APPLICATION NOTE | DAHLIA RNA FLOW ASSAYS

Screening Cell & Gene Therapy Transduction & Transfection Efficacy Using Dahlia's RNA Flow Assays

Abstract — *Developing next-generation Cell and Gene Therapies requires screening large libraries of synthetic constructs to optimize therapeutic potency and safety. A foundational requirement in this pipeline is the accurate measurement of Transduction Efficiency, Transfection Efficiency, and Vector Copy Number. Traditional analytical methods force a compromise: conventional flow cytometry requires high-affinity antibodies that do not exist for novel candidate constructs, while quantitative PCR methods provide bulk genomic data that mask cell-to-cell heterogeneity. Dahlia RNA Flow Assays resolve this dilemma by enabling high-throughput single-cell RNA analysis that plugs directly into existing flow cytometry workflows. By leveraging proprietary Proximal Two-Probe hybridization chemistry and rolling circle amplification, the platform detects transgene mRNA expression directly within heterogeneous cell populations. This eliminates antibody development bottlenecks and accelerates therapeutic candidate selection for both ex vivo and in vivo clinical environments.*

Introduction: The High Stakes of Construct Screening

The therapeutic landscape for Chimeric Antigen Receptor (CAR)-T cells, T-cell receptor (TCR)-T cells, and multiplexed gene therapies, and emerging *in vivo* CAR delivery modalities is expanding rapidly. Synthetic biology allows researchers to engineer complex, modular cassettes containing novel binding domains (scFvs), distinct hinge or transmembrane regions, and tailored co-stimulatory domains. However, translating these digital designs into viable therapies creates an acute screening bottleneck. Transduction and transfection efficiency—the percentage of target cells successfully modified—and Vector Copy Number—the average number of integrated cassettes per genome—are critical quality attributes that can significantly impact downstream therapeutic candidate function and success.

Tradeoffs between existing protein vs nucleic acid based measurements

- **The antibody barrier (Flow Cytometry):** Traditional flow cytometry is the gold standard for single-cell phenotypic analysis. However, it relies heavily on the availability of high-affinity antibodies or fluorophore-conjugated target proteins to detect surface expression. When screening completely novel binders, neoantigen scFvs, or non-coding payloads, matching antibodies do not exist. Generating custom detection reagents takes months, stalling candidate validation.
- **Structural and packaging trade-offs of reporter genes and epitope tags (Flow Cytometry)**: To facilitate monitoring by flow cytometry, investigators often integrate a fluorescent reporter like GFP to provide a robust visual validation, but this strategy expands the vector's cargo size, which can inherently suppress the transduction efficiency of the primary therapeutic transgene. Conversely, utilizing compact epitope tags like Flag or Myc minimizes payload size constraints but poses unknown structural and functional risks.
- **Lack of single-cell resolution (qPCR/ddPCR):** Quantitative PCR (qPCR) and Droplet Digital PCR (ddPCR) provide precise quantification of genomic integration. However, these methods are destructive, bulk measurements that average the signal across millions of cells. This masks vital cell-to-cell variations, making it impossible to determine if a signal stems from 100% of cells containing a single viral copy, or 10% of cells containing ten copies. Furthermore, bulk DNA assays cannot distinguish which specific cell subsets (e.g., CD4+ helper vs. CD8+ cytotoxic T cells) successfully integrated the transgene.

Dahlia RNA Flow Assays provide single-cell measurements of delivery efficiency

Streamlined process that plugs into existing workflows

Dahlia RNA Flow Assays provide ultra-throughput single-cell RNA analysis by using a streamlined workflow that bridges the gap between genetic integration and phenotypic visualization.

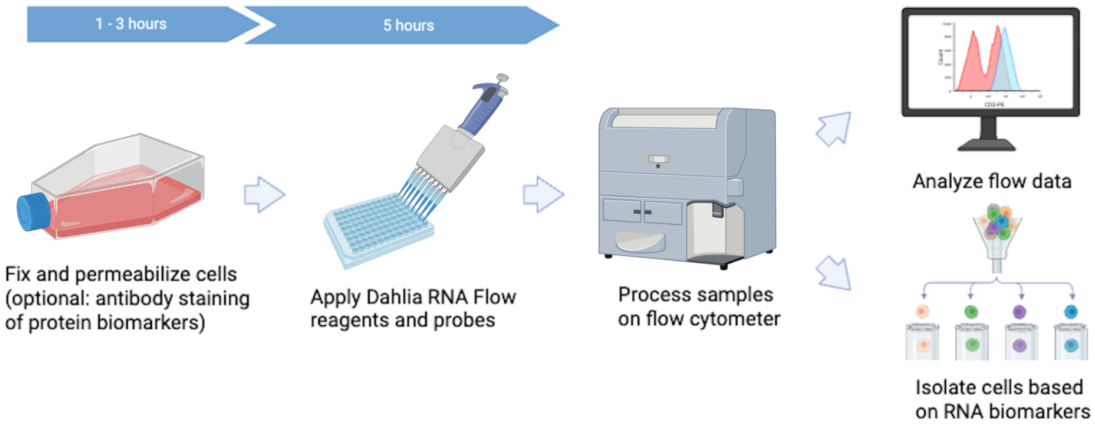


FIG 1. Dahlia RNA Flow Assay 4-step workflow. Step 1. Single-cell suspensions are fixed and permeabilized in 1.6% PFA and methanol. As an optional step, surface markers can be stained with antibodies for co-detection and cross-linked. Step 2. Dahlia RNA Flow Assays are applied to the sample. RNA detection steps can be completed in under 5 hours. Step 3. Samples are then run through flow cytometry workflow. Step 4. Further analysis or cell sorting is performed based on target RNA expression.

The Dahlia Advantage

- **Bypassing Translation Kinetics:** Dahlia detects active transgene transcription in less than a day, cutting down the traditional multi-day wait time required for protein translation, folding, and cell-surface trafficking required by standard antibody staining
- **No Antibody Required:** Dahlia's proprietary design pipeline generates fully wet-lab-validated RNA probe sets against any target sequence down to 50 nt (including splice variants and custom scFvs), circumventing antibody development entirely. It is highly valuable when screening novel, custom-engineered constructs for which no commercial antibody exists. New, custom designs are delivered within 3 weeks.
- **High-Throughput Single-Cell Resolution:** Unlike bulk qPCR or ddPCR which blend the whole sample together, RNA Flow tracks expression on a cell-by-cell basis. It accurately resolves transfection efficiency for non-viral vectors (such as LNPs) and transduction efficiency for viral delivery platforms alike, processing tens of thousands of cells per second to catch rare or low-expressing modifications.
- **Simultaneous mRNA & Protein Profiling:** It maps both the transcribed message and surface markers simultaneously. For instance, you can confirm whether the CAR mRNA is actively transcribing specifically within a CD8+ cytotoxic T cell vs. a CD4+ helper T cell subset, ensuring correct cell lineage targeting in both *ex vivo* and *in vivo* workflows.

Detecting Delivery Efficacy Without Antibodies

To validate the platform's utility in CAR-T construct screening, cell samples were subjected to Dahlia's RNA Flow protocol using custom-designed probe sets to assess gene delivery performance. When evaluating a therapeutic FMC63 CAR-T construct using a complete probe pair, Dahlia easily quantified a 22% CAR-T Positive population within the transduced sample. To confirm assay specificity, two control environments were analyzed: (1) Circular Probe Only Control dropped the signal to a baseline of 1.6%, showcasing how the Proximal Two-Probe hybridization chemistry prevents false positives from non-specific amplification; (2) Running untransduced cells against the complete probe pair showed a true negative baseline of 3.3%.

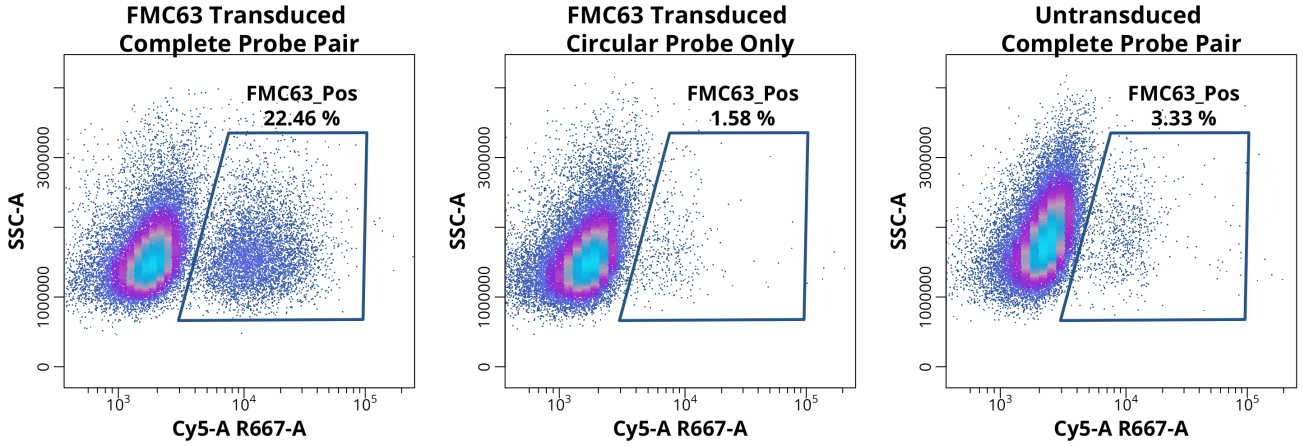


FIG.2. Detection of FMC63 CAR-T transduction efficiency by RNA Flow.

Methodology Comparison Matrix

Evaluation Metric	Flow Cytometry	Bulk ddPCR / qPCR	Dahlia RNA Flow Assays
Primary Target Analyte	Surface Protein Expression	Total DNA / RNA	**Transgene mRNA Transcripts**
Analytical Resolution	Single-Cell	Bulk Sample Average	**Single-Cell**
Detection of Novel Targets	Hard – custom antibodies	Easy	**Easy**
Time to Assay Result	4–7 Days (Requires Translation)	<1 Day	**<1 Day**
Cell Subset Multiplexing	Yes	No	**Yes**

Contact us!

Submit your RNA targets of interest or talk to our scientists.

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