invitrogen

Simple and scalable production of mRNA based on Invitrogen Dynabeads™ superparamagnetic beads

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ABSTRACT

The mRNA vaccine market is still in its infancy, and the production processes and quality requirements of the mRNA are not yet standardized. One of the challenges is the need for scalable production, ranging from low volume production for personalized cancer therapeutics to large volume production for prophylactic vaccines for larger populations. Production includes many steps in template generation, mRNA synthesis and the following purification. Magnetic beads are already known to be suitable for small scale mRNA generation and purification for personalized therapy purposes. Here we demonstrate how large scale production of mRNA can be done, using Invitrogen Dynabeads™ superparamagnetic beads.

INTRODUCTION

We present results demonstrating how scalable amounts of mRNA can be generated from a 2500bp biotinylated DNA template immobilized on streptavidin coated Dynabeads™. We compare performance of streptavidin beads with different sizes and surface chemistries, and identify the most important parameters for maximizing mRNA yield. Animal origin-free streptavidin bead prototypes of two different sizes, which are ideal for GMP production, are included, showing how bead size and template density on the bead surface affect the mRNA yield.
We also demonstrate how the need for template preparation and bead consumption can be minimized while high yields of mRNA are obtained, by reusing the solid-phase template. The template on the beads could be reused 5 times, without significant reduction in mRNA yield, indicating that the leakage of templates from the beads is minimal. The template is held back by a magnet and the need for DNase treatment is thereby most likely unnecessary or minimal.
We believe the reaction can be directly scaled up into reactors, as magnetic beads are easily manipulated in reactors with magnetic rods.

MATERIALS

- Plasmid vector with a T7 promoter and a 2.5kb insert.
- Plasmid specific primers, forward biotinylated
- SequalPrep Long PCR kit with dNTPs (cat#A10498)
- Dynabeads™ M-280 Streptavidin (SKU# 11206D, 35136)
- Dynabeads™ M-270 Streptavidin (SKU#65305)
- Dynabeads™ MyOne C1 (SKU#65001)
- Dynabeads™ M-450 E-SA (prototype, available under MTA)
- Dynabeads™ MyOne E-SA (prototype, available under MTA)
- MEGAscript™ T7 Transcription kit (Cat# AM1333)
- E-gel, 1.2% Agarose SYBR Safe (Cat#G521801)
- Qubit-DNA (dsDNA BR assay kit) (Cat#Q32850
- Qubit-RNA (RNA BR assay kit) (Cat#Q10211)

CONCLUSIONS

- Templates immobilized on Dynabeads™ Streptavidin beads yield highly efficient *in vitro* transcription.
- Immobilized template can be re-used in sequential *in vitro* transcription reactions, up to 5 times without significant loss of mRNA transcription efficiency.
- The epoxy based (MyOne and M-450) Dynabeads™ prototypes and the Dynabeads™ M-280 Streptavidin, allow the highest number of reuse.
- mRNA yield is dependent on template density on the bead surface.
- The template is easily removed from the produced mRNA by magnetic separation and may omit the need for DNase treatment.
- The typical yield is 4mg/mL mRNA produced, and we see that the most important limiting factor for mRNA production is the availability of building blocks.

CLOSING REMARKS

Magnetic bead handling is flexible, scalable and easy to automate, possibly in a continuous flow setup for large scale mRNA production.

RESULTS

How to get from 100 ng plasmid DNA to 100 mg of mRNA, by using Dynabeads™ Streptavidin

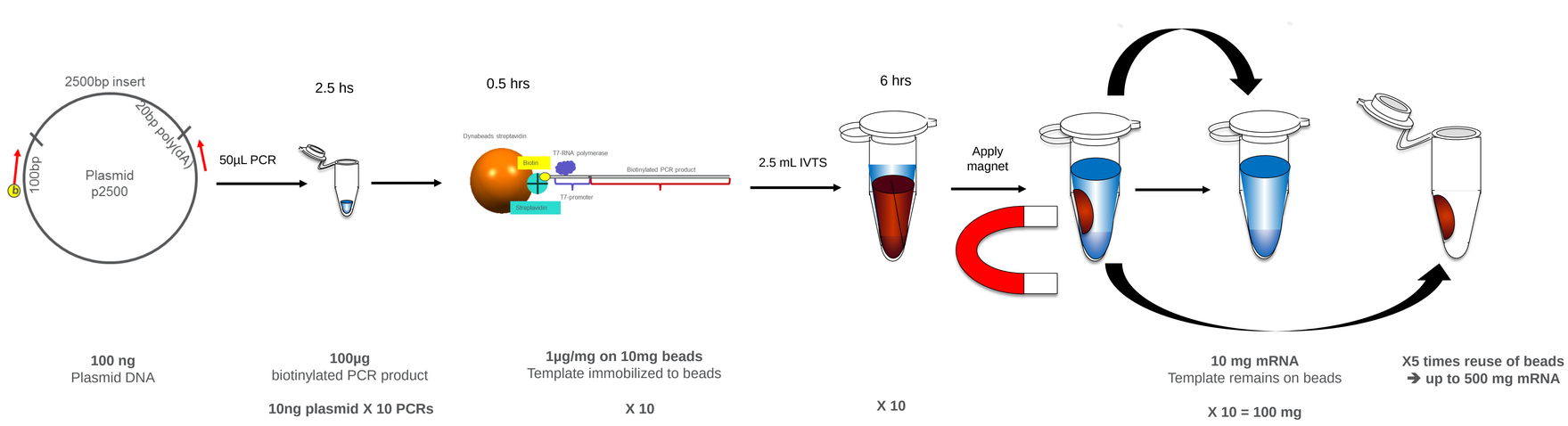


Figure 1. Solid-phase *in vitro* transcription of template immobilized on Dynabeads™ Streptavidin
PCR was performed on 10x10ng of plasmid DNA containing a 2500bp insert, using a biotinylated forward primer and a non biotinylated reverse primer, spanning the T7 promoter, the insert and the poly(A)-tail
The PCR typically yield 10x10µg of PCR product, which is immobilized on streptavidin beads at a concentration of 1µg/mg beads
In vitro transcription can be performed, using 10mg of beads in a 2.5mL IVTS for 4-6 hours, with a typical yield of 4mg/mL mRNA. This can easily be scaled up, by doing 10 reactions in parallel or 1 large reaction, with proportional outcome of mRNA. In addition, the beads with template can be reused up to 5 times, without significantly losing mRNA yield.

Choice of streptavidin bead

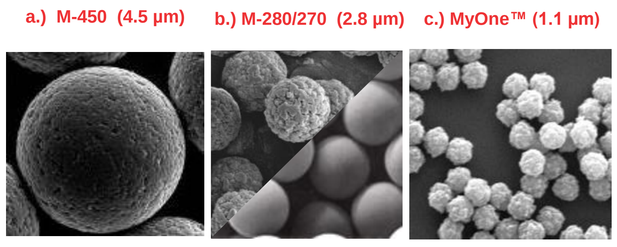


Figure 2. Streptavidin beads are made in different sizes and chemistries
Scanning electron micrographs showing the different Dynabeads™ sizes
a) Dynabeads™ M-450 Streptavidin (Epoxy based prototype bead)
b) Dynabeads™ M-280/M-270 Streptavidin (M-280 Tosyl/M-270 Carboxylic acid based, respectively, and M-270 Epoxy based prototype bead)
c) Dynabeads™ MyOne Streptavidin C1 and E (Carboxylic acid and Epoxy based beads, respectively)

Effect of bead size and chemistry during reuse of template for *in vitro* transcription

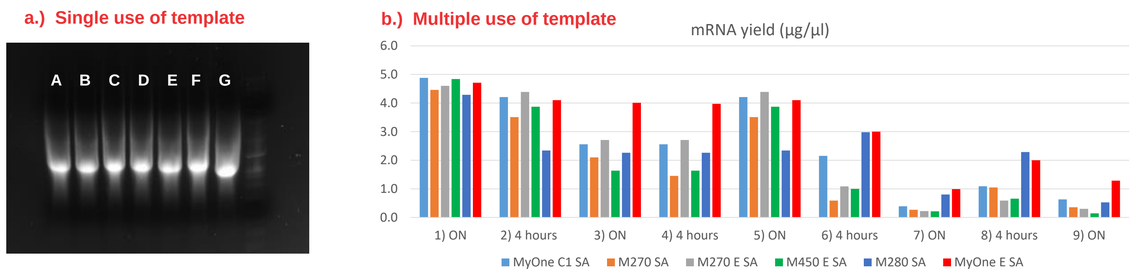
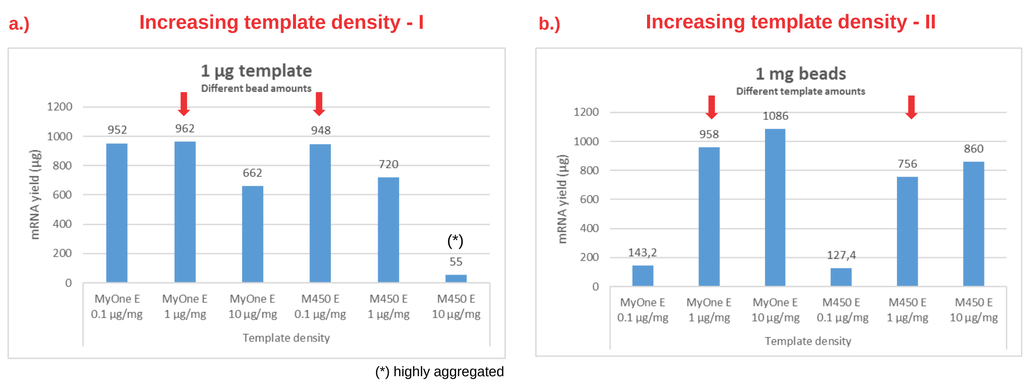


Figure 3. Comparison of performance of 6 different streptavidin beads in solid phase *in vitro* transcription
A 2500bp template was immobilized to different streptavidin beads at 1µg/mg concentration and *in vitro* transcribed in multiple rounds
a) Gel showing mRNA produced from 1µg 2500bp template immobilized to 1mg of different streptavidin beads (A:MyOne C1 SA, B: M-270 SA, C: M-270 Epoxy SA, D: M-450 Epoxy SA prototype, E: M-280 SA, F: MyOne E SA prototype. G: is the MEGAscript™ kit control
b) Reuse of the same bead-template complexes in 9 successive IVTS-reactions, showing that most of the beads can be reused at least 5 times without significant loss of template. The one micron, epoxy based MyOne Streptavidin E-SA prototype is the most stable, still yielding mRNA after 9 times re-use and 110 hours at 37ºC.

Effect of template density on bead surface



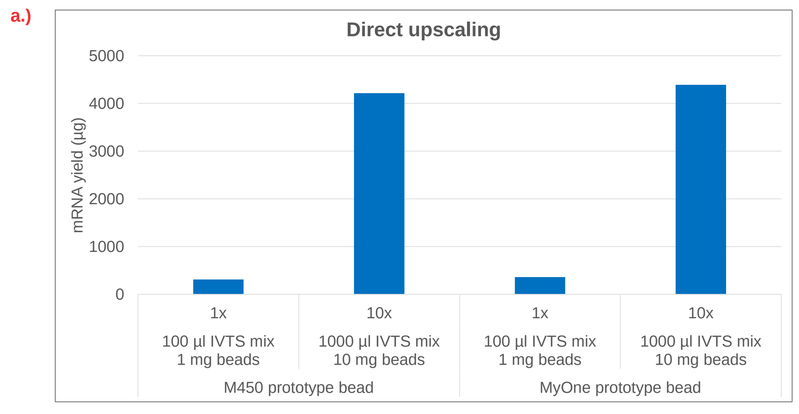
(*) highly aggregated

Figure 4. Template density on bead surface affects yield of *in vitro* transcription
a) Histogram showing the mRNA yield from 1µg of template, immobilized on two different streptavidin beads at different densities: 0.1µg/mg, 1µg/mg and 10µg/mg, showing that for MyOne beads, the 1µg/mg density gives highest template utilization. For M-450 beads, 0.1µg/mg gives higher template utilization.
b) Histogram showing the mRNA yield from the same beads as in (a), but comparing constant bead amount, thereby increasing total template added. Again, increasing template density above 1µg/mg shows decreased template utilization.
Total template amount and template density, and not total bead amount are important factors for optimal mRNA yield.

Bead	Bead size in diameter (µm)	Surface area (cm^2/mg)	% Immobilization at 1µg/mg (2500bp)	Relative template density at 1µg/mg (2500bp)
M450-E-SA	4.5	27	92	5
M280-SA	2.8	60	98	2
M270-SA	2.8	40	90	2.5
M270-E-SA	2.8	40	97	2.5
MyOne-C1-SA	1	130	96	1
MyOne-E-SA	1	130	98	1

Table 1. Dynabeads™ Streptavidin beads facts
Table showing bead size, surface area and efficiency of immobilization of the 2500bp template, when added at 1µg template per mg beads. All beads show 90% or more immobilization of biotinylated template.

Scaling up



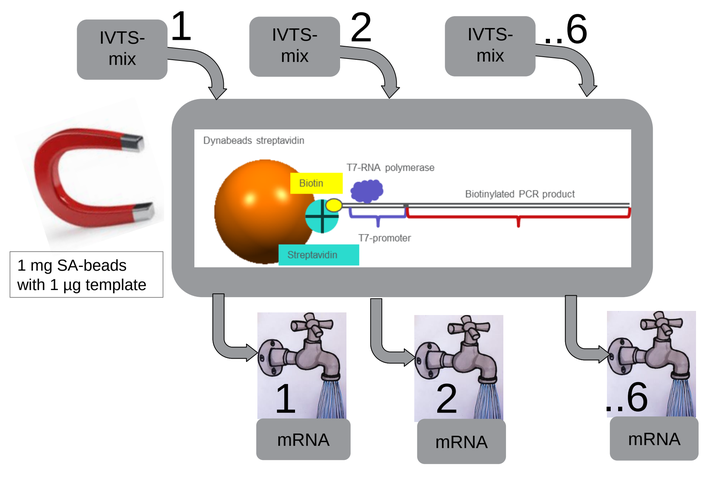


Figure 5. Solid-phase *in vitro* transcription can be scaled up in a flexible format
a) Comparison of MyOne E SA and M-450 SA performance scaled up to 1 mL, showing that reactions can be directly scaled up. A 1mL reaction using 10mg of bead-DNA complex, gives more than 4mg of mRNA. With 5 times reuse, this will produce 20mg of mRNA. This indicates that 100mg of mRNA can easily be produced in a 50mL tube.
b) Templates immobilized on Dynabeads™ Streptavidin can withstand 60 hours at 37ºC, without significantly losing transcription efficiency. This will most likely enable a continuous flow set up, where new building blocks are added and newly synthesized mRNAmay be poured off in small volume equipment.

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