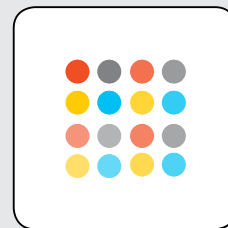


QUICK PROTOCOL

Ovation® PicoSL WTA System V2

Part No. 3312

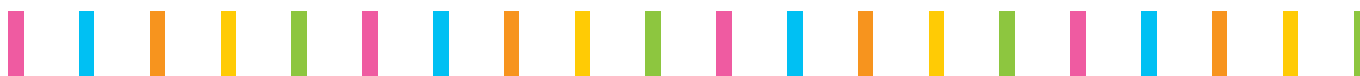
Enter the following information to automatically calculate the volumes needed to prepare each reaction. The calculated volumes include an appropriate overfill in excess of the nominal volume requirements to allow for volume loss due to handling. Simply print this document to create a working guide for your experiment, which can be kept as a record.

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

THERMAL CYCLER PROGRAMS

First Strand cDNA Synthesis	
Program 1: Primer Annealing	65 °C – 2 min, hold at 4 °C
Program 2: First Strand Synthesis	4 °C – 2 min, 25 °C – 30 min, 42 °C – 15 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 & Modification	
Program 4: SPIA Amplification	4 °C – 1 min, 47 °C – 75 min, 95 °C – 5 min, hold at 4 °C

*Number of samples field ties into embedded logic to calculate suggested master mix volumes, number of reactions.



FIRST STRAND cDNA SYNTHESIS

Step			
Thaw the **First Strand Reagents (blue)**. Mix each reagent, spin and place on ice.			
For each sample, place 2 µL of First Strand Primer Mix **A1 VER 8** into a 0.2 mL PCR tube.			
Add 5 µL of total RNA to each tube, mix and spin.			
Place the tubes in a thermal cycler running Program 1 (65 °C – 2 min, hold at 4 °C).			
After the cycler reaches 4 °C, remove tubes and place on ice.			
Prepare **First Strand Master Mix** (calculation allows for appropriate overfill). Pipet A3 enzyme slowly and rinse out tip at least five times into buffer. Per sample combine: 2.5 µL Buffer Mix **A2 VER 3** + 0.5 µL Enzyme Mix **A3 VER 1**. **Mix well.**	**No. of Samples**	**A2**	**A3**
	1	2.5 µL	0.5 µL
Add 3 µL of the **First Strand Master Mix** to each tube, mix and spin.			
Place the tubes in a thermal cycler running Program 2 (4 °C – 2 min, 25 °C – 30 min, 42 °C – 15 min, 70 °C – 15 min, hold at 4 °C).			

SECOND STRAND cDNA SYNTHESIS

Step			
Thaw the **Second Strand Reagents (yellow)**. Mix each reagent, spin and place on ice.			
Once the thermal cycler reaches 4 °C, remove tubes, spin and place on ice.			
Prepare **Second Strand Master Mix** (calculation allows for appropriate overfill). Per sample combine: 9.7 µL Buffer Mix **B1 VER 3** + 0.3 µL Enzyme Mix **B2 VER 2**. **Mix well.**	**No. of Samples**	**B1**	**B2**
	1	9.7 µL	0.3 µL
Add 10 µL of **Second Strand Master Mix** to each first strand reaction tube, mix and spin.			
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, spin and place tubes on ice.			

PURIFICATION OF DOUBLE-STRANDED cDNA

Step	Agencourt Bead Lot No.
Resuspend the Agencourt® beads and bring to room temperature.	
Remove reaction tubes from ice and place on bench at room temperature.	
Add 32 µL of beads to each reaction and mix.	
Incubate at room temperature for 10 minutes.	
Place tubes on magnet for 5 minutes to completely clear the beads.	
To minimize bead loss, remove only 45 µL of Binding Buffer before the first wash step.	
Wash the beads while still on the magnet for 30 seconds with 200 µL of freshly prepared 70% ethanol. Repeat wash 2 more times.	
Dry beads completely, at least for 15 to 20 minutes.	
Proceed with SPIA amplification.	

SPIA AMPLIFICATION

Thaw the **SPIA Amplification Reagents (red)**. Vortex **C1** and **C2**, invert **C3** 5 times. Spin all, place on ice.

Prepare **SPIA Master Mix** (calculation allows for appropriate overfill). Per sample combine:
20 µL **C2** VER **10** + 10 µL **C1** VER **9** + 10 µL **C3** VER **7**.
Mix well.

No. of Samples	C2	C1	C3
1	20 µL	10 µL	10 µL

On ice, add 40 µL of **SPIA Master Mix** to each second strand reaction tube containing the dried beads, mix and spin.

Place tubes in a thermal cycler running Program 4 (4 °C – 1 min, 47 °C – 75 min, 95 °C – 5 min, hold at 4 °C).

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

Proceed immediately to purification step or store SPIA cDNA at –20 °C.

PURIFICATION OF AMPLIFIED SPIA cDNA

Refer to the user guide and follow the method of choice for purification:	**Purification Kit Part No.**	**Purification Kit Lot No.**
Add Binding Buffer in volume of:	Spin at speed:	For a duration of:
Add Wash Buffer in volume of:	Spin at speed:	For a duration of:
Repeat for second wash.		
To elute sample use **Nuclease-free Water D1** provided with the Ovation PicoSL WTA System V2 kit.		
Add **Nuclease-free Water D1** in volume of:	Spin at speed:	For a duration of:

For Research Use Only. Not for use in diagnostic procedures.

Tecan Genomics, Inc.

USA
900 Chesapeake Drive
Redwood City, CA 94063 USA
Customer Service and Technical Support:
Toll Free Tel: 888.654.6544
Toll Free Fax: 888.296.6544
cservice-gn@tecan.com
techserv-gn@tecan.com

Europe
P.O. Box 109,
9350 AC Leek
The Netherlands
Customer Service and Technical Support:
Tel: +31-13-5780215
Fax: +31-13-5780216
europe-gn@tecan.com

Worldwide
For our international distributors contact information, visit our website
www.nugen.com

Australia +61 3 9647 4100 **Austria** +43 62 46 89 330 **Belgium** +32 15 42 13 19 **China** +86 21 220 63 206 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170 **Italy** +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 17 4 **Nordic** +46 8 750 39 40 **Singapore** +65 644 41 886 **Spain** +34 93 595 25 31 **Switzerland** +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +41 44 922 81 11

Tecan Group Ltd. makes every effort to include accurate and up-to-date information within this publication; however, it is possible that omissions or errors might have occurred. Tecan Group Ltd. cannot, therefore, make any representations or warranties, expressed or implied, as to the accuracy or completeness of the information provided in this publication. Changes in this publication can be made at any time without notice. For technical details and detailed procedures of the specifications provided in this document please contact your Tecan representative. This brochure may contain reference to applications and products which are not available in all markets. Please check with your local sales representative.

Tecan, Ovation and SPIA are registered trademarks and trademarks of Tecan Group Ltd., Männedorf, Switzerland or of Tecan Genomics, Inc., Redwood City, USA.

© 2019 Tecan Genomics, Inc., all rights reserved. For disclaimer and trademarks please visit **www.tecan.com**

www.tecan.com