Development of a new magnetic bead platform for use in GMP production of mRNA vaccines

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ABSTRACT

The success of mRNA vaccines in fighting the SARS-CoV-2 pandemic has brought the world into a new era of vaccine development. The production methods are not yet standardized and the need for flexible and highly scalable production of mRNA is still urgent.

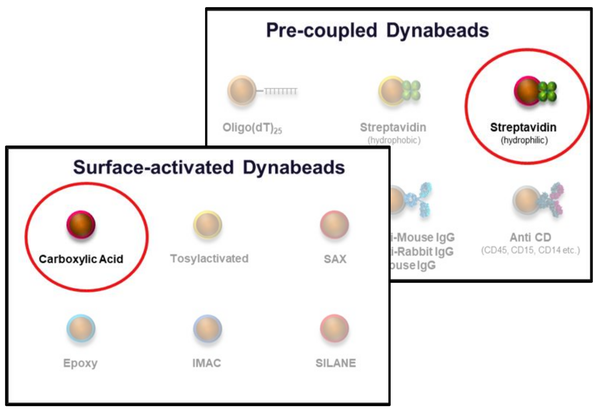
Thermo Fisher's magnetic bead technology enables simplified workflows with less bioprocessing steps and flexible strategies for scaling up mRNA synthesis and purification. The same technology is easily adapted from research scale to industrial volumes through a modular approach.

The high regulatory demands for documentation of both safety and performance of raw materials/ancillary materials being used in GMP manufacture of therapeutic agents are being addressed through development of a new Dynabeads™ magnetic bead platform and two new products.

The two Dynabeads products on the platform include a streptavidin conjugated bead for solid-phase in vitro transcription and a carboxylic acid activated bead for generic capture purification of mRNA. The beads are optimized for magnetic separation and workflow performance and are designed to be suitable as raw materials/ancillary materials for use in GMP manufacturing of mRNA vaccines.
Manuals for a complete mRNA synthesis and purification workflow with performance data and analytical data on mRNA yield, integrity and purity will be presented.

INTRODUCTION

Dynabeads have been used in both research, diagnostic platforms and cell therapy for many years, and are available in many different sizes and surface properties.



Dynabeads are recognized by high quality and reproducibility, and together with the mode of scalability, the Dynabeads magnetic beads are also showing its value in production of biomolecules. We are therefore developing a completely new magnetic bead platform to meet the regulatory requirements for the use in manufacturing of mRNA vaccines, as shown in the panel below.

Next generation products –
in development (Est launch June 2022)

Products
- Carboxylic Acid bead (generic capture)
- Streptavidin bead (solid-phase IVT)

Added value:
- Optimized to ease automation - faster to magnet, better handling and improved bead pellet
- Products developed towards mRNA therapeutics (quality, regulatory and functional/performance requirements; Non-animal derived raw materials)
- Expanded regulatory support documentation

Quality control will include Total Microbial Count, RNase detection, Endotoxin detection

Quality system: ISO 13485

MATERIALS AND METHODS

- A plasmid models system containing a T7 promoter and a 2.5kb insert
- Biotinylated PCR forward primer located upstream of the T7 promoter and nonbiotimylated reverse primer.
- Dynabeads™ MyOne Carboxylic Acid (SKU# 65012)
- Dynabeads™ MyOne Streptavidin prototypes
- Dynabeads™ MyOne Carboxylic Acid prototypes
- SequalPrep long PCR kit Cat#A10498
- MEGAscript™ T7 Transcription kit (Cat# AM1333) and bulk reagents for large scale
- Qubit 1x dsDNA HS Assay kit, Invitrogen, cat# Q33231
- Qubit RNA BR Assay kit, Invitrogen, cat# Q10211
- BioAnalyzer
- Syrris orb reactor system
- 1.5x RBB Cat# D37035D (RNA Binding Buffer)
- Agilent 2100 Bioanalyzer, prod# G2938B
- Agilent RNA 6000 Nano kit & Chip, Cat#5067-1511

RESULTS

Figure 1. The complete Dynabeads workflow: 100 micrograms of plasmid DNA can give up to 240 grams of RNA

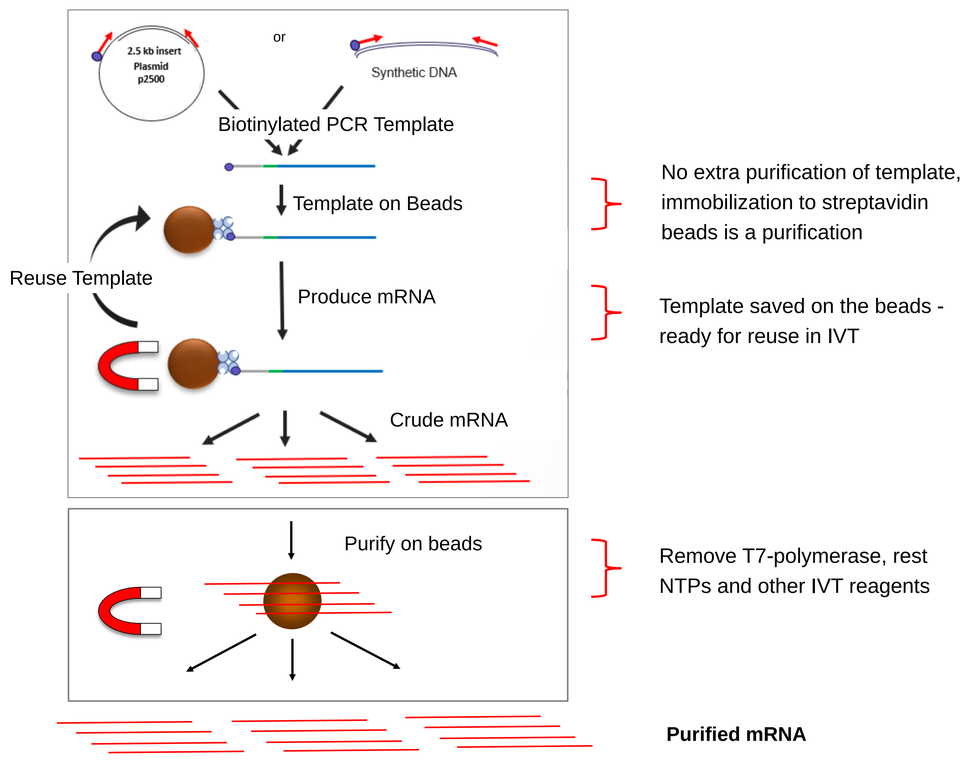


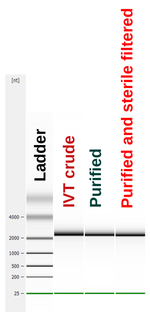
Figure 1. The solid-phase in vitro transcription and purification workflow

- A biotinylated DNA template is immobilized to Dynabeads streptavidin beads at a density of 1µg template per mg beads
- The bead-DNA template is added in vitro transcription (IVT) reagents and incubated at 37ºC, with mixing.
- After IVT completion, the DNA template is removed from the newly synthesized RNA, by applying a magnet.
- The bead-template complex can be reused 3-6 times, by added fresh IVT reagents, as shown in figure 2.
- The integrity of the crude RNA is consistent during reuse, as shown in figure 3.
- Leaching of the immobilized template DNA is low, as shown in figure 4.
- The IVT RNA is purified by generic capture on to Dynabeads carboxylic acid beads in a proprietary binding buffer.
- The recovery of RNA is high, above 90% as shown in figure 5A.
- The integrity of the purified RNA is high, as shown in figure 5B.
- Protein removal is high >99% as shown in figure 5C.
- The complete workflow is dynamically scalable from 100mL up to 1 liter in reactors, as presented in figure 6.

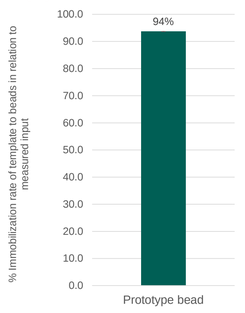
Figure 6: Scale-up of the complete workflow

(A) Reactor set-up

Template immobilization, In vitro transcription, and mRNA purification in 1L glass reactor

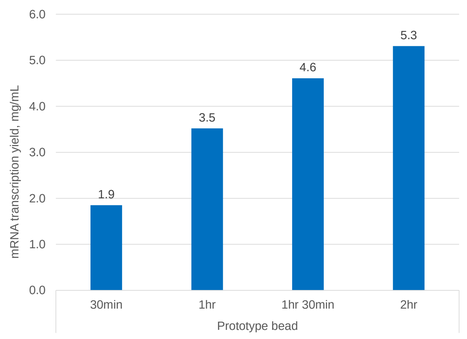


(B) Template immobilization



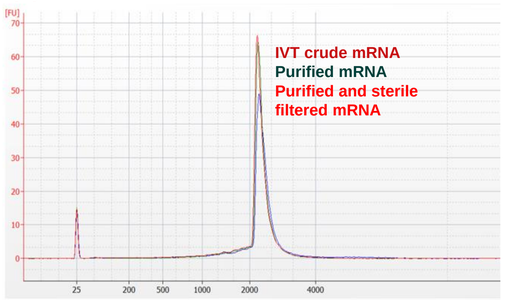
Immobilization of template DNA:
94% of template added was immobilized

(C) Solid-phase IVT



In vitro transcription «sampling» evey 30 minutes
Crude IVT-mRNA: Conc: >5mg/mL in 1L

(D) Purification of RNA by generic capture



Purified mRNA: «100%» recovery

Figure 6 The complete workflow, including template immobilization, IVT and RNA purification was dynamically scaled up to 1 L reaction volumes, in Syrris Orb glass reactor system (A). Results from this large-scale workflow show very similar results as the low-scale workflow in Eppendorf tubes, with more than 90% template immobilization (B) and an RNA yield of more than 5 mg/mL after 2 hours incubation (C). The IVT synthesis was monitored by taking out samples every 30 minutes during synthesis, as shown in the histogram. The BioAnalyzer electropherogram confirms high RNA integrity in both the crude IVT-mix, the purified RNA and the purified and sterile filtered RNA samples (D).

CONCLUSIONS

- Products manufactured under a mature quality system/ISO 13485 and designed to be suitable as raw materials/ancillary materials for use in GMP manufacturing of therapeutics; Dynabeads used by regulated markets for >25 years, including therapy
- Easily accessible, automatable, flexible and scalable workflow
- > 10,000 x higher mRNA output from plasmid preparation
- > 10,000 x reduction in antibiotics use for plasmid preparation
- Reduced number of bioprocessing steps
- Synthesis and purification steps by same technology suitable for diverse volume ranges (from µL to L scale)

Figure 2. Reuse of template in IVT

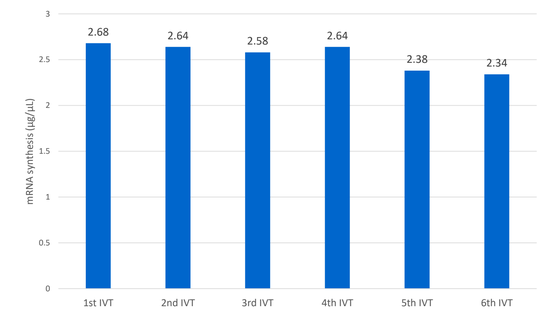


Figure 2. A 2.7 kb biotinylated PCR product, containing the T7 promoter was immobilized prototype beads at a density of 1µg/mg beads, giving more than 90% immobilization (data not shown).
The bead-DNA complex was used as template in IVT using 100µL MEGAscript mix for 1 hour at 37ºC. The bead-DNA complex was reused in a total of 6 cycles of IVT, by adding fresh reagents after transfer of the synthesized RNA to clean tubes. Each reaction gave an RNA yield of 2.7-2.3mg/mL after 1 hour.

Figure 3. Integrity of crude IVT RNA after template reuse

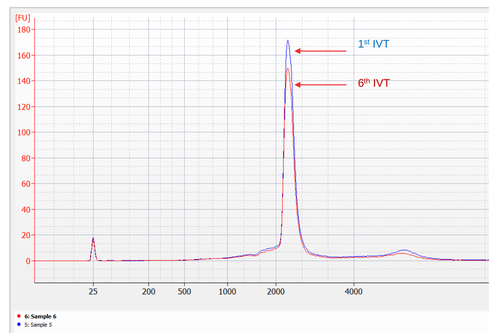


Figure 3. BioAnalyzer Electropherogram of IVT RNA from the 1st and 6th round of template reuse, confirm a consistent integrity of the synthesized RNA during template reuse.

Figure 4. Template leaching during in vitro transcription

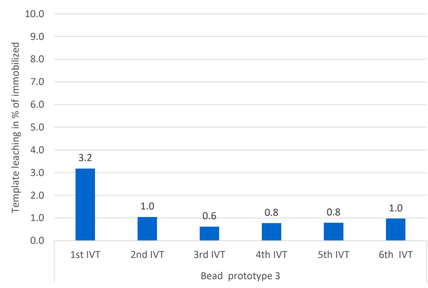


Figure 4. A TagMan qPCR assay was used to quantify the degree of template leaching from the beads during repeated cycles of IVT. Results showed that around 3% of the immobilized template leaches in the first IVT-cycle and around 1% in the successive 5 cycles of IVT.

Figure 5. Generic Capture Purification of IVT RNA

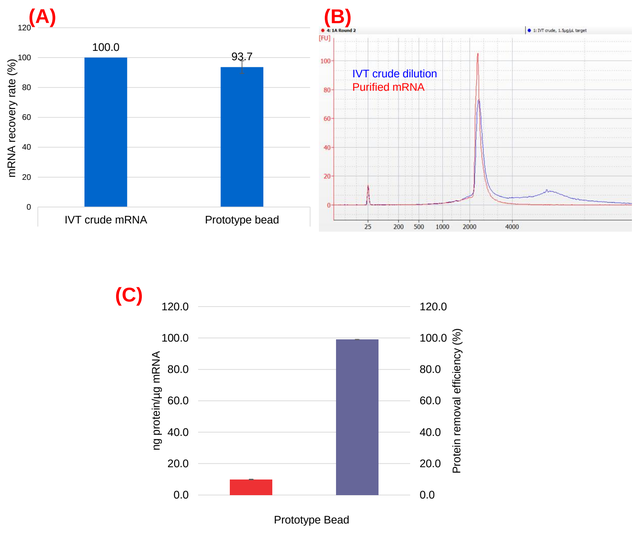


Figure 5. The IVT RNA was purified by generic capture onto the prototype beads and analyzed for (A) yield (Qubit), (B) integrity (BioAnalyzer) and (C) protein removal in blue and carryover (microBCA assay). Results showed that the yield is close to 100%, and the protein removal is close to 100% showing around 9 ng protein present in 1µg of RNA.

For research use or further manufacturing. Not for diagnostic use or direct administration into humans or animals.