PROTOCOL & PRODUCT OVERVIEW

MTP RT

RNA-seq workflow integration of Multi-Targeted Priming (MTP) Reverse Transcription (RT)

- Seamless protocol integration
- No specialized equipment necessary
- Combined target selection and off-target counter-selection
- Genome-wide scalability

The MTP RT technology

Organism-Specific Solution

We use annotation data to design custom solutions for each organism.

All we need is a RefSeq ID.

Flexibly Defined Targeting

Target Sequence Space	Off-Target Sequence Space
30,000+ unique targets	**5,000+ unique off-targets**
Typically: ✔ mRNA	*Typically:* ✖ rRNA ✖ tRNA
Can flexibly include: ✔ Non-polyadenylated mRNA ✔ lncRNA ✔ miRNA ✔ Pathogen transcripts	*Can flexibly include:* ✖ Globin mRNA ✖ Uninformative mRNAs ✖ Host background

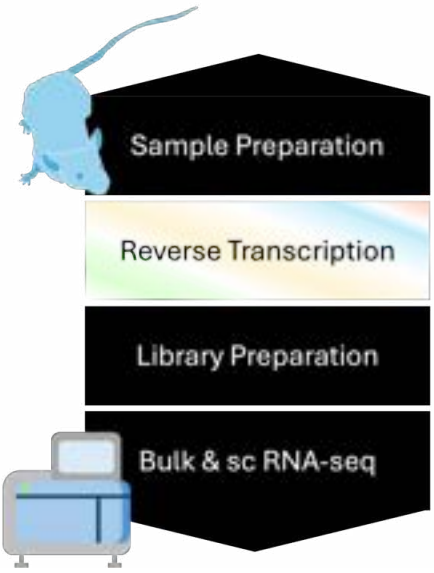
Intelligent Oligo Design

Graph Representation Learning
Enrichment Platform

Parallelized Oligo Synthesis

Proprietary Parallelized
Oligo Synthesis

MTP
Multi-Targeted Priming
Reagent



Seamless Protocol Integration

Compared to Oligo(dT), MTP gets…

Low prevalence transcripts

31–331% more

31-331% increased number of well measured genes

Differential expression

66–136% more

66-136% increased number of identified significantly differentially expressed genes (GEL_{50} threshold)

Genome-wide enrichment

30,000+ targets

Unbiased enrichment across gene pathways

No more 3′ bias

Non-PolyA transcripts

Lose the 3′ bias and target hard-to-reach mitochondrial RNA, miRNA, lncRNA, viral RNA and prokaryotic mRNA, and any other non-polyadenylated transcripts

Statistics compiled from bulk RNA sequencing, microarray profiling, and qPCR results for *S. cerivisea* and *N. crassa*.

Non-Model Organisms

Use our online product catalog or search by exact RefSeq Assembly ID at multitarga.com/search to find the non-model organism MTP offerings. These MTPs target the mRNA transcripts while counter-selecting for the rRNA and tRNA using the available sequences in RefSeq.

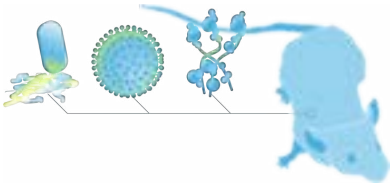
Model Organisms

Our model-organism MTPs target mRNAs while counter-selecting for rRNAs and tRNAs, using their RefSeq and/or GenBank assemblies as the sequence source material. Contact us for additional customization options including globin counter-selection, target gene-set restriction, and more.

Human MTP
Arabidopsis MTP
Mouse MTP
Drosophila MTP
Rat MTP
Zebrafish MTP

Host-Pathogen

Adaptively define target and off-target sequence spaces to enable counter-selection of background host material. Specific host-pathogen offerings coming soon. Contact us for custom collaboration on your host-pathogen or other multi-organism samples.



Pan-Onco

Comprehensive enrichment of 769 cancer-associated genes across all tissue types for precision oncology transcriptomics. Check out our website, multitarga.com/pan-onco, to find the full target gene list.

769
Cancer genes

Microbiome

We are currently developing reverse transcription and capture solutions for the human microbiome and other microbial communities. Contact us to collaborate on our metagenomic and metatranscriptomic solutions.

Overview

Multi-Targeted Priming (MTP) is a novel reverse transcription strategy that uses flexibly designed primer pools to enrich transcripts of interest. This approach augments the first step of bulk RNA-seq library preparation, reverse transcription, by replacing traditional oligo(dT) or random hexamer primers with our context specific reagent.

MTP is fully compatible with existing laboratory equipment and reagents. It is integrated seamlessly into the reverse transcription step for any Bulk RNA-seq library preparation kit without requiring changes to enzymes, buffers, or thermal cycler programs.

No new instrumentation required. MTP is a one-step swap-in replacement for randomers or oligo(dT) primers during cDNA synthesis.

Key Benefits

- Increased transcript specificity during cDNA synthesis
- Drop-in compatibility with existing RNA-seq protocols and kits
- Effective with total RNA, FFPE, and degraded samples
- No changes to equipment or enzymes
- **Cost-effective enrichment** prior to library prep
- **Flexible input amounts** (10–500 ng total RNA)
- **Supplied as lyophilized oligo pools** for long-term storage

Compatible Library Preparation Kits

MTPs can be used with any RNA-seq library preparation method that includes a first-strand cDNA synthesis step. Below are validated or compatible:

Kit Name	Company	Cat. No.	Integration Description
SuperScript IV First-Strand Synthesis System	Thermo Fisher	18091050	Targeted primers allowed for RT
Maxima H Minus Frist Strand cDNA Synthesis Kit	Thermo Fisher	K1651	Targeted primers allowed for RT
ProtoScript II First Strand cDNA Synthesis Kit	NEB	E6560	Designed for targeted primer usage
TruSeq Stranded mRNA	Illumina	20020594	Supports upstream RT prior to library prep
TruSeq Stranded Total RNA with Ribo-Zero	Illumina	20020596	Supports upstream RT prior to library prep
RNA Prep with Enrichment	Illumina	20040536	Compatible with user-prepared cDNA workflows
NEBNext Ultra II RNA Library Prep	NEB	E7770	Accepts externally Prepared cDNA
NEBNext Ultra II Directional RNA Library Prep	NEB	E7760	Accepts externally Prepared cDNA
AmpliSeq for Illumina Custom RNA Panel	Illumina	20020496	RT step separable; Randomer substitution for MTPs

Contact us if your kit is not listed. We are validating protocol integration.

Protocol: MTP Reverse Transcription

Input

- RNA input: 10–500 ng of high-quality or partially degraded total RNA
- DNase treatment to remove residual genomic DNA
- Compatible with FFPE RNA and rRNA-depleted samples

Step 1: Annealing of MTP Primers

1. In a nuclease-free PCR tube, combine the following components:

Component	Volume	Notes
Total RNA	Up to 11 µL	Adjust to 11 µL total with water
MTP primer (2 µM)	1 µL	Replace oligo(dT) or N6 primers
dNTP mix (10 mM each)	1 µL	Final 0.5 mM concentration per nucleotide

2. Mix gently by pipetting of flicking the tube
3. Spin briefly to collect liquid at the bottom
4. Place in a thermal cycler and heat to Tm + 2°C for 5 minutes (refer to the MTP vial label for Tm)
5. Immediately place the tube on ice for at least 1 minute

Step 2: First-Strand Reverse Transcription Reaction

1. Add the following to the RNA/primer mix (final volume 20 µL):

Component	Volume	Notes
5X First-Strand RT buffer	4 µL	Use buffer supplied with your RT enzyme
100 mM DTT	1 µL	Reducing agent for optimal enzyme activity
Rnase Inhibitor (40 U/µL)	1 µL	e.g., RNaseOUT or equivalent
Reverse Transcriptase	1 µL	e.g., SuperScript IV, ProtoScript II, etc.

2. Mix gently and briefly spin down
3. Incubate in a thermal cycler using the following program
    - 42–50°C for 50 minutes (based on enzyme recommendation)
    - 70°C for 15 minutes to inactivate the enzyme
    - Hold at 4°C or proceed immediately to downstream steps

Continued.

Step 3: Post-RT Workflow

- Follow the library prep workflow with the resulting cDNA.
- Continue with second-strand synthesis, adapter ligation, tagmentation, depletion, or enrichment as required by your selected kit.
- MTP-generated cDNA is fully compatible with stranded workflows, dUTP-based marking, and downstream hybrid capture.

MTP Storage & Stability

Form: Supplied as lyophilized (dried) oligo pool reagent
Storage: Store at –20°C in a desiccated container
Resuspension: Use nuclease-free water or 10 mM Tris-HCl pH 8.0
Aliquoting: Recommended to avoid freeze-thaw
Shelf life: Stable >1 year at –20°C

Reaction Yield

MTP vial quantity	Total Oligo Yield	Estimated RT Reactions (20 µL, 2 µM MTP)
25 nmole	~25,000 pmol	250-500 reactions
100 nmole	~100,000 pmol	1,000-2,000 reactions

Contact

For questions, assistance, or comments email us at contact@multitarga.com.
https://multitarga.com.