QUICK PROTOCOL

Ovation® Ultralow System V2

Part Nos. 0344, 0344NB

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:	**Kit Lot No:**
Number of Samples†:	

THERMAL CYCLER PROGRAMS

End Repair	
Program 1: End Repair	25 °C – 30 min, 70 °C – 10 min, hold at 4 °C
Ligation	
Program 2: Ligation	25 °C – 30 min, 70 °C – 10 min, hold at 4 °C
Amplification	
Program 3: Amplification	72 °C – 2 min, 95 °C – 3 min, 7–15* cycles (98 °C – 20 s, 65 °C – 30 s, 72 °C – 30 s) 72 °C – 1 min, hold 4 °C

*__Important Note:__ The number of cycles used for PCR amplification depends on the starting amount of genomic DNA. Alternatively, real-time PCR can be used to determine the appropriate number of PCR cycles. For more information, contact Tecan NGS Technical Support.

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

Remove reagents for each section from storage as needed. Return to storage promptly after use. Continue immediately to the next section unless otherwise directed.

END REPAIR

Thaw **ER1**, **ER2**, **ER3** and **D1**. Mix as needed and spin down. Place **ER1**, **ER2** and **ER3** on ice, and **D1** at room temperature.

Prepare **End Repair Master Mix** (calculation allows for appropriate overfill). Per sample combine:
3.5 µL End Repair Buffer Mix **ER1** + 0.5 µL End Repair Enzyme Mix **ER2**+ 1.0 µL End Repair Enhancer **ER3**
Mix thoroughly in a 0.5 mL capped tube.

No. of Samples	ER1	ER2	ER3
1	3.5 µL	0.5 µL	1.0 µL
0	0	0	0

Add 5 µL of **End Repair Master Mix** to each tube containing 10 µL fragmented DNA sample (10 pg–100 ng), mix by pipetting, spin and place on ice.

Place tube in a thermal cycler running Program 1:
(25 °C – 30 min, 70 °C – 10 min, hold at 4 °C)

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

Leave **D1** at room temp for use in the next step.

LIGATION

Thaw **L1**, **L2**, **L3** and **D1**. Mix as needed and spin down. Place **L1**, **L2** and **L3** on ice, and **D1** at room temperature.

If using adaptors from tubes (0344NB-08), add 6 µL of appropriate Ligation Adaptor Mix **L2** to each sample and mix thoroughly.

If using an adaptor plate (0344-32 or 0344NB-32), add the entire 15 µL End Repair mixture to the appropriate adaptor well, mix well, then transfer the reaction to a PCR tube.

Prepare **Ligation Master Mix** (calculation allows for appropriate overfill). Per sample combine:
1.5 µL Nuclease-free Water **D1** + 6.0 µL Ligation Buffer Mix **L1**+
1.5 µL Ligation Enzyme Mix **L3**
Mix thoroughly in a 0.5 mL capped tube.

No. of Samples	D1	L1	L3
1	1.5 µL	6.0 µL	1.5 µL
0	0	0	0

Add 9 µL of **Ligation Master Mix** to each tube, mix thoroughly by pipetting slowly and gently, spin and place on ice.

Place tube in a thermal cycler running Program 2:
(25 °C – 30 min, 70 °C – 10 min, hold at 4 °C)

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

Leave **D1** at room temp for use in the next step.

LIGATION PURIFICATION

Ensure the Agencourt beads have reached room temperature.
Add 70 µL of room temperature Nuclease-free Water (**D1**) to each ligation reaction.
Mix the beads by inverting several times. At room temperature, add 80 µL of Agencourt XP 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 the binding buffer and discard it.
Add 200 µL of freshly prepared 70% ethanol and let stand for 30 seconds. Remove the ethanol using a pipette.
Repeat the ethanol wash one more time for a total of two washes.
Remove all excess ethanol after the final wash and let beads air dry for 10 minutes.
Ensure the tubes have completely dried and no residual ethanol is left.
Add 40 µL 1xTE buffer (low EDTA), mix thoroughly by pipetting and let stand for 3 minutes.
Transfer the tubes to the magnet and let stand for 3 minutes.
Remove 35 µL of eluate and transfer to a fresh set of PCR tubes and place on ice.

Optional stopping point: store samples at –20 °C.

LIBRARY AMPLIFICATION

Thaw **P1**, **P2** and **P3**. Mix as needed, spin down and place on ice.

Prepare **Amplification Master Mix** (calculation allows for appropriate overfill). Per sample combine:
12.75 µL Amplification Buffer Mix **P1** + 1.25 µL Amplification Primer Mix **P2** + 1.0 µL Amplification Enzyme Mix **P3**
Mix thoroughly in a 0.5 mL capped tube.

No. of Samples	P1	P2	P3
1	12.75 µL	1.25 µL	1.0 µL
0	0	0	0

On ice, add 15 µL of **Amplification Master Mix** to each tube, mix by pipetting, spin and place on ice.

Place the tubes in a thermal cycler programmed to run Program 3. Recommended PCR cycle numbers are given to the right.
72 °C – 2 min, 95 °C – 3 min, 7–15 cycles (98 °C – 20 s, 65 °C – 30 s, 72 °C – 30 s) 72 °C – 1 min, hold 4 °C

Starting Input	PCR cycles
< 1 ng	may require optimization
1 - 10 ng	13 - 15
10 - 50 ng	10 - 12
50 - 100 ng	7 - 9

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

AMPLIFIED LIBRARY PURIFICATION

Retrieve Agencourt beads and 70% ethanol set aside previously at room temperature.	
Mix the beads by inverting several times.	
At room temperature, add 50 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 binding buffer and discard it.	
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 10 minutes.	
Ensure the tubes have completely dried and no residual ethanol is left.	
Add 33 µL 1xTE buffer (low EDTA), mix thoroughly by pipetting.	
Transfer the tubes to the magnet and let stand for 2 minutes.	
Remove 30 µL of eluate and transfer to a fresh set of PCR tubes.	 *Optional stopping point: store samples at –20 °C.*
Proceed to Qualitative and Quantitative Assessment of the library.	

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

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