Title (en)

METHODS FOR SEMI-SYNTHETICALLY PRODUCING HIGHLY PURE MINICIRCLE DNA VECTORS FROM PLASMIDS

Title (de)

VERFAHREN ZUR SEMI-SYNTHETISCHEN HERSTELLUNG HOCHREINER "MINICIRCLE" DNA-VEKTOREN AUS PLASMIDEN

Title (fr)

PROCÉDÉ DE PRODUCTION PAR SEMI-SYNTHÈSE DE VECTEURS D'ADN "MINICERCLES" À HAUT DEGRÉ DE PURETÉ À PARTIR DE PLASMIDES

Publication

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Application

EP 11767977 A 20111004

Priority

- EP 10186568 A 20101005
- EP 2011067280 W 20111004
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Abstract (en)

[origin: EP2439276A1] Producing circular DNA-vectors (c-DNA-v) in super-helical form comprises: (a) splitting parental plasmids with restriction enzymes to obtain linear DNA vector fragments with the sequence of the DNA vector; (b) separating the linear DNA vector fragments from other products of the splitting reaction; (c) ligating the linear DNA vector fragment to obtain c-DNA-v in relaxed form; (d) separating the c-DNA-v from other products of the ligation; (e) twisting the c-DNA-v of step (d) with a gyrase to obtain c-DNA-v in super-helical form; and (f) optionally purifying the c-DNA-v in super-helical form. Producing circular DNA-vectors (c-DNA-v) in super-helical form comprises: (a) splitting parental plasmids with one or more restriction enzymes to obtain linear DNA vector fragments with the sequence of the DNA vector, where the parental plasmid contains the sequence of the DNA-vectors and heterologous sequences; (b) separating the linear DNA vector fragments from other products of the splitting reaction; (c) ligating the linear DNA vector fragment to obtain c-DNA-v in relaxed form; (d) separating the c-DNA-v from other products of the ligation; (e) twisting the c-DNA-v of step (d) with a gyrase to obtain c-DNA-v in super-helical form; and (f) optionally purifying the c-DNA-v in super-helical form for the separation of byproducts. Independent claims are included for: (1) a reagent kit for producing a circular DNA vector in super-helical form, comprising a ligase, a gyrase, and optionally one or more restriction enzymes; (2) preparation of a minicircle DNA vector in super-helical form, characterized by the absence of byproducts after PCR, preferably linear or circular miniplasmid and/or parental plasmid; and (3) a method for producing super-helical minicircle DNA vectors without the use of location (sequence) specific recombinases such as flippase recombination enzyme.

IPC 8 full level

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CPC (source: EP US)

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