

# UltraMarathonRT<sup>®</sup>

20,000 U/mL | Store at -20°C

## TEMPLATE SWITCHING KIT

**UltraMarathonRT** (uMRT) undergoes an efficient and highly selective form of template switching that can be used to introduce a specific primer binding site to the 5' end of RNAs within a library. Using the Template Switching Oligonucleotides (TSO) as its new template, uMRT then adds a section of known sequence to the 3' end of the resulting cDNA. In this way, cDNA products are tagged at each end, enabling efficient PCR amplification and RNA-seq library preparation.

→ **To complete the template switching experiment, additional reagents not contained in this kit are necessary.**

### Components Not Provided

dNTP mix (10 mM each) • dATP (10 mM) • uMRT dT<sub>18</sub> & AmpPCR Primer  
RNaseOUT • KAPA HiFi™ DNA polymerase (Roche, Cat# KK2502)

uMRT dT<sub>18</sub>: RT primer:

CCCTCTCTCTCTTTCTCTCTCTTTTTTTTTTTTTTTTTTT

AmpPCR: PCR primer for cDNA amplification:

CCCTCTCTCTCTTTCTCTCTC



For product details, visit: [www.RNAConnect.com](http://www.RNAConnect.com)

# Template Switching Quick Start Protocol

## ANNEALING RT PRIMERS TO RNA TEMPLATES

1. Gently mix the following components in a nuclease-free microcentrifuge tube and collect the liquid with a quick spin.

Components		Volume (3 $\mu$ L total)	Final
Primer	uMRT-dT <sub>18</sub> (5 $\mu$ M)	0.5 $\mu$ L	2.5 pmol
Template RNA	Total RNA	variable	1 ng - 100 ng
	poly(A)-RNA	variable	0.1 ng - 20 ng
dNTP mix (10 mM each)		0.5 $\mu$ L	0.5 mM
Nuclease-free Water		added to total 3 $\mu$ L	

2. Incubate at 95°C for 30 sec and then snap cool on ice to anneal the primer to the template.

## REVERSE TRANSCRIPTION

3. Gently mix the following components in a nuclease-free microcentrifuge tube and collect the liquid with a quick spin.

Components	Volume (7 $\mu$ L total)
2x UltraMarathonRT Buffer	5 $\mu$ L
UltraMarathonRT Enzyme	0.5 $\mu$ L
Low Boost	0.5 $\mu$ L
RNase Out™ (40 U/ $\mu$ L) (optional)	0.5 $\mu$ L
Nuclease-free Water	0.5 $\mu$ L

4. Add RT reaction mix to the annealed RNA and mix gently by tapping the tube (for a total volume of 10  $\mu$ L).
5. Incubate the mixture at 30°C for 30 min and then hold the tubes at 4°C in a PCR thermocycler.



Above: *uMRT Template Switching Kit Components*

## TEMPLATE SWITCHING

- Gently mix the following components in a nuclease-free microcentrifuge tube and collect the liquid with a quick spin.

*Note: Use a heated lid (55°C) to avoid water evaporation during the reaction.*

Components	Volume (10 $\mu$ L total)
5x Template Switching Buffer	4 $\mu$ L
UltraMarathonRT Enzyme	2 $\mu$ L
uMRT-TSO (10 $\mu$ M)	2 $\mu$ L
dATP (10 mM)	2 $\mu$ L

- Add the template switching reaction mix to the RT reaction tube from step 5 (for a total volume of 20  $\mu$ L) and gently mix by tapping the tube.
- Incubate at 37°C for 15 min to catalyze the template switching reaction.
- Inactivate the enzyme by heating at 95°C for 1 min. then hold the tube at 4°C in a PCR thermocycler.

*Note: For best results, we recommend pre-heating the thermocycler to 37°C prior to incubation.*

*Note: The resulting cDNA contains adapters at both ends and can be stored at -20°C until future use.*

## PRE-AMPLIFY cDNA USING KAPA HIFI

10. Gently mix the following components in a nuclease-free microcentrifuge tube and collect the liquid with a quick spin.

Components	Volume (100 $\mu$ L total)
Unpurified cDNA product (from Step 9)	20 $\mu$ L
AmpPCR primer (20 $\mu$ M)	10 $\mu$ L
5x GC enhanced buffer	20 $\mu$ L
dNTP mix (10 mM each)	3 $\mu$ L
KAPA HiFi DNA Polymerase <sup>®</sup> (1 U/ $\mu$ L)	2 $\mu$ L
Nuclease-free Water	45 $\mu$ L

11. Run the below PCR program in a thermocycler.

KAPA HiFi PCR Conditions				
Cycle	Denature	Anneal	Extend	Hold
1	98°C, 2 min	–	–	–
11-15*	98°C, 15 s	62°C, 30 s	72°C, 6 min	–
1	–	–	72°C, 5 min	4°C
$\infty$	–	–	–	4°C

*Note: 8 -9 cycles are suggested for 10 ng total RNA or 1 ng mRNA. 12 - 13 cycles are suggested for 1 ng total RNA or 0.1 ng of mRNA.*

*Note: The resulting pre-amplified cDNA contains adapters at both ends and can be stored at -20°C.*

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