TECH NOTE

Achieving Phenotypic Profiling for Rapid Drug Discovery with High-Throughput Gene Expression Screening

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Introduction

High-throughput screening approaches are widely used to perform phenotypic profiling for drug discovery; however, these methods have significant limitations in terms of bias, cost, and scalability. To address these shortcomings, Azenta Life Sciences has developed our high-throughput gene expression (HT-GEx) screening service, a simplified RNA sequencing (RNA-Seq) workflow that eliminates the need for RNA isolation. Instead, HT-GEx screening barcodes transcripts in cell lysate to allow for pooling, thereby creating a cost-effective, unbiased assay for phenotypic profiling suited for drug discovery applications.

The Challenge

Drug discovery research relies heavily on high-throughput screening assays for measurement of phenotypic responses following compound or genetic perturbations of cell cultures. However, traditional lower-cost screening assays, such as microarrays, are highly targeted and thus highly biased. Other technologies, such as standard whole-transcriptome RNA-Seq, are typically the preferred approach for unbiased measurement of phenotypic responses but are often overlooked due to limited scalability and high sample screening costs.

The Solution

Based on traditional RNA-Seq methods, Azenta Life Sciences has developed HT-GEx screening as an optimal approach for drug discovery, compound treatment phenotyping, CRISPR treatment phenotyping, and cell response screening. This assay provides a standardized workflow (Figure 1) for customers to achieve optimal sequencing results from hundreds to thousands of samples. The keys to this process are:

1. **Removing the need for RNA purification.**
2. **Method optimization for library construction of pooled samples.**
3. **Utilizing 3' end counting for a reduction in sequencing depth coverage without compromising gene detection sensitivity.**

Once lysed cells are provided by the customer in a 384-well format, Azenta Life Sciences begins the HT-GEx screening workflow with library preparation, where transcripts are tagged with Unique Molecular Identifiers (UMIs) and barcodes to distinguish samples. Following library preparation, sequencing is performed using 3' end counting, which reduces sequencing depth to be sufficient for gene detection saturation (Figure 2). Azenta Life Sciences can then perform advanced, downstream bioinformatics analysis utilizing differential gene expression and clustering by compound/treatment for a complete, sample-to-data solution. Altogether, this optimized workflow enables unbiased sequencing of high sample numbers to be performed quickly and at a reduced cost compared to other approaches to enable faster target discovery.

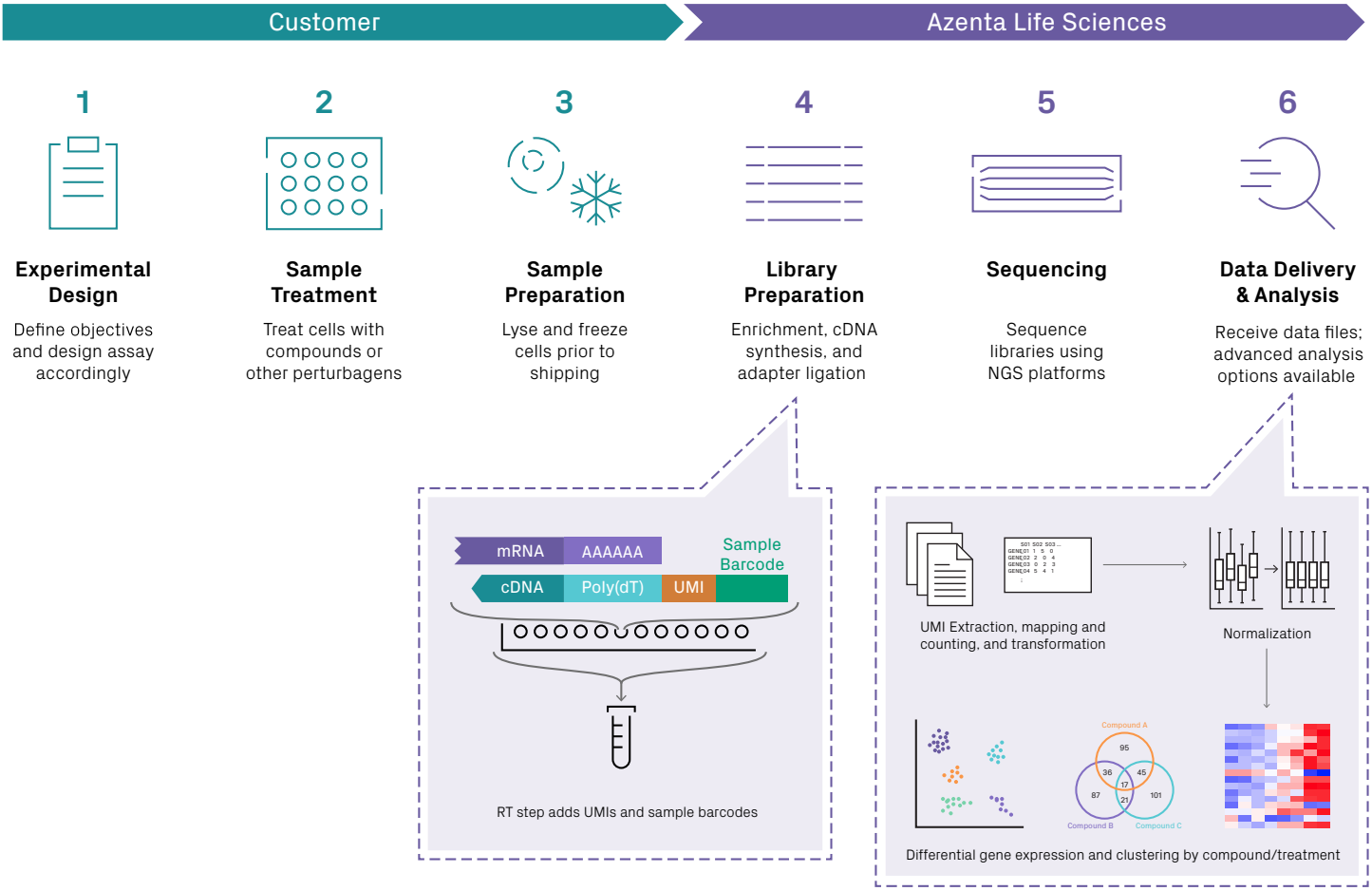

Figure 1. Azenta Life Sciences' HT-GEx screening end-to-end workflow.

The customer:

1. Designs the experiment.
2. Treats samples with a myriad of conditions.
3. Lyses and freezes cells in a 384-well plate format and ships to Azenta Life Sciences.

Once samples are received, Azenta Life Sciences performs:

4. Library preparation through enrichment, cDNA synthesis, and adapter ligation.
5. Sequencing using NGS platforms.
6. Data analysis, including Unique Molecular Index (UMI) extraction for rapid mapping, differential gene expression, and clustering by treatment, and delivery.

The Results

Azenta Life Sciences has defined gene detection sensitivity and reproducibility via HT-GEx screening for both cell lysate and isolated RNA samples by testing sample types in parallel at various sequencing depths. At 1-2 million reads per sample, cell lysate samples yielded gene detection sensitivity comparable to alternative high-throughput compound screening methods such as PLATE-Seq[1] and DRUG-Seq[2]. Gene detection begins to plateau at around 3-5 million reads, indicating a diminishing return for sequencing depths greater than 2 million reads (Figure 2). Azenta Life Sciences also tested reproducibility by evaluating the number of detected genes of replicate lysate samples and isolated RNA samples. Compared to isolated RNA, cell lysate samples do not significantly compromise reproducibility (Figure 3). Ultimately, the usage of cell lysate bolsters both cost and time savings when processing hundreds to thousands of samples in sequencing reactions while retaining the reproducibility required for comparative analysis.

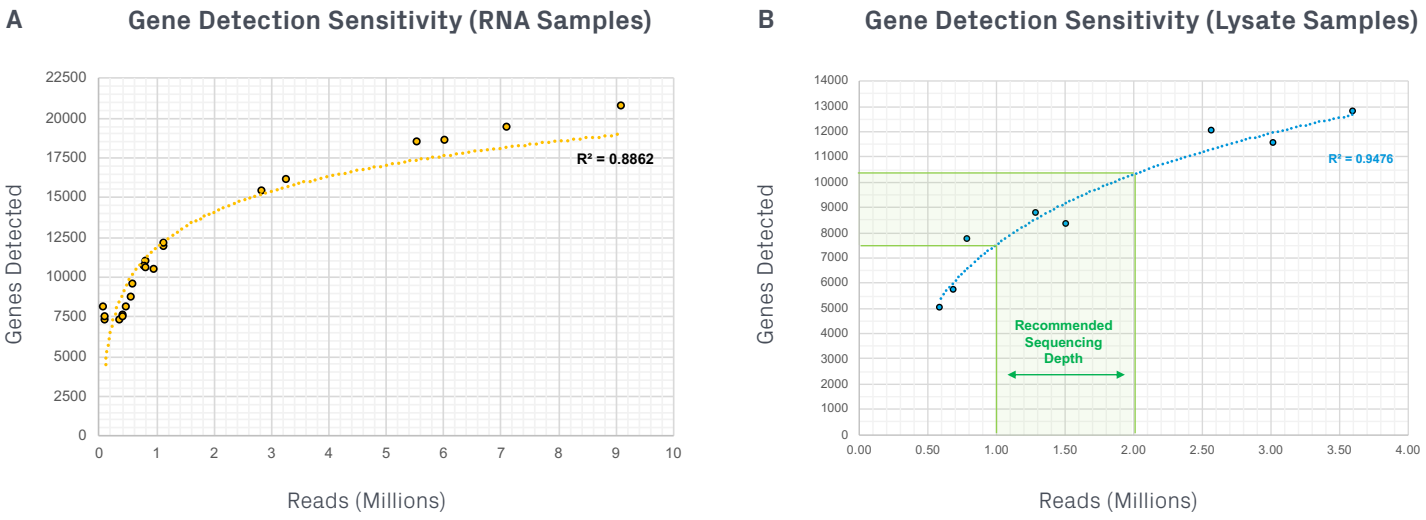

Figure 2: Results of sensitivity tests using RNA samples and cell lysates performed by Azenta Life Sciences demonstrate lysate sensitivity is sufficient for phenotypic profiling.

A. Gene Detection Sensitivity (RNA Samples). Extracted purified RNA samples were processed using Azenta Life Sciences' HT-GEx screening workflow. Gene detection sensitivity followed a logarithmic trend and began to plateau at ~3-5 million reads per sample.

B. Gene Detection Sensitivity (Lysate Samples). Cell lysate samples were processed directly using the same workflow; however, RNA extraction and purification were not performed. Gene detection sensitivity saturated at 2 million reads, similar to RNA samples, and is comparable to other high-throughput screening methods making it sufficient for phenotypic screening.

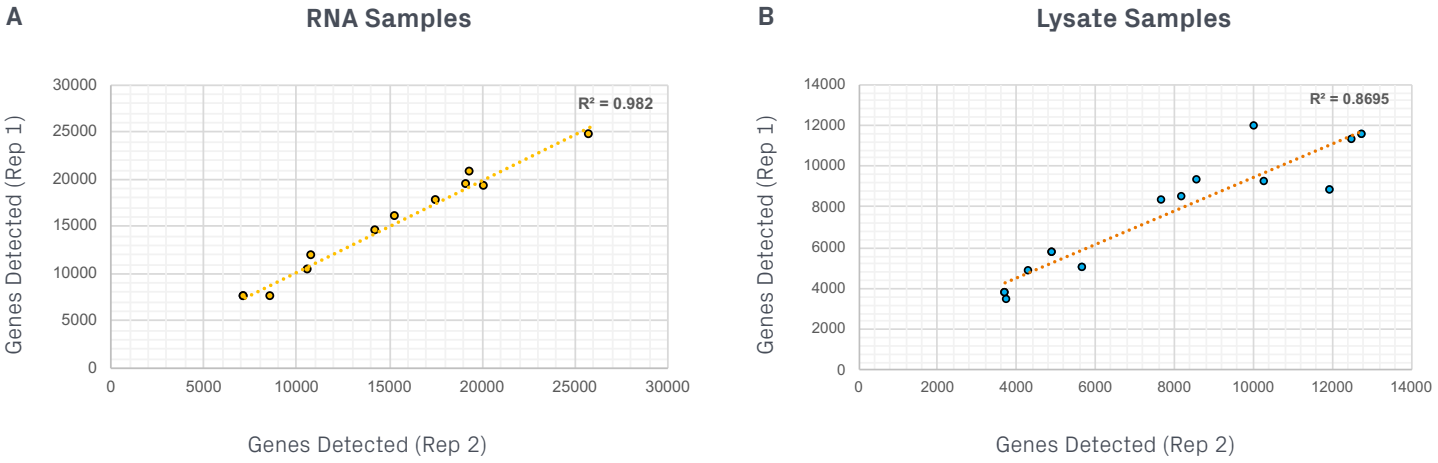

Figure 3: Results of reproducibility tests using RNA samples and cell lysate performed by Azenta Life Sciences reveal reproducibility is not significantly reduced when using lysate samples.

A. Reproducibility (RNA Samples). The number of genes detected between replicate purified RNA samples demonstrated a strong linear correlation which implies results were highly reproducible.

B. Reproducibility (Lysate Samples). The number of genes detected between replicate cell lysate samples also demonstrated a linear correlation. This suggests that foregoing RNA extraction and purification does not significantly reduce reproducibility using lysate samples.

The Conclusion

Leveraging its extensive experience optimizing DNA and RNA sequencing protocols, Azenta Life Sciences has developed HT-GEx screening to perform unbiased measurement of phenotypic responses for drug discovery applications at a reduced cost and turnaround time compared to RNA-Seq. By testing both RNA and cell lysate samples at varying sequencing depths, it was determined that this approach generated comparable results to that of other high-throughput compound screening methods in terms of sensitivity and reproducibility. This screening method, combined with Azenta Life Sciences' downstream analysis capabilities, makes Azenta Life Sciences' HT-GEx screening workflow an ideal end-to-end approach for rapid target discovery.

References

1 Bush, E.C., Ray, F., Alvarez, M.J. et al. PLATE-Seq for genome-wide regulatory network analysis of high-throughput screens. *Nature Communications* 8, 105 (2017). https://doi.org/10.1038/s41467-017-00136-z

2 Ye, C., Ho, D.J., Neri, M. et al. DRUG-seq for miniaturized high-throughput transcriptome profiling in drug discovery. *Nature Communications* 9, 4307 (2018). https://doi.org/10.1038/s41467-018-06500-x

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