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Non-Confidential Description - PSU No. 4464
"A Novel Reverse Transcriptase Enzyme"

Keywords:

Research Tools & Devices, Medical Diagnostics, double-stranded RNA, Reverse transcriptase, cDNA,

Links:

Inventor Website: <https://roossincklab.com/>

Inventor Webpage: <http://plantpath.psu.edu/directory/mjr25>

Inventors:

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Background

The ability of reverse transcriptase (RT) to copy RNA into DNA enables many molecular biology applications, including cloning, RT-polymerase chain reaction (PCR), diagnostics, RNA sequencing, and the expression of many important pharmaceuticals that were first isolated as messenger RNA. However, double-stranded RNA (dsRNA) has posed a particular challenge to copy into DNA because RT enzymes are only active on single-stranded RNA (ssRNA). While several methods are available to convert dsRNA into ssRNA, most of the techniques are inefficient and/or inconsistent, or require the use of extremely toxic chemicals. This is a significant problem for research in virology, siRNA work, and CRISPR work where dsRNA must be converted to ssRNA before being copied into complementary DNA (cDNA). Furthermore, commercially available RT enzymes have a relatively low fidelity, i.e., lower replication accuracy. This has been particularly problematic for studies in RNA virus populations and transcriptome single nucleotide polymorphisms (SNPs). Therefore, there is an ongoing and unmet need for improved technology that converts dsRNA molecules into cDNA, as well as a tool helpful in the discovery and diagnostics of RNA viruses and RNA and DNA sequences. The present disclosure addresses these needs.

Invention Description

The invention includes a RNA dependent RNA polymerase (RdRp) comprising RT activity for use in producing cDNA from double stranded RNA (dsRNA) and methods thereof. The RdRp, which originated from a virus, contains a domain that is similar to a domain found in RT enzymes. The RdRp functions as a RT enzyme that works on dsRNA at room temperature and will be suitable for use as a RT without the inconveniences of a retrovirus-derived RT enzyme. The invention also includes methods of making RT proteins. Such methods comprise both isolating the virus that comprises the RdRp from the host where it is found normally and using isolated particles for performing reverse transcription, or by producing the protein recombinantly. Producing the protein recombinantly generally comprises initially introducing an expression vector encoding the RdRp into any suitable host cells by any method known in the art. Accordingly, it is the expectation of the inventors that any dsRNA can be used as a template for cDNA production using approaches of this invention.

Advantages/Applications

- Technique to convert dsRNA into cDNA is more efficient than other methods
 - No need for toxic chemicals
 - No need to reach or maintain high temperature levels
- Greater maintenance of enzyme fidelity during conversion to DNA compared to other techniques
- Greater efficiency and consistency in converting dsRNA into ssDNA than other techniques

IP Status: U.S. provisional patent application filed on April 10, 2017.

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