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Trna Handles

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OTHER INFORMATION

KEYWORDS

tRNA sequencing, sequencing
adapter, tRNA, nanopore
sequencing, RNA, RNAseq, long
read sequencing

CATEGORIZED AS

▶ Research Tools

  ▶ Nucleic Acids/DNA/RNA

RELATED CASES

2014-725-0

BACKGROUND

tRNA is notoriously difficult to manipulate . Sequencing of tRNA presents various problems because of complex and tightly bound secondary structures and associated proteins. Current methods of tRNA analysis include RNA sequencing, microarray analysis and mass spectrometry. Each has limitations, however. RNA sequencing requires extensive library preparation and PCR amplification followed by reverse transcription. This results in the loss of the original RNA strand and its secondary structure. Reverse transcription is also impeded by the structural and nucleotide modifications that commonly occur in tRNA's. These so-called RTstops result in truncated cDNAs that do not reflect natural tRNA's.

tRNA can be sequenced with nanopore sequencers, so long as they can be unfolded and electrically attracted to the nanopore. So a mechanism to capture tRNA molecules, unfold them, and initiate threading them into a nanopore is needed.

TECHNOLOGY DESCRIPTION

The invention includes attaching DNA or RNA "handles" to a tRNA molecule. These handles allow manipulation of the tRNA molecule, including unfolding its structure and acting as targets for attaching other molecules to the tRNA.

One example is a double stranded oligonucleotide adapter that can be ligated to a tRNA from a biological sample. Such an adepter can be a Y shaped double stranded DNA-RNA adapter with a 3' RNA overhang complementary to the CCA tail present in tRNA. The adapter can also include a cholesterol tag within its 3' end.
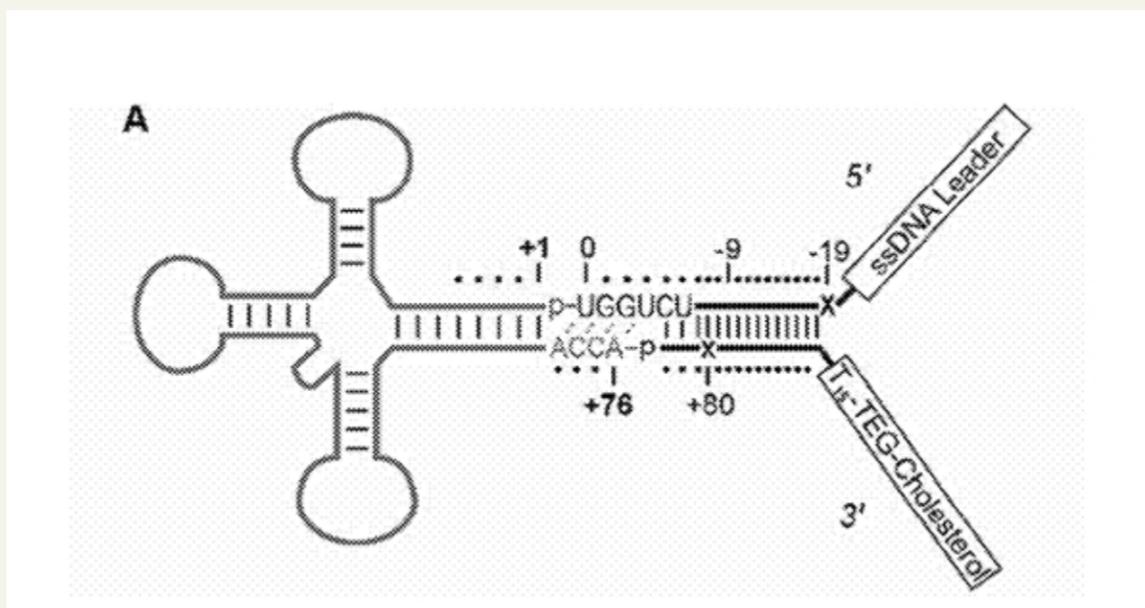
Examples of sequences used in oligonucleotide adaptors include:

CTCACCTATCCTTCCACXCATACTATCATTATCTXTCAGATCTCACTAUCUGGU

GATXGTGAGATCTGATTTTTTTTTTTTTTTTZ

GATAGTGAGATCTGATTTTTTTTTTTTTTTTZ

CTCACCTATCCTTCCACTCATACTATCATTATCTCTCAGATCTCACTAUCUGGU

GATXGTGAGATCTGATTTTTTTTTTTTTTTTZ

CTCACCTATCCTTCCACTCATACTATCATTATCTCTCAGATCTCACTAUCUGGU

X indicates an abasic 1'2' dideoxyribose; Z indicates a triethylene glycol cholesterol

APPLICATIONS

▷ Identification of tRNA
▷ Sequencing of tRNA

▶ Nanopore sequencing of tRNA

ADVANTAGES

▶ Molecular adaptor that facilitates tRNA seqeuncing by nanopore

▶ Rapid identification of tRNA species in a biological sample

INTELLECTUAL PROPERTY INFORMATION

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	10,131,944	11/20/2018	2014-725
Patent Cooperation Treaty	Published Application	WO 2015/148567	10/01/2015	2014-725

RELATED MATERIALS

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

▶ Software Tool for Generating Optimized Gene Sequences

▶ Software Tool for Predicting Sequences in a Genome that are Subject to Restriction or Other Surveillance Mechanisms