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Flexible and Scalable Clean-up of in vitro Transcribed mRNA, Using Invitrogen Dynabeads™ Superparamagnetic Bead Technology

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ABSTRACT

We have developed a mRNA purification protocol based on Invitrogen Dynabeads™ MyOne™ Carboxylic Acid and a proprietary binding buffer, which gives higher yield than traditional purification methods. The workflow can be scaled up proportionally to desired reaction volumes. The yield is at least one mg of mRNA per mg of beads. In addition, prototype beads with higher binding capacity have been developed.
Our data shows binding capacity, recovery and purity of mRNA, produced by solid-phase *in vitro* transcription of PCR-templates immobilized on Dynabeads coupled with Streptavidin. Magnetic beads are easily handled by automatic work stations for small scale mRNA production. The scale-up capabilities that we have demonstrated, together with the availability of large magnets and single use equipment, shows that this technology can handle both the solid-phase mRNA synthesis and the purification of grams of mRNA in a closed system.

INTRODUCTION

The growing field of mRNA therapeutics development requires highly variable production scales, from small scale production of personalized vaccines to large scale production of prophylactic vaccines. This has put pressure on the development of flexible production workflows. Generation of mRNA by *in vitro* transcription includes several clean-up steps. Traditionally, purification techniques like phenol:chloroform followed by precipitation or spin columns are used for enzyme removal, mRNA purification and concentration. These techniques have limited capacity, need centrifugation steps and are thereby not easily automated. Chromatographic techniques like HPLC are cumbersome and expensive and need thorough optimization for each transcript type and production scale. Magnetic bead based technologies on the other hand are flexible, directly scalable and automation friendly.

MATERIALS

- Plasmid vector with a T7 promoter and a 2.5kb insert.
- Plasmid-specific PCR primers, forward primer biotinylated
- SequalPrep Long PCR kit with dNTPs (cat#A10498)
- Dynabeads™ MyOne™ E-SA (prototype, available under MTA)
- MEGAscript™ T7 Transcription kit (Cat# AM1333-reagents bought in bulk volumes)
- Dynabeads™ MyOne™ Carboxylic Acid (Cat#65012) with proprietary RNA Binding Buffer
- E-gel, 1.2% Agarose SYBR Safe (Cat#G521801)
- Qubit RNA BR assay kit (Cat#Q10211)
- Qubit dsDNA BR assay kit (Cat#Q32853)
- Qubit Protein assay kit (Cat#Q33212)

CONCLUSIONS

Solid phase *in vitro* transcription from biotinylated template immobilized to Dynabeads Streptavidin

- Template is re-usable, and removed by a magnet
- Less than 1% template leaching during IVTS (preliminary data on Streptavidin prototype bead)
- Template density on bead surface affects mRNA yield
- Distance between bead surface and T7-promoter affects yield
- Amount of T7-polymerase and NTPs, affects yield the most

mRNA purification/upconcentration by generic capture on Dynabeads MyOne Carboxylic Acid and prototype beads

- High yield ➔ more than 1.6 mg mRNA/mg beads
- No protein detected by Qubit
- Less than 0.4% of original template carry over to purified mRNA, when omitting DNase treatment

Up-scaling of complete workflow from microtubes to large reactors

- Complete workflow is directly scalable from microliters to liters

RESULTS

Solid-phase in vitro transcription and mRNA purification workflow

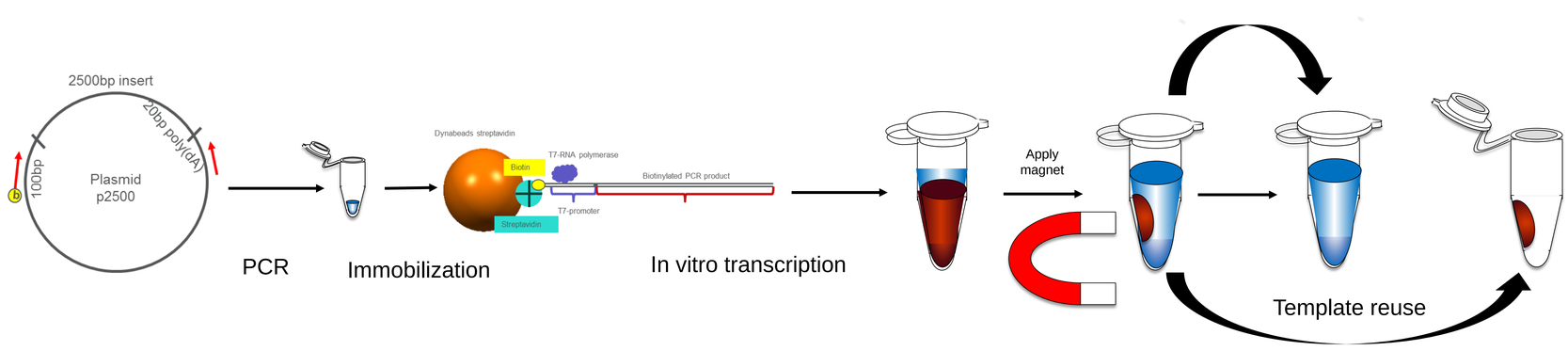


Figure 1. Synthesis of mRNA by Solid-phase *in vitro* transcription (IVTS) of template immobilized on Dynabeads™ Streptavidin
PCR was performed on 10 x 10 ng of plasmid DNA containing a 2,500 bp insert, using a biotinylated forward primer and a non-biotinylated reverse primer, spanning the T7 promoter, the insert and the poly(A)-tail. The PCR typically yields 10 x10 µg of PCR product, which is immobilized on streptavidin beads at a concentration of 1 µg/mg beads.
In vitro transcription has been performed in scales reaching from 1 mg of beads in 50 µL IVTS to 100 mg of beads in 100 mL IVTS, for 6 hours to over night. Typical yields are more than 4 mg/mL of mRNA.

mRNA purification

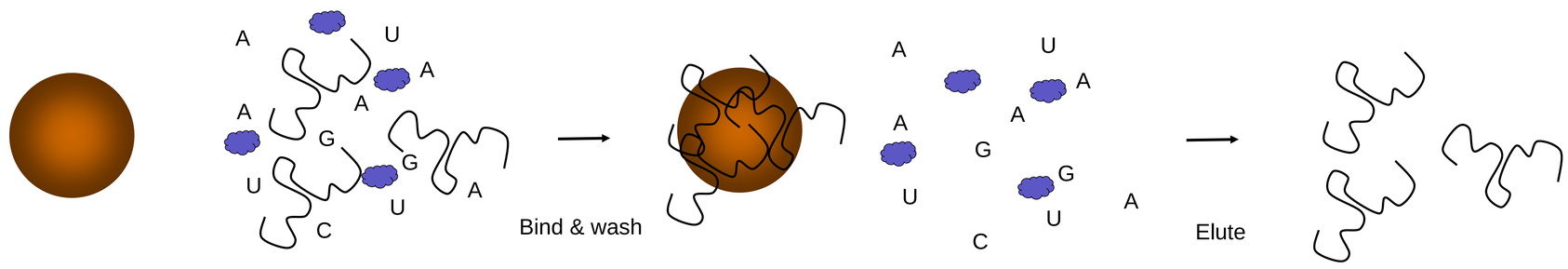
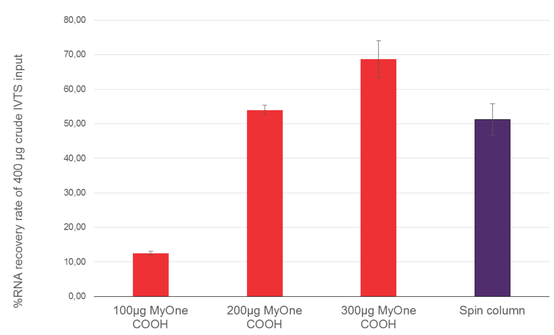


Figure 2. Purification of *in vitro* transcribed mRNA by generic capture onto Dynabeads™ MyOne™ Carboxylic Acid
Dynabeads MyOne Carboxylic Acid are mixed with *in vitro* transcribed mRNA at approximately 1:1 mg:mg ratio, and added 1.5 x RNA Binding Buffer.
Binding occurs at room temperature under moderate shaking conditions. The bead-RNA complex is washed 3 times, and supernatant discarded. The bead pellet is optionally left to dry for 5 minutes, before adding elution buffer, and the purified mRNA is eluted by vigorous shaking for 5 minutes.

Yield and purity of mRNA purified by different amounts of Dynabeads MyOne Carboxylic Acid, compared to spin column

a)



b)

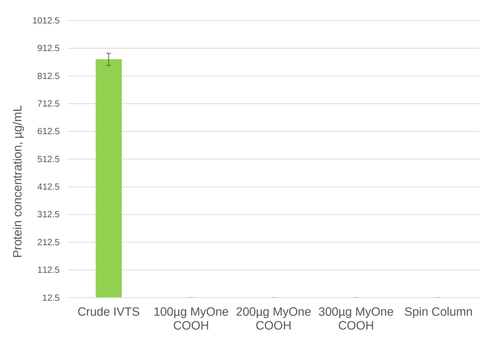


Figure 3. Yield and purity of mRNA, Dynabeads™ MyOne™ Carboxylic Acid versus spin column
Histogram showing the mRNA yield from 1 µg of template purified by different amounts of Dynabeads MyOne COOH, in comparison to spin column. The results show that magnetic bead purification is scalable, while spin columns have an upper limitation in small scale.
a.Yield of mRNA from 400 µg crude IVTS
b.Purity of mRNA determined by Qubit

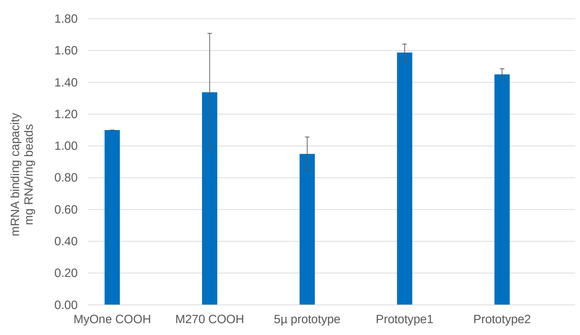


Figure 4. Increased recovery with prototype beads
Histogram showing the recovery of mRNA by purification with Dynabeads MyOne COOH prototypes, in comparison to Dynabeads MyOne Carboxylic Acid. Prototype 1 beads yield about 1.6 mg mRNA per mg beads, and further improvement is being investigated.

Scaling up the complete workflow – from micrograms to grams

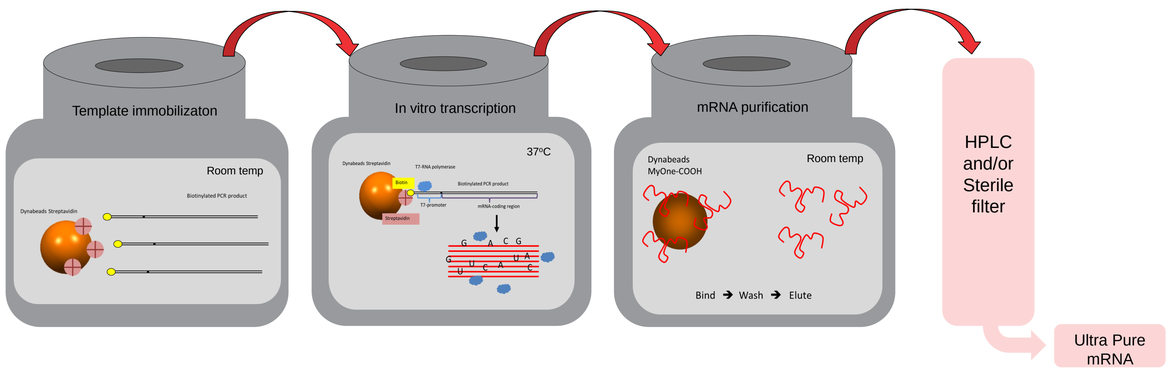
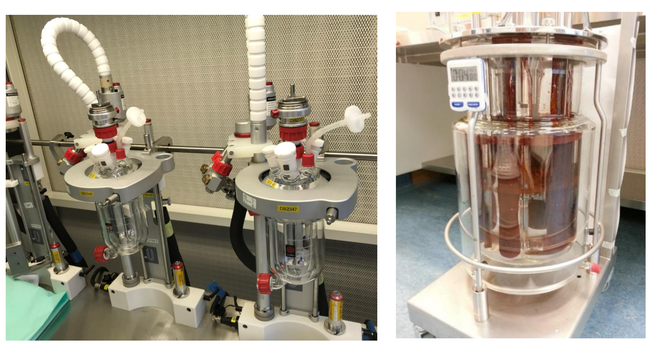


Figure 5. Solid-phase *in vitro* transcription and mRNA purification can be scaled up in a flexible format, using reactors with external magnets or internal magnetic rods.



Scalable volumes 50 mL - 500 mL to several liters, depending on choice of reactor – with external magnet or magnetic rods

CLOSING REMARKS

Magnetic bead handling is flexible, scalable, and easy to automate, from high throughput small scale production to large production volumes in closed systems

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