

Affinity-Based Polishing Enables Efficient Removal of dsRNA Contaminants from IVT mRNA

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Introduction

In vitro-transcribed (IVT) mRNA must support robust protein expression while minimizing activation of innate immune pathways. A major obstacle is the formation of double-stranded RNA (dsRNA) by-products during IVT (Figure 1).

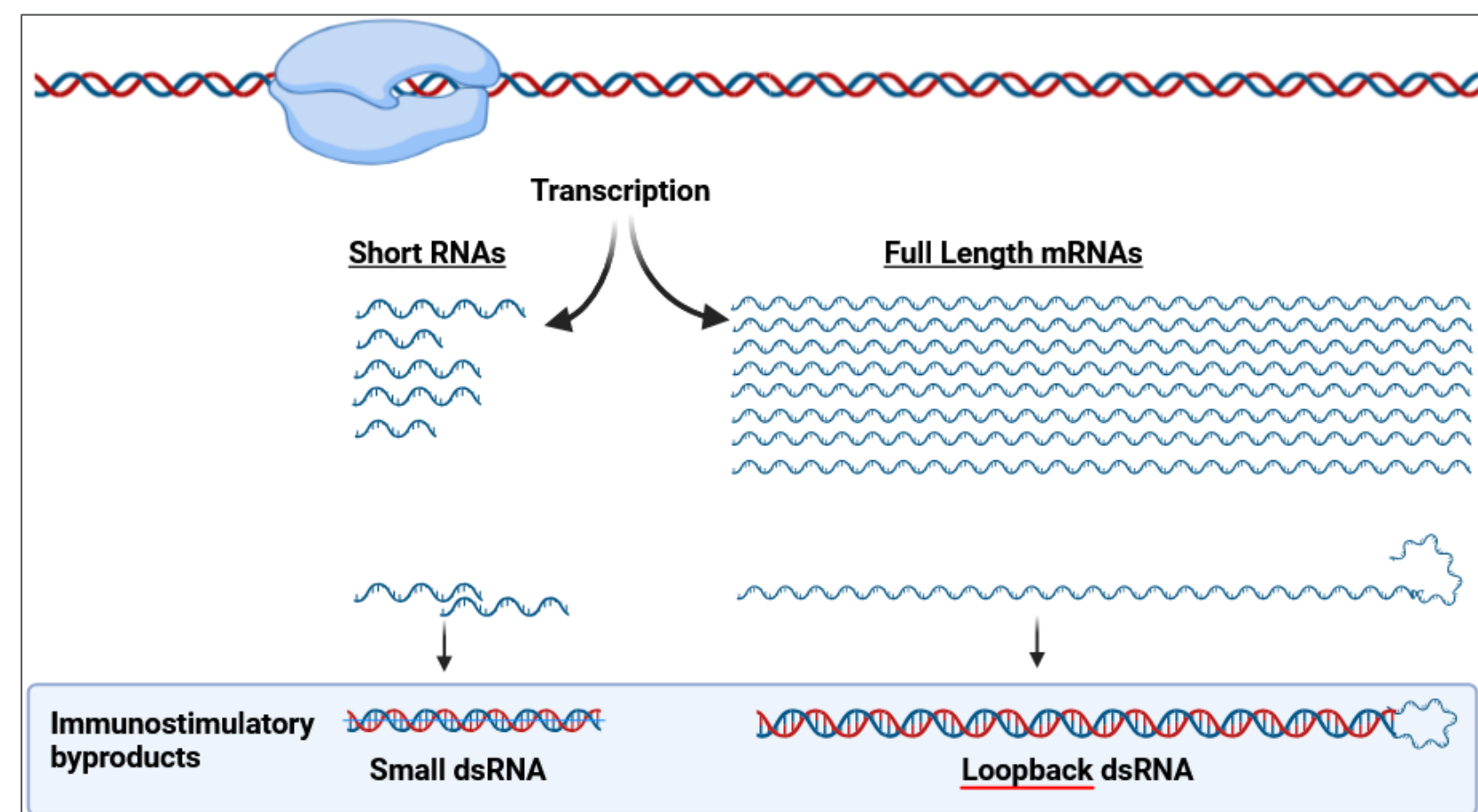


Figure 1. Mechanism of dsRNA formation in IVT reactions.

dsRNA activates Toll-like and cytosolic RNA sensors, reducing translation and promoting inflammatory responses that can compromise safety and efficacy (Figure 2).

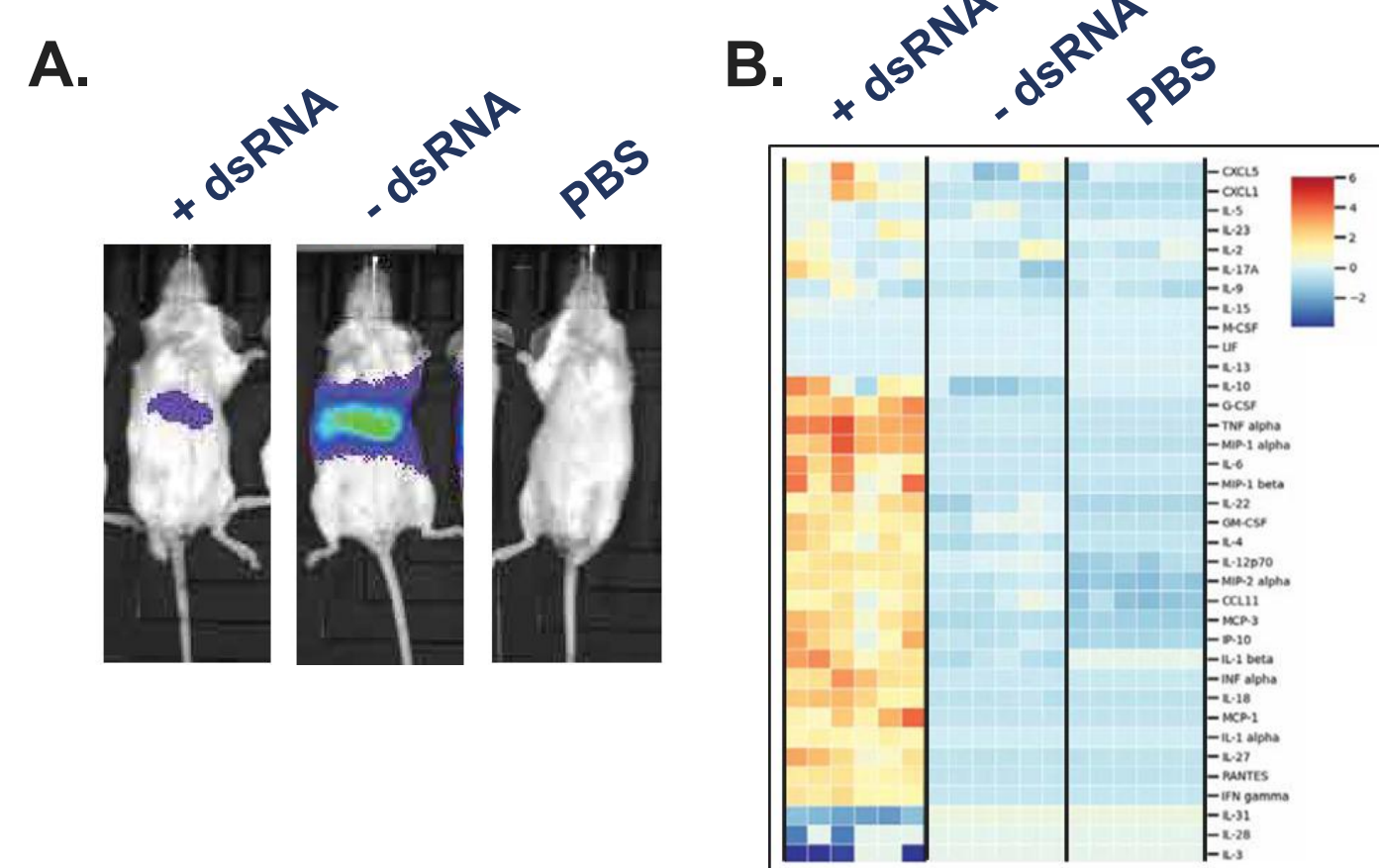


Figure 2. dsRNA removal improves translation & reduces toxicity. (A) Removal of dsRNA increased luciferase signal 11-fold following FLuc mRNA administration. (B) Toxicity panel. Data from our App Note: Generating high-quality mRNA for in vivo delivery which is available on our website.

Current mitigation strategies such as engineered RNA polymerases, IVT optimization, enzymatic treatment, cellulose purification, and ion-paired RP-HPLC can require specialized workflows, reduce yield, damage RNA, or leave residual dsRNA (Figure 3).

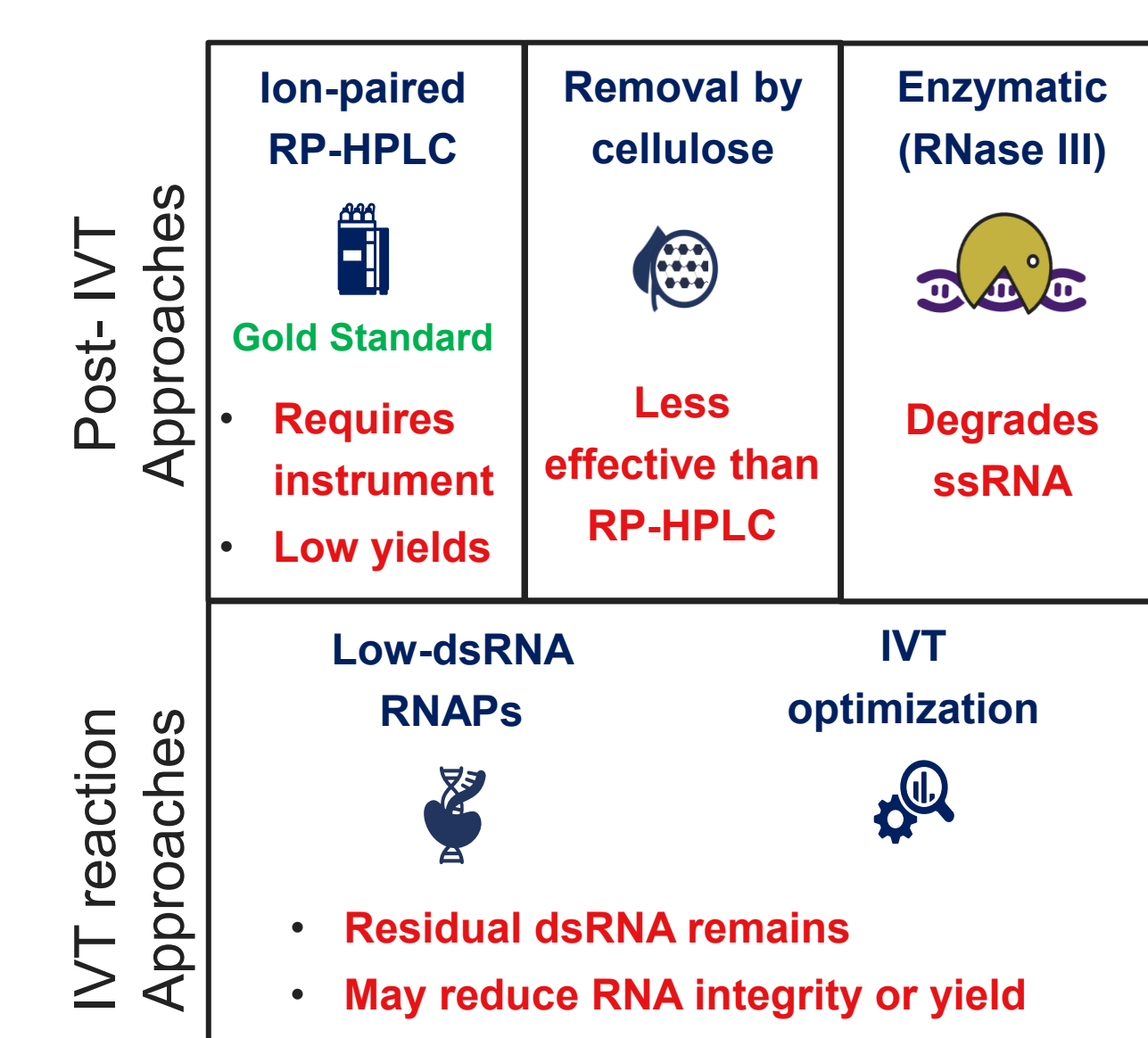


Figure 3. Current dsRNA removal methods have significant disadvantages

This highlights the critical need for scalable, robust, and selective purification technologies that can eliminate dsRNA. Here, we evaluate a post-IVT affinity-polishing workflow based on an engineered dsRNA-binding protein (DRBP) and determine whether it can improve mRNA expression, innate immune response, and recovery of viable base-edited cells.

Materials and methods

Key reagents

- DRBP clean up: MEGAclean dsRNA Removal Kit - Available in September 2026
- mMESSAGE mMACHINE™ T7 mRNA Kit with CleanCap™ Reagent AG (Cat. #A57620)
- Dynabeads™ RNA Purification Kit (Cat. #65032D)
- Lipofectamine™ MessengerMAX™ Transfection Reagent (Cat. #LMRNA008)
- J2 antibody (Cat. #10010200-200UG)
- K1 antibody (Cat. #10020500-500UG)

Results

1. Engineered dsRNA-binding protein (DRBP) is specific for dsRNA

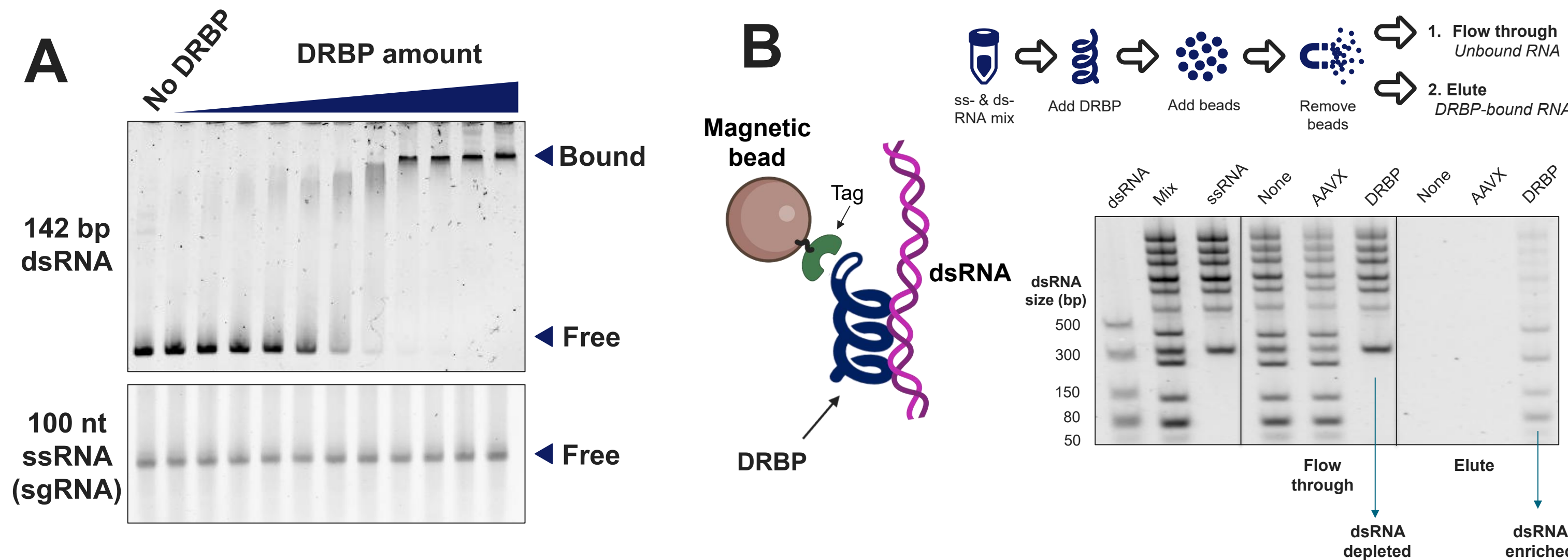


Figure 4. DRBP selectively binds dsRNA. (A) Electrophoretic mobility-shift assays using dsRNA or ssRNA substrates. A mobility shift is observed only with dsRNA. (B) Schematic of affinity capture of the DRBP-dsRNA complex. Agarose-gel analysis shows selective removal of dsRNA from an ssRNA/dsRNA mixture; AAVX is a negative control.

2. DRBP integrates easily into the IVT workflow to remove dsRNA

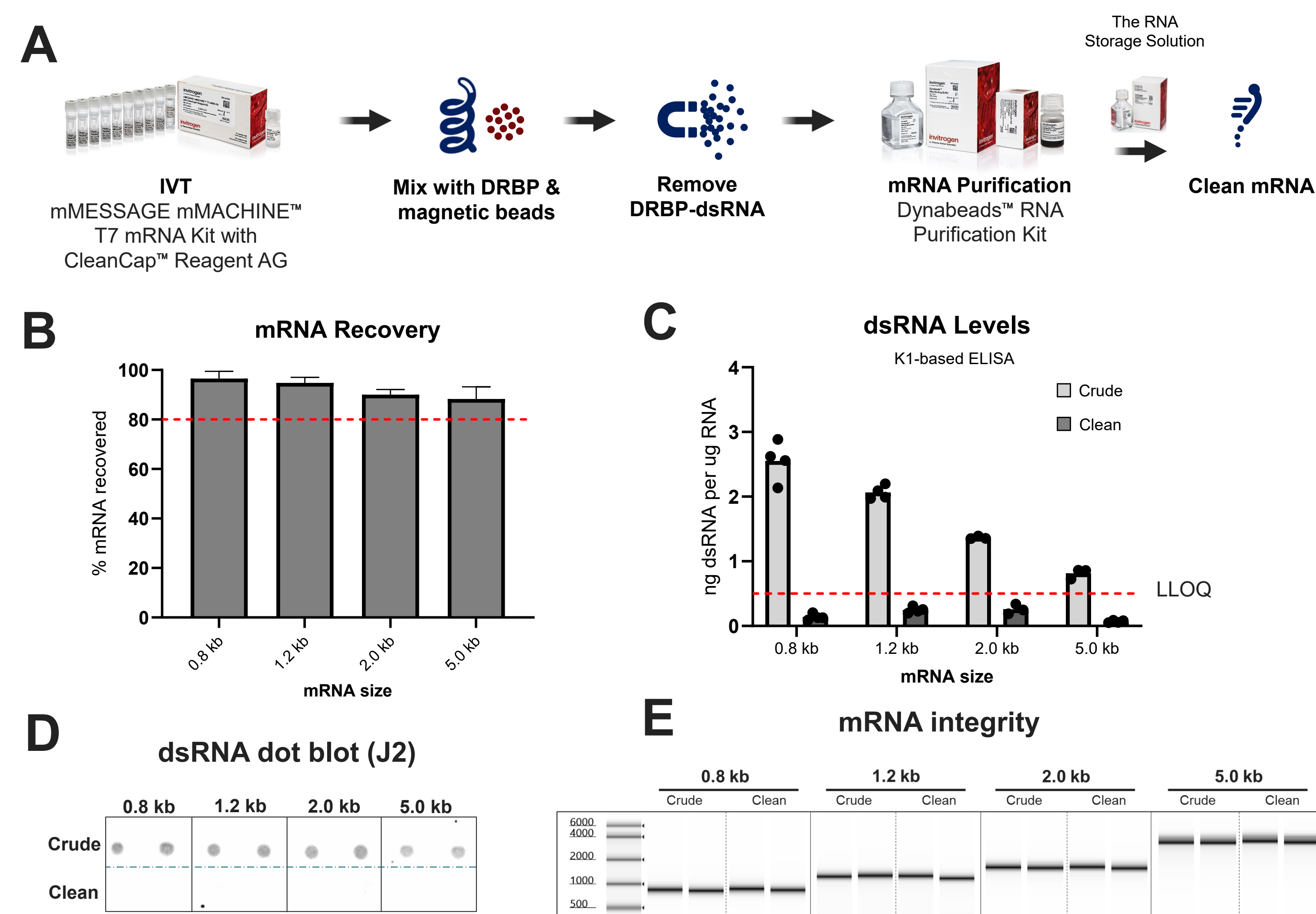


Figure 5. DRBP efficiently removes dsRNA across mRNA transcript lengths with minimal impact on recovery or integrity. (A) Post-IVT DRBP workflow. (B) mRNA recovery remains above 80%. (C,D) dsRNA is reduced to near or below the assay LLOQ. (E) mRNA integrity is maintained. "Crude" indicates mRNA not subjected to DRBP polishing. All mRNAs contain m1Ψ.

3. DRBP maintains high mRNA recovery, reduces dsRNA, increases reporter expression, and suppresses cytokine induction to near-background levels

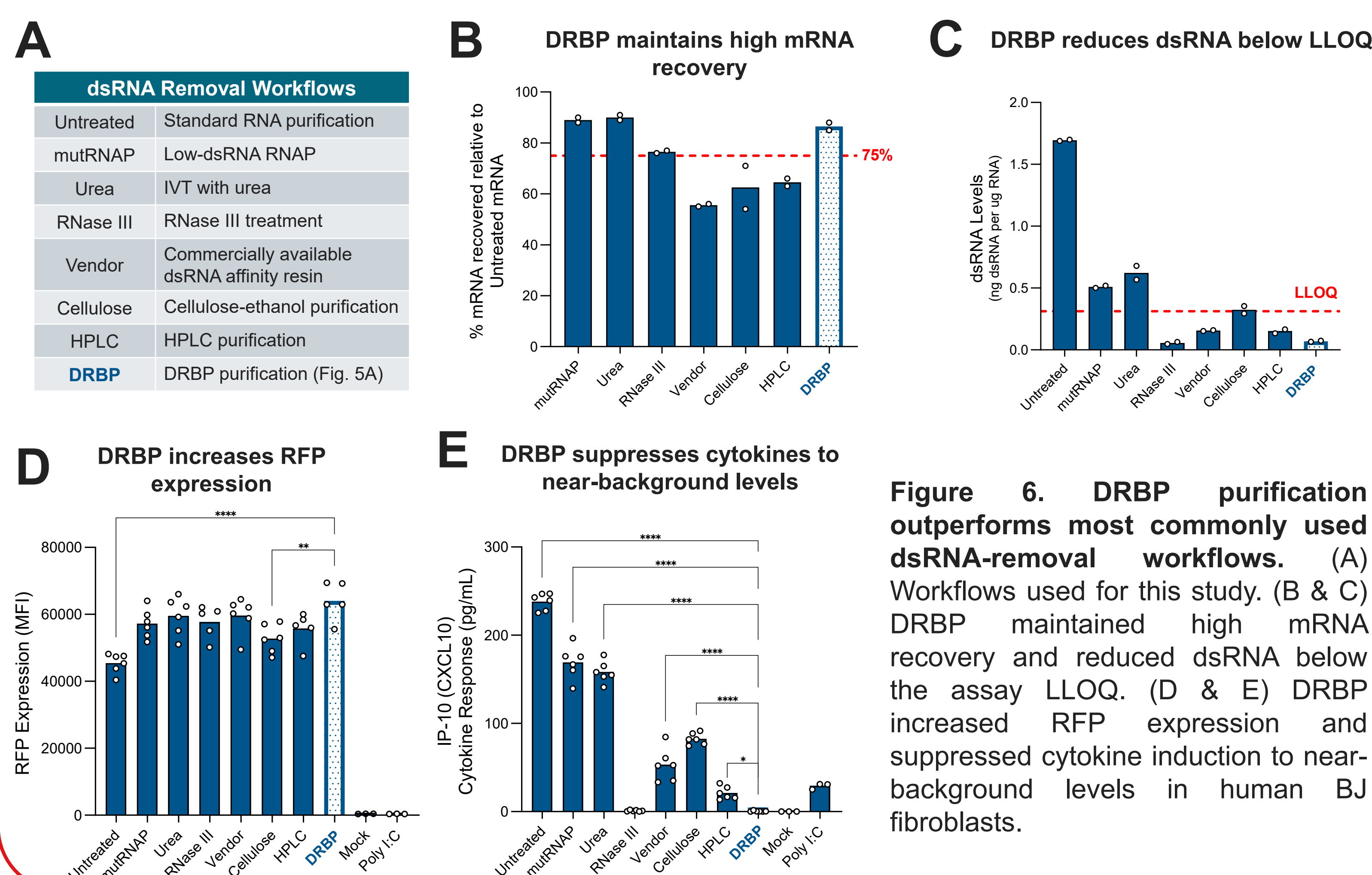


Figure 6. DRBP purification outperforms most commonly used dsRNA-removal workflows. (A) Workflows used for this study. (B & C) DRBP maintained high mRNA recovery and reduced dsRNA below the assay LLOQ. (D & E) DRBP increased RFP expression and suppressed cytokine induction to near-background levels in human BJ fibroblasts.

4. DRBP-purified mRNA increases recovery of viable gene-edited THP-1 cells

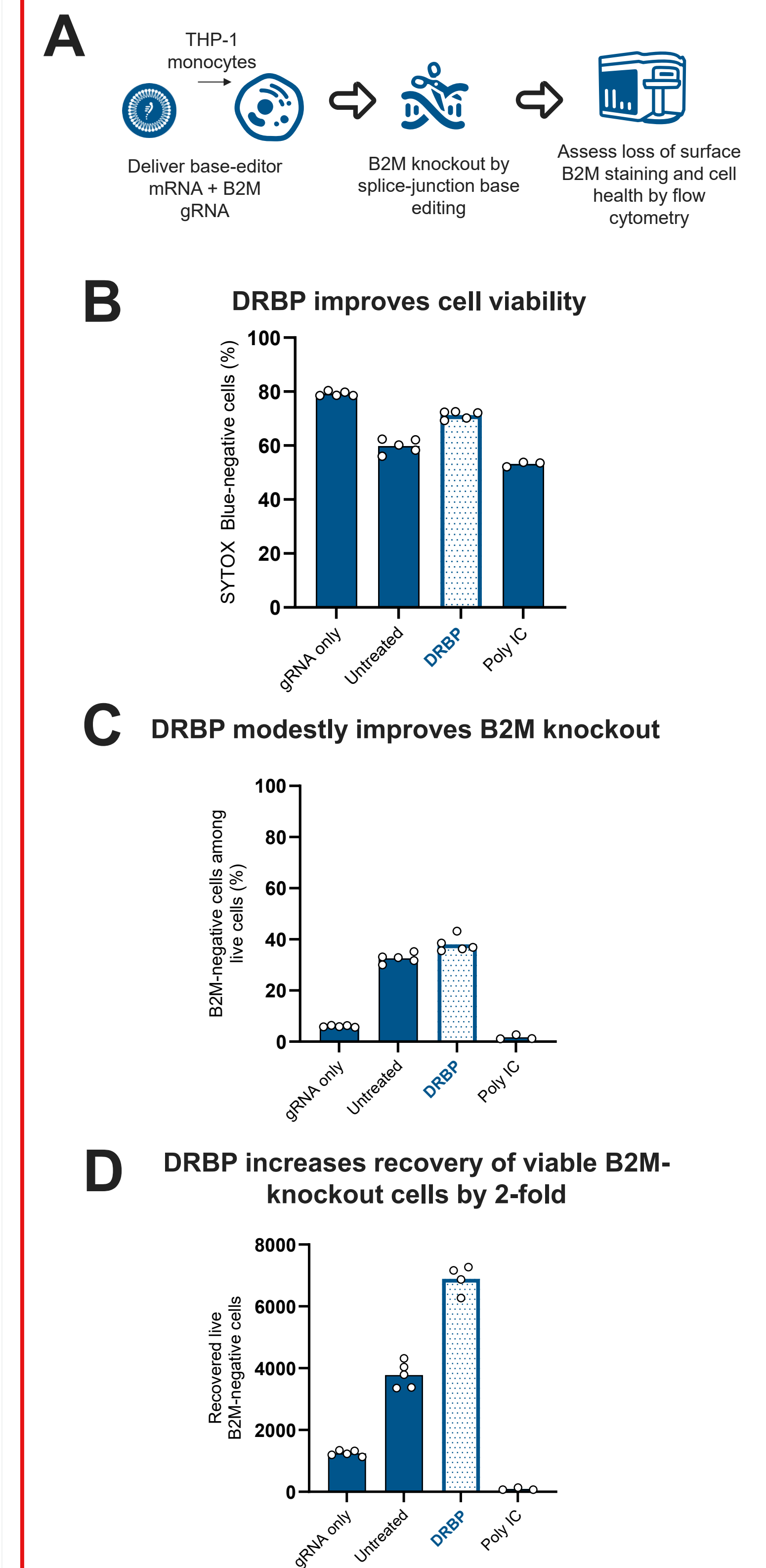


Figure 7. DRBP-purified base-editor mRNA improves recovery of viable B2M-edited THP-1 cells. (A) THP-1 cells were delivered base-editor mRNA and a guide RNA targeting a B2M splice junction, followed by flow-cytometric assessment of surface B2M expression and cell viability. (B-D) DRBP purification improved cell viability, modestly increased B2M knockout, and approximately doubled recovery of viable B2M-negative cells, highlighting the value of high-purity mRNA for gene-editing applications.

Conclusions

The engineered DRBP selectively removes dsRNA contaminants while preserving mRNA integrity and recovery.

The post-IVT polishing step is rapid, scalable, and compatible with existing mRNA production workflows.

DRBP-purified mRNA improves expression, reduces cytokine induction, and increases recovery of viable base-edited cells.

More information

Interested in the DRBP clean up step? Contact us about dsRNA removed mRNA through our mRNA Synthesis Services:



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