Element Biosciences

Technical Note

Teton Atlas™ Custom Primer Design

Primer Design Guidance for Targeted Direct In Sample Sequencing

Introduction

Teton Atlas on the AVITI24™ System enables highly multiplexed co-detection of cell morphology, gene expression, and protein expression directly in cells at subcellular spatial resolution.

Direct In Sample Sequencing (DISS) chemistry on Teton Atlas supports native RNA profiling through two complementary approaches: 3′ transcriptome method using poly-T probes to capture polyadenylated mRNA, and a targeted approach employing probes custom designed against specific RNA features of interest. Both methods leverage a single-sided probe chemistry for cDNA extension, circularization, and rolling-circle amplification, followed by sequencing using avidite base chemistry (ABC). RNA and protein targets are detected in sequential sequencing batches, each using unique batch-specific primers to overcome optical crowding and increase the number of detection events per cell.

The ElemBio Cloud Custom Designer™ allows you to flexibly build Teton Atlas workflows with fixed and custom targets for cell morphology, RNA, and protein analysis. This technical note outlines primer design strategies for custom targeted RNA sequencing using DISS.

The ElemBio Cloud Custom Designer is initially validated for designing primers to target CRISPR guide RNAs but enables broader applications through the Advanced Non-Guide RNA Sequencing Applications setting when creating a Teton Atlas custom workflow. Examples of advanced applications include sequencing viral transcripts, gene fusions, and more.

Guide RNA Sequencing

The ElemBio Cloud Custom Designer guide RNA sequencing application primer design algorithm accounts for guide RNA secondary structures and binding energies. A dual-primer design is used to improve specificity for guide RNA sequencing. In this scheme, a reverse transcription (RT) primer binds to the 3′ flanking region of the target RNA sequence (i.e., downstream of the target region, toward the 3′ end of the RNA). Reverse transcription proceeds toward the 5′ end of the RNA, synthesizing cDNA that contains the intended target sequence.

After cDNA circularization and rolling circle amplification (Figure 2), a sequencing primer binds upstream of the target. Sequencing begins immediately at the end of the sequencing primer and proceeds in the 3′ to 5′ direction relative to the parent RNA. This orientation ensures that both the RT primer and the sequencing primer must bind successfully at opposite ends of the target for sequencing to occur. This dual-binding requirement increases specificity and helps prevent off-target reads.

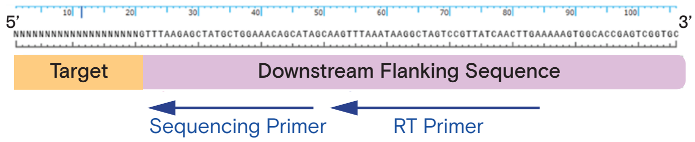

Figure 1. Primer Design Scheme for Guide RNA Sequencing

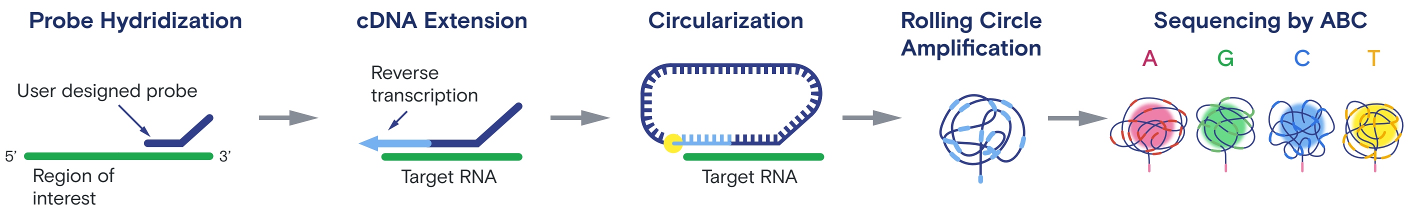

Figure 2. Targeted Direct In Sample Sequencing Workflow

Custom Applications

DISS chemistries for targeted RNA sequencing are designed for flexible customization, allowing you to tune primer design and cycle lengths to address a variety of applications beyond guide RNA sequencing. For these advanced applications, custom primers can be designed using third-party tools and then sequences can be uploaded directly to the ElemBio Cloud Custom Designer.

Teton Atlas enables sequencing of custom targets up to 100 bp in length, allowing custom applications from targeting exogenous sequences to viral genes and gene splice sites. Custom applications use the same primer design where RT and sequencing primers are designed to bind to the downstream flanking region of your target of interest. However, additional primer optimization outside of the ElemBio Cloud Custom Designer may be required to account for the target specificity, secondary structure, binding energies, and GC content.

Custom Primer Considerations

Before initiating a custom design, consider critical factors, such as target expression, cDNA length distribution, and target plexity.

- **Target Expression**—Evaluate target expression with orthogonal methods, such as qPCR, FISH, or bulk RNA-seq. Targets with an expression level like GAPDH (~1000 copies per cell) are detected with a single primer. Low abundance targets can require multiple primers to boost detection.
- **cDNA Length Distribution**—Consider the distribution of cDNA lengths (defined as the transcribed sequence from the end of the RT primer) with an average length of 90–100 nucleotides when sequencing longer target regions.
- **Target Plexity**—Consider the number of targets and abundance of each target to avoid optical crowding in the batch. If multiple highly expressed genes are targeted in a single batch, assay performance can be impacted. With Teton Fixed Panels, Element uses < 100 genes per batch to achieve high per-gene sensitivity.

Primer Design

When designing RT and sequencing primers for advanced applications, use following guidelines to maximize success.

RT primer requirements

- Element recommends that the RT primer hybridization site is 25–35 nucleotides in length with Tm ≥ 50°C (50 mM Na+) and 35–65% GC content.
- Place the RT primer hybridization site 30-50 nucleotides away from the start of your intended sequencing region.
- Append the adapter sequence (CAGCCGCATCTTCTTTTGC) to the 5’ end of the oligonucleotide.
- Each RT Primer requires a 5’ phosphate and a 3’ hydroxyl.
- Minimum yield for the RT primer should be > 10 fmol.
- Element recommends 1-10 nM per target, depending on the expected target abundance.

Sequencing primer requirements

- Element recommends that the sequencing primer hybridization site is 25–35 nucleotides in length with Tm ≥ 50°C (50 mM Na+) and with 35–65% GC content.
- Place the sequencing primer immediately downstream of the intended sequencing region (non-overlapping with RT primer).
- Minimum yield for the sequencing primer should be > 1 nmol.
- Element recommends a final concentration of 1 µM per sequencing primer.

Additional guidance

- Each sequence you plan to target requires a dedicated RT primer and sequencing primer.
- Use Nucleotide BLAST (blastn) to check for off-target homology.
- Avoid homopolymer repeats or sequences with low complexity.
- Avoid heterodimers between RT and sequencing primers. Consider using third-party tools to optimize primer designs:
    - **IDT Oligo Analyzer**—A web-based tool from Integrated DNA Technologies (IDT) for primer analysis, Tm predictions, secondary structure, self-dimers, and hetero-dimers.
    - **mFold**—A web-based tool for secondary structure predictions of DNA and RNA.
    - **ViennaRNA**—A suite of computational tools to predict and analyze RNA thermodynamics, including secondary structure (RNAfold) and duplex formation (RNAcofold).
- If multiple primers are designed for the same target, space the primers at least 300 nucleotides apart.

Input & Ordering Guidance

When primer designs are finalized, a CSV file with the target name and sequences of all RT and sequencing primers can be uploaded to the ElemBio Cloud Custom Designer using the Advanced Non-Guide RNA Sequencing Applications setting. When designing your primers, ensure the Element adapter sequence, 5' phosphate, and 3' hydroxyl is appended to every RT primer.

Order oligos directly from your preferred oligo vendor with the following requirements:

- Desalted oligos
- Minimum yield for RT primer, 10 fmol
- Minimum yield for sequencing primer, 1 nmol
- Suspended to 100 µM concentration as stock

Summary

Teton Atlas with DISS chemistry is designed for fast and flexible customization, featuring easy-to-design primer schemes and an open format that allows you to order and spike-in primers to any Teton Atlas cartridge. To get started, log in to your ElemBio Cloud account and access the Custom Designer.

To explore custom applications beyond guide RNA sequencing, contact support@elembio.com or your Element representative.

To learn more, visit elementbiosciences.com

Element Biosciences

Telephone: 619.353.0300
Email: info@elembio.com