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Summit Pharmaceuticals Europe

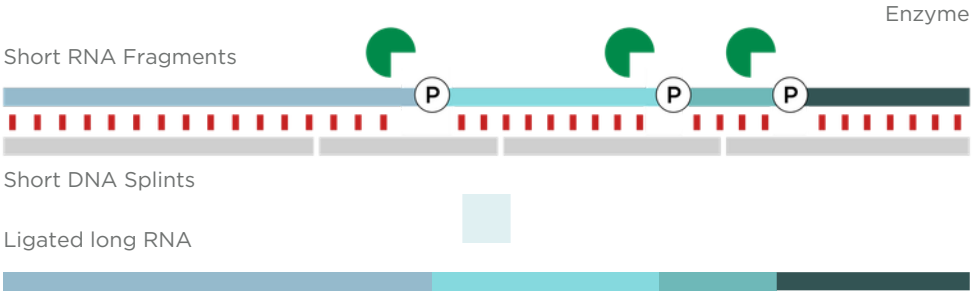
Enzymatic RNA Production

Long chain RNA production

Over 600 mer RNA production with chemical modification.

Summary of Production:

• Concept: To obtain long chain RNAs in high yield & quality



WO2024048684 Method For Producing Nucleic Acid Molecule

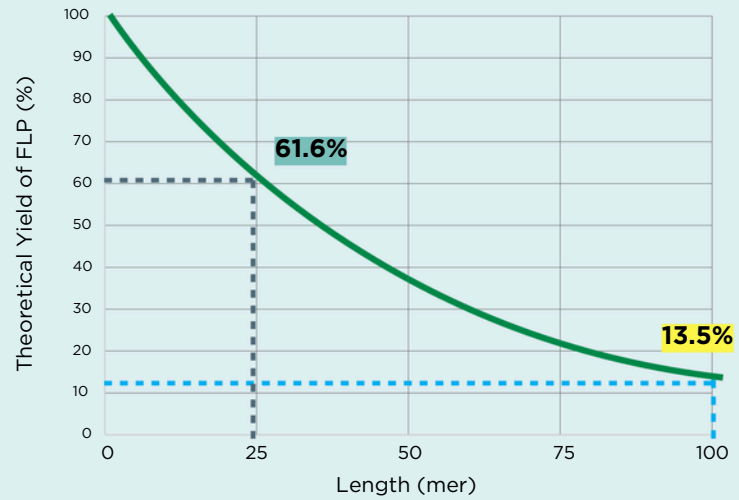
Providing long RNAs such as sgRNA and pre-miRNA
• With chemical modification (2'-F, 2'-MOE, LNA etc.).
• For analytical standard etc.

• Introduction: Chemical Synthesis vs Enzymatic Ligation

Long chain RNAs are obtained in higher yield by enzymatic ligation.

Yield of Chemical Synthesis

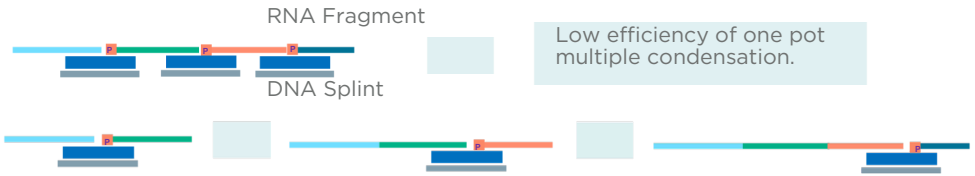
(Coupling efficiency: 98.0%/elongation)



100 MER SYNTHESIS	
Solid Phase	**13.5%** yield
Enzymatic Ligation	Solid phase synthesis of the 25 mer fragments 61.6% p p p 25 mer 90% 61.6% x 0.9 x 0.9 x 0.9 = **44.9% yield**

• Conventional Ligation Methods of Long RNA Production from Fragments

Conventional Method - 1



Low efficiency of one pot multiple condensation.

Need purification in each ligation step.

Conventional Method - 2

X

Huge burden to produce >150 mer DNA splint in high quality.

Disruption of secondary structure of RNA by long DNA splint.

Kurschat et al., RNA. 11: 1909-1914. (2005)

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• New Method of Long RNA Production from Fragments

Short RNA Fragments

Short DNA Splints

Ligated Long RNA

- DNA splints are segmented, covering all RNA sequences complementarily.
- Designed on nicked junction like cohesive ends of restriction enzyme digestion.
- Splint DNAs can be easily removed by liquid chromatography.

High Efficiency by One Pot Reaction.

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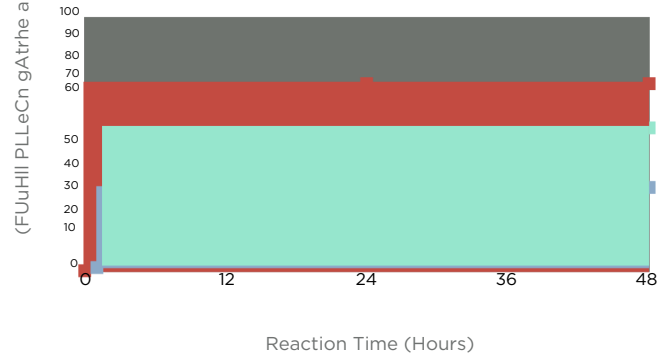
• 100 mer ssRNA Synthesis: UHPLC Analysis of the Products

New method
Conventional 1
Conventional 2



This new method for multiple ligation on long RNA production is superior to conventional methods.