No. 3 September 2003.
- US publication no. 20040214329 and 20060223778
- US Published Patent Application no. 20100075401 "Minicircle DNA Vector Preparations and Methods of Making and Using the Same"

Patents

- Published Application: 20100075401
- Published Application: WO2010002470
- Published Application: 20130034882
- Published Application: 20150217000
- Issued: 8,236,548 (USA)
- Issued: 8,945,885 (USA)
- Issued: 9,233,174 (USA)

Innovators

- Zhi-Ying Chen
- Mark Kay

Licensing Contact

Sam Rubin

Licensing Associate, Life Science

Email