USC LIBRARY PREP AND CDNA SYNTHEIS PROTOCOL VER. 3

Eberwine Complete aRNA Protocol Hugo Version 2

Part I-I. First Round, First Strand cDNA synthesis

1) Start from 5ul samples

2) Make following mastermix(FSMM1)

	2.4	µl	5x First Strand buffer
	1.2	µl	dNTP's (2.5 mM each)
	0.3	µl	dT-T7 oligo (10ng/µl)
	0.45	µl	DTT (100mM)
Total:	**4.35**	µl	

3) Add 4.35ul of FSMM1 and Run Program **JEPR1**(Volume:9.35ul): 5 minutes at 70°C
4) Immediately place on ice (ice water bath with float)
5) Add 0.9µl water to bring volume up to 10.25 ul with DEPC treated water
6) Make mastermix(FSMM2)

	0.3	µl	Rnasin (avoid temperatures > 50 C)
	0.45	µl	Superscript III (can synthesize first strand up to 55 C)
	1	µl	DEPC water
Total:	**1.75**	µl	

7) Add 1.75ul of FSMM2 to samplesmix and spin briefly
8) Run Program **JEPR2**(Volume:12ul):

a. Incubate 30 minutes at 42°C
b. Incubate 15 minutes at 70°C

9) Store at –20 or -80°C or continue on to next steps

Part I-II. First Round, Second Strand cDNA synthesis

1) Make following MasterMix(SSMM).

	8.25	µl	RNase Free Water
	5.56	µl	5X Second strand buffer
	0.75	µl	dNTP mix (2.5mM each/10mM)
	1	µl	DNA polymerase I (10U/µl)
	0.25	µl	RNase H (2U/µl)
Total:	**15.81**	µl	

2) Add 15.81ul of SSMM to the samples(20ul) and ,mix thoroughly by pipetting and spin briefly
3) Run Program **JEPR3**(Volume:27.81ul): Incubate 2 hours at 16°C

4) After exactly 2hrs, add 1µl of T4 DNA polymerase (5 U/ml)
5) Mix by pipetting and spin briefly
6) Run Program **JEPR3.5**: Incubate 10 more minutes at 16°C
7) Clean up reaction with Minelute kit (Add ethanol (96–100%) to Buffer PE concentrate before use. If it is already added previously, you can just use it). (see bottle label for volume),

a. Load rotor Adaptors with collection tube and filter
b. Load samples on 2ml collection tubes
c. Load Buffer ERC, PE, and water
d. Load 200ul and 1000ul tips and run MinElute Program
e. When the program is finished, Move the liquid to 200ul tubes

8) Concentrate sample to ~8 µl using Speedvac

Part I-III. First Round, IVT using Ambion MEGAscript T7 Kit

1) Bring cDNA volume up with Minelute elution buffer
2) Make following mastermix(IVTMM):

2 µl	ATP (75 mM)-on ice
2 µl	CTP (75 mM)-on ice
2 µl	UTP (75 mM)-on ice
2 µl	GTP (75 mM)-on ice
2 µl	10X reaction buffer-RT while assembling rxn
	Mix well to fully resuspend pellet and add
2 µl	10X enzyme mix (on ice)
Total: 12 µl	

3) Run Program **JEPR4** (Volume:20ul):

a. with heated lid
b. 37° C **14 hours**
c. 4°C hold

4) Clean up reaction with MEGAclear 96 kit

a. Add 70µl of Binding Solution to Concentrate to the sample. Mix gently by pipetting.
b. Add 50 µL of 100% ethanol to the sample. Mix gently by pipetting.
c. Apply samples to 96 filters
d. Apply vacuum for 2mins or Centrifuge 1900 g for 3mins. While doing this, heat elution solution up to 95 degrees

e. Wash 2 times with 300ul of Wash Solution (Apply Vacuum for 2 mins)
f. Elute sample with preheated elution solution (Apply solution and vacuum 3 mins) X2. So that the final volume is 100ul
g. Precipitate with 5 M Ammonium Acetate. To concentrate the RNA, precipitate as follows. If the sample was eluted with 100 µL Elution Solution as suggested, add

10	µl	(0.1 volumes) ammonium acetate (5 M)
2	µl	glycogen (5 mg/mL)
250	µL	(2.5 volumes) **cold ethanol** (100%)

h. incubate at -80°C for 1-2hrs in a precooled rack
i. Microcentrifuge at top speed for 20 min at 4°C (Pre-cooled)
j. Carefully remove and discard the supernatant and wash the pellet with 800 µL 70% ethanol (in nuclease free water). Be sure to fully dislodge the pellet to remove all of the excess salt.
k. To remove the last traces of ethanol, quickly re-spin the tube, and aspirate any residual fluid with a very fine tipped pipette, or with a syringe needle.
l. Air dry the pellet for no longer than 15 min (~10mins).

5) Resuspend pellet in **4 µL DEPC–H2O** and proceed immediately to the appropriate round of first-strand synthesis

Part II-I. Second Round, First Strand synthesis

1) Bring aRNA volume to 4 uL with MEGAclear elution buffer
2) Add 1ul of Random primers (0.05 mg/ml)
3) Run Program **JEPR5** (Volumne:5ul): Heat at 70°C for 10 minutes
4) Immediately place on ice for at least 2 minutes
5) Make following MasterMix(SR-FSMM)

	2 µl	5x First Strand buffer
	0.5 µl	dNTP's (2.5 mM each/10mM)
	0.5 µl	RNasin
	1 µl	DTT (100mM)
	1 µl	Superscript III
Total:	**5** µl	

6) Add 5ul of SR-FSMM to smaples and Run Program **JEPR6**(Volume :10ul)

a. Allow to sit at RT(25°C) for 10 minuntes
b. Incubate 30 minutes at 42°C
c. Heat 5 minutes at 95°C to denature

7) Place on ice for at least 2 minutes (water ice bath)
8) Spin briefly

Part II-II. Second Round, Second strand cDNA synthesis

1) Add 2µl of dT-T7 oligo (10 ng/ml) to the first strand mix
2) Run Program **JEPR7** (Volume : 12ul): Heat for 6 minutes at 70°C
3) Immediately place on ice for at least 2 minutes
4) Spin briefly
5) Make following MasterMix(SR-SSMM)

	44.5	µl	DEPC water
	15	µl	5X Second strand buffer
	1.5	µl	dNTP mix (2.5 mM each/10mM)
	2	µl	DNA polymerase I
Total:	**63**	µl	

6) Add 63ul of SR-SSMM and Mix thoroughly by pipetting and spin briefly
7) Run Program **JEPR3**(Volume : 75ul): Incubate 2 hours at 16°C
8) Add 2µl of T4 DNA polymerase (5U/ml)
9) Mix thoroughly by pipetting and spin briefly
10) Run Program **JEPR3.5**(Volume : 77ul): Incubate 10 more minutes at 16°C
11) Clean up reaction with Minelute kit (Add ethanol (96–100%) to Buffer PE concentrate before use. If it is already added previously, you can just use it). (see bottle label for volume),

a. Load rotor Adaptors with collection tube and filter
b. Load samples on 2ml collection tubes
c. Load Buffer ERC, PE, and water
d. Load 200ul and 1000ul tips and run MinElute Program
e. When the program is finished, Move the liquid to 200ul tubes

12) Concentrate sample to ~8 µl using Speedvac

Part II-III. Second Round, IVT using Ambion MEGAscript T7 Kit

1) Bring cDNA volume up with Minelute elution buffer to 8ul
2) Make following mastermix(IVTMM):

2	µl	ATP (75 mM)-on ice
2	µl	CTP (75 mM)-on ice

2	µl	UTP (75 mM)-on ice
2	µl	GTP (75 mM)-on ice
2	µl	10X reaction buffer-RT while assembling rxn

Mix well to fully resuspend pellet and add

2	µl	10X enzyme mix (on ice)
Total: 12	µl	

3) Run Program **JEPR4** (Volume:20ul):

a. with heated lid
b. 37˚ C **14 hours**
c. 4˚C hold

4) Clean up reaction with MEGAclear 96 kit

a. Add 70µl of Binding Solution to Concentrate to the sample. Mix gently by pipetting.
b. Add 50 µL of 100% ethanol to the sample. Mix gently by pipetting.
c. Apply samples to 96 filters
d. Apply vacuum for 2mins or Centrifuge 1900 g for 3mins. While doing this, heat elution solution up to 95 degrees
e. Wash 2 times with 300ul of Wash Solution (Apply Vacuum for 2 mins)
f. Elute sample with preheated elution solution (Apply solution and vacuum 3 mins) X2. So that the final volume is 100ul
g. Precipitate with 5 M Ammonium Acetate. To concentrate the RNA, precipitate as follows. If the sample was eluted with 100 µL Elution Solution as suggested, add

10	µl	(0.1 volumes) ammonium acetate (5 M)
2	µl	glycogen (5 mg/mL)
250	µL	(2.5 volumes) **cold ethanol** (100%)

h. incubate at -80°C for 1-2hrs in a precooled rack
i. Microcentrifuge at top speed for 20 min at 4°C (Pre-cooled)
j. Carefully remove and discard the supernatant and wash the pellet with 800 µL 70% ethanol (in nuclease free water). Be sure to fully dislodge the pellet to remove all of the excess salt.
k. To remove the last traces of ethanol, quickly re-spin the tube, and aspirate any residual fluid with a very fine tipped pipette, or with a syringe needle.
l. Air dry the pellet for no longer than 15 min (~10mins).

5) Resuspend pellet in **4 µL DEPC–H2O** and proceed immediately to the appropriate round of first-strand synthesis

Part III-I. Third Round, First Strand synthesis

1) Bring aRNA volume to 4 uL with MEGAclear elution buffer
2) Add 1ul of Random primers (0.05 mg/ml)

3) Run Program **JEPR5** (Volumne:5ul): Heat at 70°C for 10 minutes
4) Immediately place on ice for at least 2 minutes
5) Make following MasterMix(TR-FSMM)

2	µl	5x First Strand buffer
0.5	µl	dNTP's (2.5 mM each/10mM)
0.5	µl	RNasin
1	µl	DTT (100mM)
1	µl	Superscript III
Total: **5**	µl	

6) Add 5ul of TR-FSMM to smaples and Run Program **JEPR6** (Volume :10ul)

a. Allow to sit at RT(25°C) for 10 minuntes
b. Incubate 30 minutes at 42°C
c. Heat 5 minutes at 95°C to denature

7) Place on ice for at least 2 minutes (water ice bath)
8) Spin briefly

Part III-II. Third Round, Second strand cDNA synthesis

1) Add 2µl of dT-T7 oligo (10 ng/ml) to the first strand mix
2) Run Program **JEPR7** (Volume : 12ul): Heat for 6 minutes at 70°C
3) Immediately place on ice for at least 2 minutes
4) Spin briefly
5) Make following MasterMix(TR-SSMM)

44.5	µl	DEPC water
15	µl	5X Second strand buffer
1.5	µl	dNTP mix (2.5 mM each/10mM)
2	µl	DNA polymerase I
Total: **63**	µl	

6) Add 63ul of TR-SSMM and Mix thoroughly by pipetting and spin briefly
7) Run Program **JEPR3**(Volume : 75ul): Incubate 2 hours at 16°C
8) Add 2µl of T4 DNA polymerase (5U/ml)
9) Mix thoroughly by pipetting and spin briefly
10) Run Program **JEPR3.5**(Volume : 77ul): Incubate 10 more minutes at 16°C
11) Clean up reaction with Minelute kit (Add ethanol (96–100%) to Buffer PE concentrate before use. If it is already added previously, you can just use it). (see bottle label for volume),

a. Load rotor Adaptors with collection tube and filter
b. Load samples on 2ml collection tubes
c. Load Buffer ERC, PE, and water
d. Load 200ul and 1000ul tips and run MinElute Program
e. When the program is finished, Move the liquid to 200ul tubes

12) Concentrate sample to ~8 µl using Speedvac

Part III-III. Third Round, IVT using Ambion MEGAscript T7 Kit

1) Bring cDNA volume up with Minelute elution buffer to 8ul
2) Make following mastermix(IVTMM):

2 µl	ATP (75 mM)-on ice
2 µl	CTP (75 mM)-on ice
2 µl	UTP (75 mM)-on ice
2 µl	GTP (75 mM)-on ice
2 µl	10X reaction buffer-RT while assembling rxn
	Mix well to fully resuspend pellet and add
2 µl	10X enzyme mix (on ice)
Total: 12 µl	

3) Run Program **JEPR4** (Volume:20ul):

a. with heated lid
b. 37˚ C **14 hours**
c. 4˚C hold

4) Clean up reaction with MEGAclear 96 kit

a. Add 70µl of Binding Solution to Concentrate to the sample. Mix gently by pipetting.
b. Add 50 µL of 100% ethanol to the sample. Mix gently by pipetting.
c. Apply samples to 96 filters
d. Apply vacuum for 2mins or Centrifuge 1900 g for 3mins. While doing this, heat **DEPC treated water** up to 95 degrees
e. Wash 2 times with 300ul of Wash Solution (Apply Vacuum for 2 mins)
f. Elute sample with preheated **DEPC treated water** (Apply solution and vacuum 3 mins) X2. So that the final volume is 100ul

5) Take out 1ul of each sample, mix with 4ul of Agilent RNA tapestation buffer and heat at 70˚ C for 3 mins.

6) Run Agilent 2200 Tapestation to check concentration.
7) Based on concentration, aliquot samples and dry down by Sample to ~0.5ul and proceed to Library construction. Keep the rest of samples in -80˚C

Part IV-I. Illumina Truseq, cDNA synthesis

1) Add 17 µl of Fragment, Prime, Finish Mix to each well of the RBP plate. Gently pipette the entire volume up and down 6 times to mix thoroughly.

2) Centrifuge the thawed First Strand Synthesis Act D Mix tube to 600 × g for 5 seconds.

3) Add 50 µl SuperScript II to the First Strand Synthesis Act D Mix tube. If you are not using the entire contents of the First Strand Synthesis Act D Mix tube, add SuperScript II at a ratio of 1 µl SuperScript II for each 9 µl First Strand Synthesis Act D Mix. Mix gently, but thoroughly, and centrifuge briefly. Label the First Strand Synthesis Act D Mix tube to indicate that the SuperScript II has been added.

4) Add 8 µl of First Strand Synthesis Act D Mix and SuperScript II mix to each well of the CDP plate. Gently pipette the entire volume up and down 6 times to mix thoroughly

5) Seal the CDP plate with a Microseal 'B' adhesive seal and centrifuge briefly.

6) Return the First Strand Synthesis Act D Mix tube to -15°C to -25°C storage immediately after use.

7) Place the sealed CDP plate on the pre-programmed thermal cycler. Close the lid, and then select and run the **Synthesize 1st Strand program**.

a. Choose the pre-heat lid option and set to 100°C
b.25°C for 10 minutes
c. 42°C for 15 minutes
d. 70°C for 15 minutes
e. Hold at 4°C

8) Do one of the following:
• If using the in-line control reagent:
— Centrifuge the thawed End Repair Control tube to 600 × g for 5 seconds.
— Dilute the End Repair Control to 1/50 in Resuspension Buffer (For example, 2 µl End Repair Control + 98 µl Resuspension Buffer) before use. Discard the diluted End Repair Control after use.
— Add 5 µl of diluted End Repair Control to each well of the CDP plate.

• If not using the in-line control reagent, add 5 µl of Resuspension Buffer to each well of the CDP plate.

9) Centrifuge the thawed Second Strand Marking Master Mix to 600 × g for 5 seconds.

10) Add 20 µl of thawed Second Strand Marking Master Mix to each well of the CDP plate. Gently pipette the entire volume up and down 6 times to mix thoroughly.

11) Seal the CDP plate with a Microseal 'B' adhesive seal.

12) Place the sealed CDP plate on the pre-heated thermal cycler. Close the lid and incubate at 16°C for 1 hour.

13) Remove the CDP plate from the thermal cycler and place it on the bench.
14) Remove the adhesive seal from the CDP plate.
15) Let the CDP plate stand to bring it to room temperature.

16) Vortex the AMPure XP beads until they are well dispersed.

17) Add 90 µl of well-mixed AMPure XP beads to each well of the CDP plate containing 50 µl of ds cDNA. Gently pipette the entire volume up and down 10 times to mix thoroughly.

18) Incubate the CDP plate at room temperature for 15 minutes.

19) Place the CDP plate on the magnetic stand at room temperature, for 5 minutes to make sure that all of the beads are bound to the side of the wells.

20) Remove and discard 135 µl supernatant from each well of the CDP plate.

21) With the CDP plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.

22) Incubate the CDP plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.

23) Repeat steps 21) and 22) one time for a total of two 80% EtOH washes.

24) Let the CDP plate stand at room temperature for 15 minutes to dry, and then remove the plate from the magnetic stand

25) Centrifuge the thawed, room temperature Resuspension Buffer to 600 × g for 5 seconds.

26) Add 17.5 µl Resuspension Buffer to each well of the CDP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

27) Incubate the CDP plate at room temperature for 2 minutes.

28) Place the CDP plate on the magnetic stand at room temperature for 5 minutes.

29) Transfer 15 µl supernatant (ds cDNA) from the CDP plate to the new 96-well 0.3 ml PCR plate labeled with the ALP barcode.

Part IV-II. Illumina Truseq, Library Construction

1) Do one of the following:
• If using the in-line control reagent:
— Centrifuge the thawed A-Tailing Control tube to 600 × g for 5 seconds.

— Dilute the A-Tailing Control to 1/100 in Resuspension Buffer (For example, 1 µl A-Tailing Control + 99 µl Resuspension Buffer) before use. Discard the diluted A-Tailing Control after use.
— Add 2.5 µl of diluted A-Tailing Control to each well of the ALP plate.

• If not using the in-line control reagent, add 2.5 µl of Resuspension Buffer to each well of the ALP plate.

2) Add 12.5 µl of thawed A-Tailing Mix to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

3) Seal the ALP plate with a Microseal 'B' adhesive seal.

4) Place the sealed ALP plate on the pre-programmed thermal cycler. Close the lid, then select and run the **ATAIL70 program**.

a. Choose the pre-heat lid option and set to 100°C
b.37°C for 30 minutes
c. 70°C for 5 minutes d Hold at 4°C

5) Centrifuge the thawed RNA Adapter tubes to 600 × g for 5 seconds.

6) Centrifuge the Ligation Control (if using Ligation Control) and Stop Ligation Buffer tubes to 600 × g for 5 seconds.

7) Immediately before use, remove the Ligation Mix tube from -15°C to -25°C storage.

8) Remove the adhesive seal from the ALP plate.

9) Do one of the following:

• If using the in-line control reagent:

— Dilute the Ligation Control to 1/100 in Resuspension Buffer (For example, 1 µl Ligation Control + 99 µl Resuspension Buffer) before use. Discard the diluted Ligation Control after use.

— Add 2.5 µl of diluted Ligation Control to each well of the ALP plate.

• If not using the in-line control reagent, add 2.5 µl of Resuspension Buffer to each well of the ALP plate.

10) Add 2.5 µl of Ligation Mix to each well of the ALP plate.

11) Return the Ligation Mix tube to -15°C to -25°C storage immediately after use.

12) Add 2.5 µl of the thawed RNA Adapter Index to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

13) Seal the ALP plate with a Microseal 'B' adhesive seal.

14) Centrifuge the ALP plate to 280 × g for 1 minute.

15) Place the sealed ALP plate on the pre-heated thermal cycler. Close the lid and incubate at 30°C for 10 minutes.

16) Remove the adhesive seal from the ALP plate.

17) Add 5 µl of Stop Ligation Buffer to each well of the ALP plate to inactivate the ligation. Gently pipette the entire volume up and down 10 times to mix thoroughly.

18) Vortex the AMPure XP Beads for at least 1 minute or until they are well dispersed.

19) Add 42 µl of mixed AMPure XP Beads to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

20) Incubate the ALP plate at room temperature for 15 minutes.

21) Place the ALP plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

22) Remove and discard 79.5 µl supernatant from each well of the ALP plate. Take care not to disturb the beads

23) With the ALP plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.

24) Incubate the ALP plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well. Take care not to disturb the beads.

25) Repeat steps 6 and 7 one time for a total of two 80% EtOH washes.

26) With the ALP plate on the magnetic stand, let the samples air-dry at room temperature for 15 minutes.

27) Remove the ALP plate from the magnetic stand.

28) Add 52.5 µl Resuspension Buffer to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly or until the beads are fully resuspended.

29) Incubate the ALP plate at room temperature for 2 minutes.

30) Place the ALP plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

31) Transfer 50 µl supernatant from each well of the ALP plate to the corresponding well of the new 0.3 ml PCR plate labeled with the CAP barcode. Take care not to disturb the beads.

32) Vortex the AMPure XP Beads until they are well dispersed.

33) Add 50 µl of mixed AMPure XP Beads to each well of the CAP plate for a second cleanup. Gently pipette the entire volume up and down 10 times to mix thoroughly.

34) Incubate the CAP plate at room temperature for 15 minutes.

35) Place the CAP plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

36) Remove and discard 95 µl supernatant from each well of the CAP plate. Take care not to disturb the beads.

37) With the CAP plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well. Take care not to disturb the beads.

38) Incubate the CAP plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well. Take care not to disturb the beads.

39) Repeat steps 20 and 21 one time for a total of two 80% EtOH washes.

40) With the CAP plate on the magnetic stand, let the samples air-dry at room temperature for 15 minutes, and then remove the plate from the magnetic stand.

41) Add 22.5 µl Resuspension Buffer to each well of the CAP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly or until the beads are fully resuspended.

42) Incubate the CAP plate at room temperature for 2 minutes.

43) Place the CAP plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

44) Transfer 20 µl supernatant from each well of the CAP plate to the corresponding well of the new 0.3 ml PCR plate labeled with the PCR barcode. Take care not to disturb the beads.

Part IV-III. Illumina Truseq, PCR enrichment

1) Add 5 µl of thawed PCR Primer Cocktail to each well of the PCR plate.

2) Add 25 µl of thawed PCR Master Mix to each well of the PCR plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

3) Seal the PCR plate with a Microseal 'B' adhesive seal.

4) Place the sealed PCR plate on the pre-programmed thermal cycler. Close the lid, then select and run **PCR to amplify the plate**.

a. Choose the pre-heat lid option and set to 100°C
b. 98°C for 30 seconds

c. 15 cycles of:
— 98°C for 10 seconds
— 60°C for 30 seconds
— 72°C for 30 seconds
d. 72°C for 5 minutes
e. Hold at 4°C

5) Remove the adhesive seal from the PCR plate.

6) Vortex the AMPure XP Beads until they are well dispersed.

7) Add 50 µl of the mixed AMPure XP Beads to each well of the PCR plate containing 50 µl of the PCR amplified library. Gently pipette the entire volume up and down 10 times to mix thoroughly.

8) Incubate the PCR plate at room temperature for 15 minutes.

9) Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

10) Remove and discard 95 µl supernatant from each well of the PCR plate.

11) With the PCR plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.

12) Incubate the PCR plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.

13) Repeat steps 7 and 8 one time for a total of two 80% EtOH washes.

14) With the PCR plate on the magnetic stand, let the samples air-dry at room temperature for 15 minutes, and then remove the plate from the magnetic stand.

15) Add 32.5 µl Resuspension Buffer to each well of the PCR plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

16) Incubate the PCR plate at room temperature for 2 minutes.

17) Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

18) Transfer 30 µl supernatant from each well of the PCR plate to the corresponding well of the new 0.3 ml PCR plate labeled with the TSP1 barcode.

19) Take 1µl of library and add 3µl of D1000 Tapestation buffer & Run tapestation.

20) Also, take another 1µl and dilute 1:10,000, run qPCR to check for concentration.