Solid-phase *in vitro* Transcription and mRNA Purification using Dynabeads™ Superparamagnetic Beads

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ABSTRACT

mRNA-based therapeutics has shown great promise in prevention and treatment of multiple diseases. New tools and scalable workflows for mRNA vaccine development and production will be critical for bringing this promising technology to the clinic.
Here we present a proof-of-concept using magnetic beads to simplify the *in vitro* transcription of mRNA, downstream of mRNA purification.

Streptavidin functionalized Invitrogen Dynabeads™ supermagnetic beads were used for solid-phase *in vitro* transcription, which generates high quality mRNA, starting with a re-usable biotinylated DNA template immobilized to the beads. The template was easily removed after mRNA transcription by applying a magnet. The immobilized template could be stored for repeated *in vitro* transcription reactions.

After removal of the magnetic beads, the *in vitro* transcript (polyadenylated mRNA) was free of template. Further purification and up-concentration was performed by hybridization to Dynabeads™ Oligo(dT)$_{25}$, providing high quality mRNA with intact poly-A tail. The demonstrated workflow using magnetic beads to immobilize the DNA template, perform *in vitro* transcription and mRNA isolation, is scalable and easily automated for high throughput and high reproducibility.

Bringing mRNA therapy to the clinic will include increasing regulatory requirements. Invitrogen Dynabeads™ have proven track-records with more than 30 years experience in customer partnership and know-how within the field of immune therapy and current Good Manufacturing Practice (GMP).

MATERIALS

- CK19 cDNA cloned into pGEM 4Z-polyA(*) vector
- pCMV-Red Firefly luc plasmid
- Dynabeads™ M-280 Streptavidin (SKU# 11206D, 35136)
- Dynabeads™ mRNA purification kit (SKU# 65005, 61006)
- Dynabeads™ Oligo(dT)$_{25}$ (SKU# 61005)
- Dynabeads™ MyOne SILANE (SKU# 37002D)
- Dynabeads™ M-270 Carboxylic acid (SKU# 14306D)
- Dynabeads™ MyOne Carboxylic Acid (SKU# 65012)
- MEGAscript™ T7 Transcription kit (Cat# AM1333)
- mMESSAGE mMACHINE™ T7 ULTRA Transcription Kit (AM1345)
- Poly(A) Tailing Kit (AM1350M)
- MEGAClear™ Transcription Clean-Up Kit (Cat# AM1908)
- Invivofectamine mRNA™ Reagent (custom formulation)
- BALB/C Mice, female, 4-6 weeks

CONCLUSIONS

- *In vitro* transcription can be efficiently performed from a biotinylated template immobilized on Dynabeads™ Streptavidin beads.
- Immobilized template can be re-used in sequential *in vitro* transcription reactions.
- Magnetic removal of template can omit the need for DNase treatment.
- *In vitro* transcribed mRNA purified by Dynabeads™ Oligo(dT)$_{25}$, is functional *in vivo*.
- Higher concentrations of mRNA can be obtained by including a concentration step, using different types of Dynabeads™ for generic binding and elution.
- Prototype beads with higher capacity for mRNA hybridization are under development.
- Magnetic bead handling is flexible, scalable and easy to automate.

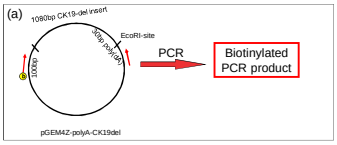
ACKNOWLEDGEMENTS

(*) The pGEM4Z-polyA vector was kindly provided by Professor Dr. Joakim Lundeberg, Division of Gene Technology, KTH Royal Institute of Technology, Sweden.

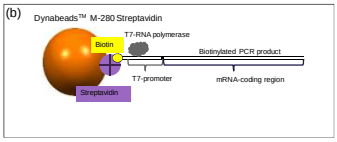
RESULTS

Solid-phase *in vitro* transcription work flow

Template generation



Template immobilization and *in vitro* transcription



Crude *in vitro* transcript

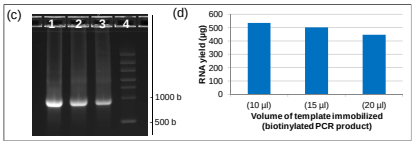
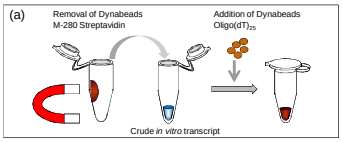
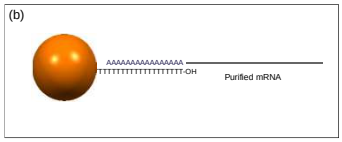


Figure 1. Solid-phase *in vitro* transcription of template immobilized on Dynabeads M280 streptavidin
(a) The vector region spanning the T7 promoter, and the cloned cDNA insert including 30 adenine bases, was amplified by PCR using a biotinylated forward primer and a non-biotinylated reverse primer.
(b) The biotinylated template was immobilized on Dynabeads™ M-280 Streptavidin and *in vitro* transcription performed by resuspending the beads with template in a transcription mix added T7 RNA polymerase.
(c) Gel and (d) histogram showing crude *in vitro* transcript yield (measured by A260 on the NanoDrop One C instrument) of three 50 µL reactions, using 1 mg of Dynabeads™ M-280 Streptavidin immobilized with 10, 15 and 20 µL of the PCR product. Highest yield obtained with lowest template amount. Further titration ongoing.

Magnetic removal of immobilized template



mRNA purification by oligo(dT) hybridization



Yield of mRNA in scalable protocols

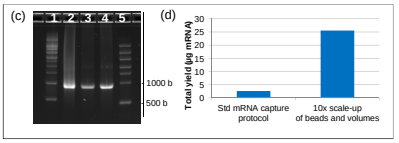


Figure 2. Purification of mRNA by hybridization to Dynabeads™ Oligo(dT)$_{25}$
(a) DNA-template was removed from the *in vitro* transcript by applying a magnet, and Dynabeads™ Oligo(dT)$_{25}$ were added to the *in vitro* transcript in the supernatant.
(b) Hybridization was performed for 5 minutes and the beads were washed twice. The mRNA was eluted at 80° C for 2 minutes, and the yield measured by A260 on the NanoDrop One C instrument.
(c) Gel showing crude *in vitro* transcript (lane 2), mRNA purified using Dynanbeads™ Oligo(dT)$_{25}$ standard protocol (lane 3), and 10 times increased bead amount in standard binding volume (lane 4). Lanes 1 and 5 are Millenium marker and RiboRuler ladder, respectively.
(d) Histogram, showing the yield of mRNA isolated using standard protocol and a 10 times direct up-scaling of both bead amount and buffer volumes.

Re-use of solid-phase template in sequential reactions

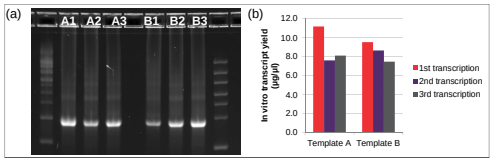


Figure 3 Re-use of solid-phase template in sequential *in vitro* transcription reactions
Gel (a) and histogram (b) showing the *in vitro* transcript yield from two templates (A and B) immobilized on 1 mg streptavidin beads, re-used in three sequential 50 µL reactions.

Up-concentration of captured mRNA using Dynabeads™ MyOne Silane

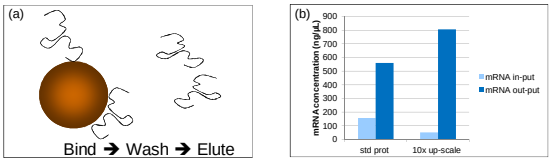
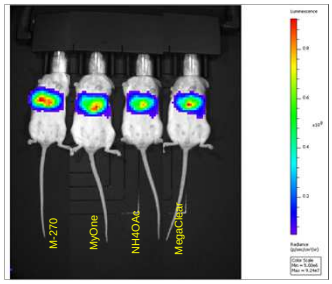


Figure 4. Up-concentration of mRNA eluate from Dynabeads™ Oligo(dT)$_{25}$
(a) Generic capture workflow, using Dynabeads™ MyOne SILANE to up-concentrate the mRNA
(b) Concentration of mRNA , measured by A260 on the NanoDrop One C instrument, before and after the up-concentration step. The mRNA was from the standard and the up-scaled isolation protocol in Figure 2(d).

Functionality of oligo(dT)-purified mRNA

(a) ***In vivo* Imaging Systems – 4hr post injection**



(b) **Luciferase signal quantification**

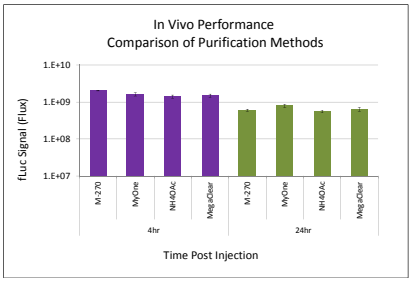
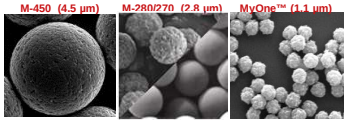


Figure 5. Functionality comparison between mRNA isolated using different oligo(dT)$_{25}$ bead prototypes and traditional purification methods
In paralell with solid-phase *in vitro* transcription, a standard *in vitro* transcription experiment was performed: *in vivo* delivery and functionality of mRNA purified by prototypes of Dynabeads™ M-270 and Dynabeads™ MyOne Carboxylic Acid coupled with oligo(dT)$_{25}$ using proprietary coupling protocols were studied using a luciferase template model. Purified mRNA was intraveneously injected into mice in Invivofectamin, using 0.5 mg/kg and two mice per group.
(a) Image showing *in vivo* bioluminiscense of 4 different mice, injected with mRNA purified by Dynabeads™ M-270 and Dynabeads™ MyOne prototypes, NH_4OAc precipitation and MegaClear™, respectively, demonstrating that all mRNAs had similar activity.
(b) Quantification of the luciferase activity after 4 hrs and 24 hrs, again showing similar activity between purification methods.

CLOSING REMARKS

With more than 30 years experience in customer partnership and know-how within the field of immune therapy and GMP, we enable our customers to develop high quality work-flows. Our tools are based on a comprehensive range of Dynabeads™ monosized superparamagnetic beads, and we offer custom made solutions with beads of different sizes and surface functionalities.



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