

An Optimized Active Decapping Complex for Transcription Start Site Mapping

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INVENTION NOVELTY

This invention describes an optimized, constitutive active decapping complex from *S. pombe* for efficient transcription start site (TSS) mapping.

VALUE PROPOSITION

Changes in 5' untranslated regions (UTRs) can have profound effects on a gene's translational efficiency, resulting in both qualitative and quantitative differences in expressed proteins. Therefore, mapping the 5' UTRs in organisms ranging from single cell organisms to humans has rewritten our understanding of genetic plasticity and its impact on various human diseases including cancer.

RNA-seq methods are constantly being innovated to reproducibly map TSS. Most of these methods rely on the enzymatic removal of m⁷G cap structure and subsequent ligation of an adaptor sequence to the reactive 5' monophosphate. Though bacterial RNA 5' Pyrophosphohydrolase (RppH) is currently being used for this reaction, its specific activity is directed towards triphosphorylated, uncapped bacterial RNAs, and not eukaryotic 5' capped RNAs.

The decapping complex discovered by scientists at UCSF has the following advantages:

- ▶ Can be readily purified from standard *E. coli* protein production systems using affinity columns and size exclusion chromatography.
- ▶ Shows 10,000-fold higher catalytic efficiency in eukaryotic m⁷G cap removal as compared to RppH.
- ▶ Demonstrates greater decapping activity in sequencing relevant scenario of total yeast RNA.
- ▶ The monophosphorylated RNA produced using this complex is suitable for standard downstream RNA cloning methods.

TECHNOLOGY DESCRIPTION

This invention describes an IPTG inducible single plasmid expression system designed to purify a constitutively active *S. pombe* decapping complex using standard production and purification methodologies. The expressed active decapping enzyme is catalytically more efficient (V_{max}/K_m) than RppH in multiple turnover conditions for 5' m⁷G cap removal from eukaryotic RNA. The end product suitability for downstream cloning applications has been verified using splinted-ligation procedure. Therefore, this active "super" decapping complex can be used for both small-scale and large-scale sequencing methods for efficient TSS mapping.

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OTHER INFORMATION

KEYWORDS

TSS mapping, 5' Decapping, 5' m⁷G cap removal, RNA sequencing

CATEGORIZED AS

- ▶ **Research Tools**
- ▶ Nucleic Acids/DNA/RNA

RELATED CASES

2018-001-0

LOOKING FOR PARTNERS

To develop & commercialize the technology as a specific and highly efficient eukaryotic alternative to commercially available bacterial decapping enzymes.

STAGE OF DEVELOPMENT

Proof of Concept

DATA AVAILABILITY

Under a CDA/ NDA

RELATED MATERIALS

► Manuscript available under a CDA/NDA

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