Improving mRNA Quality by Removal of Truncated mRNA Species by Oligodt Purification


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Due to low quality of the DNA template, or high complexity of the transcribed sequence, it is possible that transcription is prematurely terminated. When this occurs, it gives rise to truncated RNA fragments. While the presence of high levels of truncated RNA impurities can often be resolved using codon optimization or increasing the quality of the DNA template, there is cases where the presence of truncated RNA impurities has to be addressed in the downstream process. Affinity purification of mRNA using oligo(dT) chromatography is a well-established process that – besides its capacity to potently remove components of the IVT reaction – is especially suited to the removal of truncated mRNA impurities [1].

The technique utilizes short, 2′-deoxythymidine (dT) oligonucleotides, usually 18- or 25-mers, attached to a stationary phase using a linker. Oligo(dT) purification is a highly flexible and scalable chromatographic process that can be performed using magnetic beads or column chromatography and can be applied at a wide range of scales ranging from early R&D to commercial mRNA manufacturing [2, 3]. Purification is performed by balancing electrostatic repulsion of the negatively charged phosphate groups and base pairing between the oligo(dT) sequence and the poly(A) tail of the in vitro transcribed mRNA. By adding a moderate concentration of salt, the RNA relaxes and electrostatic repulsion between the phosphate groups of the oligo(dT) sequence and the mRNA is significantly reduced. This allows base pairing to occur between the thymidine groups fixed on the stationary phase and the adenosines in the poly(A) tail of the mRNA (Figure 1).

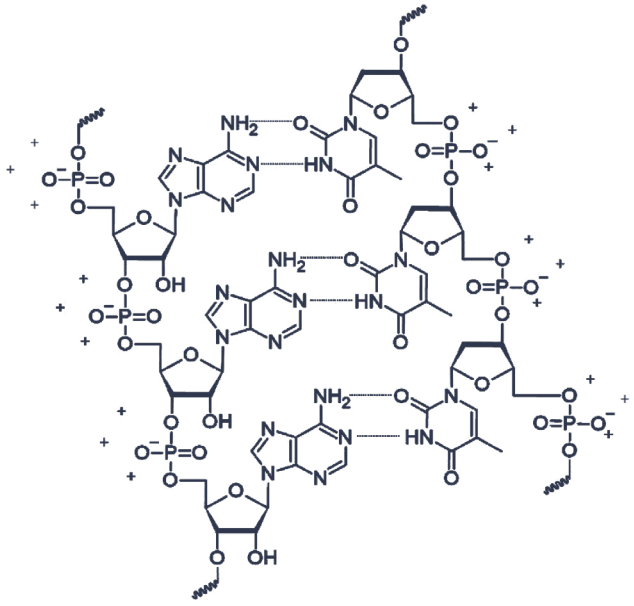

Figure 1

Base pairing between the poly(A) tail of mRNA and oligo(dT) in the presence of a salt.

Under these conditions, truncated RNA fragments that do not contain a poly(A) tail but do contain longer sequences of adenosine, may have sufficient affinity for the oligo(dT) resin. The presence of non-canonical base pairs other than the Watson-Crick A-T base pair may also give rise to low levels of non-specific binding [4]. In order to remove these undesired fragments, the salt content of the mobile phase can be reduced. This increases the electrostatic repulsion between the oligo(dT) sequence and the mRNA backbone. By finetuning the salt content during this wash step, a process can be developed wherein only molecules that strongly bind to the oligo(dT) sequence are finally eluted.

When performing oligo(dT) purification on a novel construct, it is advisable to perform the procedure using a stepwise reduction of the salt content of the mobile phase, as demonstrated in Figure 2. This approach allows multiple wash fractions to be collected and analyzed, facilitating product characterization and optimization of the purification process.

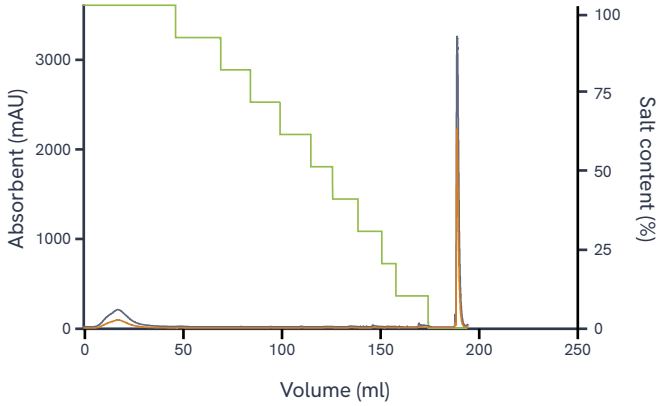

Figure 2

Chromatogram for oligo(dT) purification using stepwise reduction of salt concentration. Black: Abs(260nm), Pink: Abs(280nm), Teal: Salt concentration.

As shown in figures 2 and 3, a peak in absorbance at 260 and 280 nm is often observed during loading of the mRNA on the oligo(dT) column. The material that causes this peak in absorption, present in the flowthrough fraction of chromatography, corresponds to the truncated RNA fragments that do not possess a poly(A) tail and therefore do not bind to the stationary phase. As shown in Figure 3, however, material also elutes from the column during the washing steps. Whenever the salt content of the mobile phase is reduced, a small amount of mRNA elutes from the column. The material eluting during these wash steps was only weakly bound to the stationary phase, likely through non-specific interactions between the oligo(dT) sequence and adenosine-rich stretches in the mRNA. Finally, when the concentration of salt is reduced below a critical level, the negative charges of the phosphate backbones are no longer sufficiently shielded. At this point, the electrostatic repulsion between the phosphate backbones of the oligo(dT) sequence and the mRNA grows stronger than the hydrogen bonds between the nucleobases, and the mRNA elutes from the column.

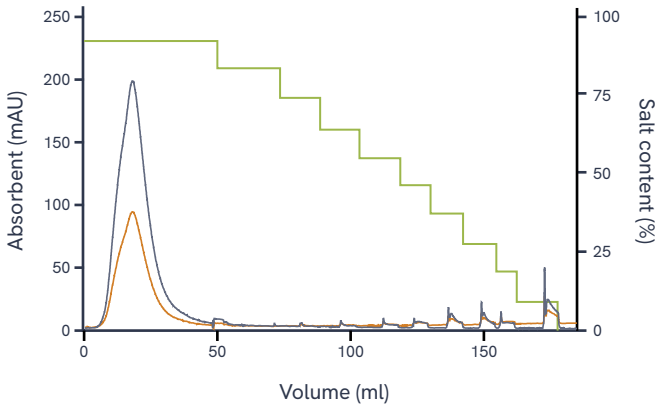

Figure 3

Lower absorbance range of chromatogram for oligo(dT) purification using stepwise reduction of salt concentration. Black: Abs(260nm), pink: Abs(280nm), teal: salt concentration.

The process described above was used to purify an impure mRNA product containing a notable population of truncated mRNA fragments. Whereas this truncated material is clearly visible in the unpurified mRNA (red trace in Figure 4), this impurity is no longer significantly present after oligo(dT) purification (green trace in Figure 4). Furthermore, the material present in the flowthrough fraction of oligo(dT) chromatography corresponds to the truncated fragments present in the unpurified and absent from the purified mRNA. By performing this procedure, the purity of the mRNA product could be increased from ±74 to ±91%.

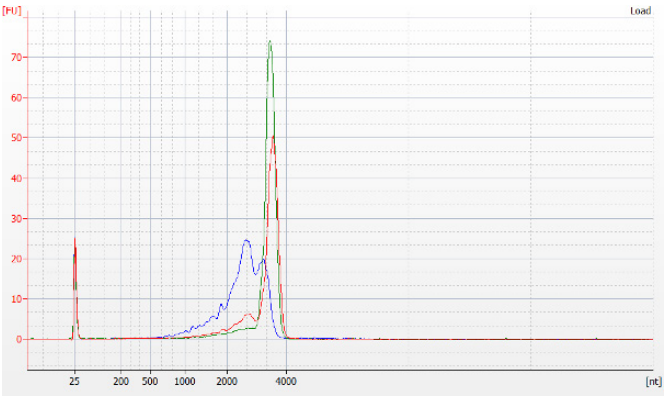

Figure 4

Overlay of the CGE electropherogram of mRNA containing a clear population of truncated mRNA fragments, visible as a secondary peak (red), the material in the flowthrough fractions of oligo(dT) chromatography (blue), and the purified mRNA (green). The CGE markers are shown at 25nt.

Conclusion

The quality of mRNA can be improved by removal of truncated mRNA species by chromatographic Oligo(dT) purification which is a versatile and well-established process. The purification method is fully scalable, can be used in the manufacturing of microgram to multi-gram scales of polyadenylated RNA and is a valuable tool to increase mRNA purity when the presence of truncated fragments cannot be prevented using codon optimization or increasing the quality of the DNA template.

References

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