QUICK PROTOCOL

Ovation® RNA-Seq System V2

Part No. 7102

Enter the number of reactions you are running in the provided field to automatically calculate the volumes needed to prepare each master mix. The calculated volume includes an appropriate overfill in excess of the nominal volume requirements (typically 10%) to allow for loss due to handling. Simply print this document to create a working guide for your experiment, which can be kept as a record.

Operator's Name:	**Date:**
Kit Part No: 7102-	**Kit Lot No:**
Number of Samples*:	

THERMAL CYCLER PROGRAMS

First Strand cDNA Synthesis	
Program 1: First Strand Primer Annealing	(For RNA inputs ≤1 ng) 65 °C – 2 min, hold at 4 °C (For RNA inputs >1 ng) 65 °C – 5 min, hold at 4 °C
Program 2: First Strand Synthesis	4 °C – 1 min, 25 °C – 10 min, 42 °C – 10 min, 70 °C – 15 min, hold at 4 °C
Second Strand cDNA Synthesis	
Program 3: Second Strand Synthesis	4 °C – 1 min, 25 °C – 10 min, 50 °C – 30 min, 80 °C – 20 min, hold at 4 °C
SPIA® Amplification	
Program 4: SPIA Amplification	4 °C – 1 min, 47 °C – 60 min, 80 °C – 20 min, hold at 4 °C

*Number of samples field ties into embedded logic to calculate suggested master mix volumes.
Tecan recommends processing a minimum of 4 samples at a time.

FIRST STRAND cDNA SYNTHESIS

Thaw the **First Strand cDNA Synthesis reagents (blue)** and **Nuclease-free Water (green)**.

Spin **A3 VER 7** briefly and place on ice. Vortex **A1 VER 4** and **A2 VER 3**, spin and place on ice.
Leave **Nuclease-free Water** at room temperature.

On ice, mix 2 µL of **A1** and 5 µL of total RNA sample (500 pg to 100 ng) in a 0.2 mL PCR tube.

Place the tubes in a thermal cycler running Program 1 (65 °C – 2 min, hold at 4 °C or 65 °C – 5 min, hold at 4 °C).

Once the thermal cycler reaches 4 °C, remove tubes and place on ice.

Prepare **First Strand Master Mix** (calculated volumes allow for appropriate overfill). Be sure to pipet **A3** enzyme slowly and rinse out tip at least five times into buffer.
Per sample combine:
2.5 µL Buffer Mix **A2** + 0.5 µL Enzyme Mix **A3**.
Mix well.

No. of Samples	A2	A3
1	2.5 µL	0.5 µL
0	0.0	0.0

Add 3 µL of **First Strand Master Mix** to each tube, mix by pipetting, spin and place on ice.

Place the tubes in a thermal cycler running Program 2
(4 °C – 1 min, 25 °C – 10 min, 42 °C – 10 min, 70 °C – 15 min, hold at 4 °C).

Once the thermal cycler reaches 4 °C, remove tubes, spin and place on ice.

Continue immediately with Second Strand cDNA Synthesis.

SECOND STRAND cDNA SYNTHESIS

Resuspend the Agencourt® beads provided with the Ovation RNA-Seq System V2 and leave at room temperature for use in the next step.

Thaw the **Second Strand cDNA Synthesis reagents (yellow)**.

Spin **B2 VER 2** briefly and place on ice. Vortex **B1 VER 3**, spin and place on ice.

Prepare **Second Strand Master Mix**. Be sure to pipet **B2** enzyme slowly.
Per sample combine:
9.7 µL Buffer Mix **B1** + 0.3 µL Enzyme Mix **B2**.
Mix well.

No. of Samples	B1	B2
1	9.7 µL	0.3 µL
0	0.0	0.0

Add 10 µL of **Second Strand Master Mix** to each reaction tube, mix by pipetting, spin and place on ice.

Place the tubes in a thermal cycler running Program 3
(4 °C – 1 min, 25 °C – 10 min, 50 °C – 30 min, 80 °C – 20 min, hold at 4 °C).

Once the thermal cycler reaches 4 °C, remove tubes, spin and place on bench top.

Continue immediately with Purification of cDNA.

PURIFICATION OF cDNA

Ensure the Agencourt beads have reached room temperature.
Mix the beads by inverting several times.
At room temperature, add 32 µL of Agencourt beads to each reaction tube and mix by pipetting 10 times.
Incubate at room temperature for 10 minutes.
Transfer the tubes to the magnet and let stand for an additional 5 minutes
Remove only 45 µL of the binding buffer.
Add 200 µL of **freshly prepared** 70% ethanol and let stand for 30 seconds. Remove the ethanol using a pipette.
Repeat the ethanol wash 2 more times.
Remove all excess ethanol after the final wash and let beads air dry for 15 to 20 minutes.
Ensure the tubes have completely dried and no residual ethanol is left.
Continue immediately with SPIA Amplification, with the cDNA bound to the dry beads.

SPIA AMPLIFICATION

Thaw the **SPIA Amplification Reagents (red)**.				
Invert **C3 VER 7** 5 times to mix, spin and place on ice. Vortex **C1 VER 9** and **C2 VER 11**, spin and place on ice.				
Prepare **SPIA Master Mix**. Per sample combine: 20 µL Buffer Mix **C2** + 10 µL Primer Mix **C1** + 10 µL Enzyme Mix **C3**. **Mix well.**	**No. of Samples**	**C2**	**C1**	**C3**
	1	20 µL	10 µL	10 µL
	0	0	0	0
Add 40 µL of **SPIA Master Mix** to each reaction tube and resuspend beads thoroughly by pipetting. Place on ice.				
Place the tubes in a thermal cycler running Program 4 (4 °C – 1 min, 47 °C – 60 min, 80 °C – 20 min, hold at 4 °C).				
Once the thermal cycler reaches 4 °C, remove tubes, spin and place on ice.				
Transfer tubes to the magnet and let stand 5 minutes to completely clear the solution of beads.				
Carefully transfer 40 µL of the cleared supernatant to a fresh tube.				
Continue immediately with Purification of SPIA cDNA or store SPIA cDNA at -20 °C.				

PURIFICATION OF SPIA cDNA

We recommend using the QIAGEN® MinElute® Reaction Cleanup Kit for best results.	**Purification Kit Part No.**	**Purification Kit Lot No.**
Add Buffer ERC in volume of:	Spin at speed:	For a duration of:
Add Buffer PE in volume of:	Spin at speed:	For a duration of:
To elute sample use 1X TE or Buffer EB.		
Add 1X TE or Buffer EB in volume of:	Spin at speed:	For a duration of:

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