

Title (en)

METHODS FOR DNA MANIPULATION

Title (de)

METHODEN ZUR DNA-MUTAGENESE UND DNA-KLONIERUNG

Title (fr)

PROCEDES DE MANIPULATION D'ADN

Publication

**EP 1432797 A2 20040630 (DE)**

Application

**EP 02779456 A 20021002**

Priority

- DE 10148607 A 20011002
- EP 0211086 W 20021002

Abstract (en)

[origin: DE10148607A1] Mutagenesis and cloning of nucleic acids (NA), is new. Mutagenesis and cloning of a nucleic acid (NA), comprises: (a) preparing a circular, double-stranded (ds) NA matrix to be mutated; (b) preparing two oligonucleotides (ON) that each hybridize to one strand of the matrix and have at the 5'-ends a 2-4 base pair (bp) segment, mutually complementary, where at least one ON contains a mutated target sequence; (c) amplifying the matrix using an ON with the mutated target sequence as a primer to create a mutated ds amplicon; (d) trimming the ends of the amplicon to create a product with mutually complementary 5'-overhangs; and (e) ligating the trimmed amplicon to produce a mutated circular NA construct. Independent claims are also included for: (1) a reagent kit for the new method, comprising agents for: (i) NA amplification; (ii) trimming ds NA to create complementary ends; (iii) for ligation; and (iv) optionally for selective removal of methylated DNA; (2) cloning NA fragments into a vector; and (3) reagent kits for the method of (2).

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**C12N 15/10; C12N 15/66**

IPC 8 full level

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