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(54) Title: CIRCULAR RNA FOR TRANSLATION IN EUKARYOTIC CELLS

(57) Abstract: Disclosed are methods and constructs for engineering circular RNA. Disclosed is a vector for making circular RNA, said vector comprising the following elements operably connected to each other and arranged in the following sequence: a.) a 5' homology arm, b.) a 3' group I intron fragment containing a 3' splice site dinucleotide, c.) optionally, a 5' spacer sequence, d.) a protein coding or noncoding region, e.) optionally, a 3' spacer sequence, f.) a 5' Group I intron fragment containing a 5' splice site dinucleotide, and g.) a 3' homology arm, said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells. In another embodiment, the vector can comprise the 5' spacer sequence, but not the 3' spacer sequence. In yet another embodiment, the vector can comprise the 3' spacer sequence, but not the 5' spacer sequence. Also disclosed is a method for purifying the circular RNA produced by the vector and the use of nucleoside modifications in circular RNA produced by the vector.

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