TruSeq RNA Preparation with Modification for Four Rounds of Poly-A Selection

Dr. Daniel Richter

Abstract

March, 2012; based on sample preparation guide v2, catalog # RS-930-1021, part # 15026495 Rev. A, August 2011

Modifications: samples in PCR tubes (because a plate/seal will not be used, some numbered steps are skipped); include two rounds of poly-A selection (with two separate sets of beads); repeat clean-up step after PCR to remove additional adapters; elute with 1.5 ul less volume off of beads (to leave 1 ul behind, not 2.5 ul)

Citation: Dr. Daniel Richter TruSeq RNA Preparation with Modification for Four Rounds of Poly-A Selection. **protocols.io** dx.doi.org/10.17504/protocols.io.imucc6w
Published: 04 Dec 2017

Guidelines

PCR Programs

mRNA Denaturation: 65ºC for 5 min, 4ºC hold

mRNA Elution 1: 80ºC for 2 min, 25ºC hold

80ºC: 80ºC forever (pre-heat, then run for 2 min)

Elution 2 – Frag – Prime: 94ºC for 8 min, 4ºC hold

1st Strand: 25ºC for 10 min, 42ºC for 50 min, 70ºC for 15 min, 4ºC hold

2nd Strand: 16ºC forever (pre-heat, then run for 1 hour)

End Repair: 30ºC forever (pre-heat, then run for 30 min)

A Tail: 37ºC forever (pre-heat, then run for 30 min)

Ligate Adapters: 30ºC forever (pre-heat, then run for 10 min)

PCR: 98ºC for 30 sec; 15 cycles: 98ºC for 10 sec, 60ºC for 30 sec, 72ºC for 30 sec; 72ºC for 5 min, 10ºC hold

Protocol

Purify and Fragment mRNA - Make RBP
Step 1.

Start with 50 µL total RNA

Purify and Fragment mRNA - Make RBP
Step 2.

Vortex RNA Purification Beads (RPB) to resuspend

Purify and Fragment mRNA - Make RBP
Step 3.

Add 50 µL RPB to RNA

Purify and Fragment mRNA - Make RBP
Step 4.

Gently pipette up and down 6 times to mix thoroughly

Purify and Fragment mRNA - Incubate 1 RBP
Step 5.

Pre-heat thermal cycler lid to 100ºC

Purify and Fragment mRNA - Incubate 1 RBP
Step 6.

Run the mRNA Denaturation program

NOTES
Alyssa Alsante 26 Jun 2017

Program details can be found in the guidelines

Purify and Fragment mRNA - Incubate 1 RBP
Step 7.

Remove PCR tubes when the cycler reaches 4ºC

Purify and Fragment mRNA - Incubate 1 RBP
Step 8.

Incubate tubes at room temperature (RT) on the bench for 5 min

NOTES

Alyssa Alsante 26 Jun 2017

This allows the RNA to bind to the beads

Purify and Fragment mRNA - Wash RBP

Step 9.

Place tubes on a magnetic stand at RT for 5 min

NOTES

Alyssa Alsante 26 Jun 2017

This allows seperation of the poly-A RNA beads from the solution

Purify and Fragment mRNA - Wash RBP

Step 10.

Remove and discard the supernatant from each tube. Take care not to disturb the beads.

Purify and Fragment mRNA - Wash RBP

Step 11.

Remove tubes from the magnetic stand

Purify and Fragment mRNA - Wash RBP

Step 12.

Wash beads with Bead Washing Buffer (BWB)

AMOUNT

200 µl Additional info:

Purify and Fragment mRNA - Wash RBP

Step 13.

Gently pipette up and down 6 times to mix thoroughly

Purify and Fragment mRNA - Wash RBP

Step 14.

Place tubes on a magnetic stand at RT for 5 min

Purify and Fragment mRNA - Wash RBP

Step 15.

Remove and discard the supernatant from each tube. Take care not to disturb the beads.

NOTES

Alyssa Alsante 26 Jun 2017

The supernatant contains the majority of the ribosomal and other non-messenger RNA

Purify and Fragment mRNA - Wash RBP
Step 16.

Remove tubes from the magnetic stand

Purify and Fragment mRNA - Wash RBP
Step 17.

Add Elution Buffer (ELB) to each tube

AMOUNT
50 µl Additional info:

Purify and Fragment mRNA - Wash RBP
Step 18.

Gently pipette up and down 6 times to mix thoroughly

Purify and Fragment mRNA - Incubate 2 RBP
Step 19.

Pre-heat the thermal cycler lid to 100ºC

Purify and Fragment mRNA - Incubate 2 RBP
Step 20.

Run the mRNA Elution 1 program

NOTES
Alyssa Alsante 26 Jun 2017

Program details can be found in the guidelines

Purify and Fragment mRNA - Incubate 2 RBP
Step 21.

Remove the tubes from the thermal cycler when it reaches 25ºC

Purify and Fragment mRNA - Incubate 2 RBP
Step 22.

Place the tubes on the benchtop at RT

Purify and Fragment mRNA - Make RFP
Step 23.

Add Bead Binding Buffer (BBB) to each tube

AMOUNT
50 µl Additional info:

NOTES
Alyssa Alsante 28 Jun 2017

This allows mRNA to specifically rebind the beads, while reducing the amoung of rRNA that non-specifically binds.

Purify and Fragment mRNA - Make RFP
Step 24.

Gently pipette up and down 6 times to mix thoroughly

Purify and Fragment mRNA - Make RFP
Step 25.

Incubate the tubes at room temperature for 5 minutes

Purify and Fragment mRNA - Make RFP
Step 26.

Place the tubes on a magnetic stand at RT for 5 min

Purify and Fragment mRNA - Make RFP
Step 27.

Remove and discard supernatant from each tube. Take care not to disturb the beads.

Purify and Fragment mRNA - Make RFP
Step 28.

Remove the tubes from the magnetic stand

Purify and Fragment mRNA - Make RFP
Step 29.

Wash the beads with Bead Washing Buffer (BWB)

AMOUNT
200 µl Additional info:

Purify and Fragment mRNA - Make RFP
Step 30.

Gently pipette up and down 6 times to mix thoroughly

Purify and Fragment mRNA - Make RFP
Step 31.

Place the tubes on a magnetic stand for 5 min

Purify and Fragment mRNA - Make RFP

Step 32.

Remove and discard all of the supernatant from each tube. Take care not to disturb the beads.

NOTES

Alyssa Alsante 28 Jun 2017

The supernatant contains residual rRNA and other contaminants, and were released in the first elution and did not rebind the beads.

Purify and Fragment mRNA - Make RFP

Step 33.

Remove the tubes from the magnetic stand

Purify and Fragment mRNA - Make RFP

Step 34.

If this is the first time through the protocol: continue to repeat Poly-A Selection.

Purify and Fragment mRNA - Make RFP

Step 35.

If this is the second time through the protocol: add Elute, Prime, Fragment Mix (EPF) to each tube

AMOUNT

18 µl Additional info:

NOTES

Alyssa Alsante 03 Jul 2017

The Elute, Prime, Fragment Mix contains random hexamers for RT priming and serves as the first strand cDNA synthesis reaction buffer.

Purify and Fragment mRNA - Make RFP

Step 36.

Gently pipette up and down 6 times to mix thoroughly

NOTES

Alyssa Alsante 28 Jun 2017

The Elute, Prime, Fragment Mix contains random hexamers for RT priming and serves as the first strand cDNA synthesis reaction buffer. After adding 18 uL Elute, Prime, Fragment Mix, continue to **Incubate RFP**.

Purify and Fragment mRNA - Repeat Poly-A Selection with New Beads

Step 37.

Add Elution Buffer (ELB) to each tube

AMOUNT
50 µl Additional info:

Purify and Fragment mRNA - Repeat Poly-A Selection with New Beads
Step 38.

Gently pipette up and down 6 times to mix thoroughly

Purify and Fragment mRNA - Repeat Poly-A Selection with New Beads
Step 39.

Pre-heat the thermal cycler to 80ºC with the program 80ºC

NOTES
Alyssa Alsante 28 Jun 2017

Program details can be found in the guidelines

Purify and Fragment mRNA - Repeat Poly-A Selection with New Beads
Step 40.

Incubate the tubes for 2 min at 80ºC

Purify and Fragment mRNA - Repeat Poly-A Selection with New Beads
Step 41.

Immediately transfer the tubes to the magnetic stand

NOTES
Alyssa Alsante 28 Jun 2017

This prevents mRNA from re-binding to the beads

Purify and Fragment mRNA - Repeat Poly-A Selection with New Beads
Step 42.

Transfer supernatant to a new set of tubes immediately after the beads have aggregated on the magnetic stand

AMOUNT
50 µl Additional info:

Purify and Fragment mRNA - Repeat Poly-A Selection with New Beads
Step 43.

Repeat the entire protocol from step 2 (Purify and Fragment mRNA - Make RBP / Vortex RNA Purification Beads to resuspend) to step 35 (Purify and Fragment mRNA - Make RFP / Add Elute, Prime, Fragment Mix)

Purify and Fragment mRNA - Incubate RFP (RNA Fragmentation Plate)

Step 44.

Pre-heat the thermal cycler lid to 100ºC

Purify and Fragment mRNA - Incubate RFP (RNA Fragmentation Plate)

Step 45.

Run the program Elution 2 - Frag - Prime

NOTES

Alyssa Alsante 28 Jun 2017

Program details can be found in the guidelines

Purify and Fragment mRNA - Incubate RFP (RNA Fragmentation Plate)

Step 46.

Remove the tubes from the thermal cycler when it reaches 4ºC and centrifuge briefly

NOTES

Alyssa Alsante 28 Jun 2017

Proceed immediately to Synthesize First Stand cDNA

Synthesize First Strand cDNA - Make CDP

Step 47.

Place the tubes on the magnetic stand at RT for 5 min

Synthesize First Strand cDNA - Make CDP

Step 48.

Transfer supernatant (fragmented and primed mRNA) to new tubes

AMOUNT

17 µl Additional info:

Synthesize First Strand cDNA - Make CDP

Step 49.

Add First Strand Master Mix (FSM)/SuperScript II to each tube

AMOUNT

8 µl Additional info:

Synthesize First Strand cDNA - Make CDP

Step 50.

Gently pipette up and down 6 times to mix thoroughly

Synthesize First Strand cDNA - Make CDP

Step 51.

Return the First Strand Master Mix/SuperScript II tube back to -20ºC immediately after use

Synthesize First Strand cDNA - Incubate 1 CDP

Step 52.

Pre-heat the thermal cycler lid to 100ºC

Synthesize First Strand cDNA - Incubate 1 CDP

Step 53.

Run the first strand program

NOTES

Alyssa Alsante 28 Jun 2017

Program details can be found in the guidelines

Synthesize First Strand cDNA - Incubate 1 CDP

Step 54.

When the thermal cycler reaches 4ºC, remove the tubes and proceed immediately to Synthesize Second Strand cDNA.

Synthesize Second Strand cDNA - Add SSM

Step 55.

Add thawed Second Strand Master Mix (SSM) to each tube

AMOUNT

25 µl Additional info:

Synthesize Second Strand cDNA - Add SSM

Step 56.

Gently pipette up and down 6 times to mix thoroughly

Synthesize Second Strand cDNA - Incubate 2 CDP

Step 57.

Pre-heat the thermal cycler to 16ºC with the second strand program

NOTES

Alyssa Alsante 28 Jun 2017

Program details can be found in the guidelines

Synthesize Second Strand cDNA - Incubate 2 CDP

Step 58.

Incubate the tubes at 16ºC for 1 hr

Synthesize Second Strand cDNA - Incubate 2 CDP

Step 59.

Remove the tubes from the thermal cycler and let stand to bring to RT

Synthesize Second Strand cDNA - Clean Up CDP

Step 60.

Vortex the AMPure XP beads until they are well dispersed

Synthesize Second Strand cDNA - Clean Up CDP

Step 61.

Add 90 µL beads to each tube containing 50 µL ds cDNA

Synthesize Second Strand cDNA - Clean Up CDP

Step 62.

Gently pipette the entire volume up and down 10 times to mix thoroughly

Synthesize Second Strand cDNA - Clean Up CDP

Step 63.

Incubate the tubes at RT for 15 min

Synthesize Second Strand cDNA - Clean Up CDP

Step 64.

Place the tubes on the magnetic stand at RT for 5 min

Synthesize Second Strand cDNA - Clean Up CDP

Step 65.

Remove and discard 135 µL supernatant from each tube. Take care not to disturb the beads.

Synthesize Second Strand cDNA - Clean Up CDP

Step 66.

With the tubes remaining on the magnetic stand, add 80% EtOH to each tube without disturbing the beads.

AMOUNT

200 µl Additional info:

Synthesize Second Strand cDNA - Clean Up CDP

Step 67.

Incubate tubes at RT for 30 sec

Synthesize Second Strand cDNA - Clean Up CDP
Step 68.

Remove and discard the supernatant from each tube without disturbing the beads

Synthesize Second Strand cDNA - Clean Up CDP
Step 69.

Repeat steps 66-68 for a total of two 80% EtOH washes

Synthesize Second Strand cDNA - Clean Up CDP
Step 70.

Let the tubes stand at RT for 15 min to dry

Synthesize Second Strand cDNA - Clean Up CDP
Step 71.

Remove them from the magnetic stand

Synthesize Second Strand cDNA - Clean Up CDP
Step 72.

Add Resuspension Buffer (RSB) to each tube

AMOUNT
61 µl Additional info:

Synthesize Second Strand cDNA - Clean Up CDP
Step 73.

Gently pipette the entire volume up and down 10 times to mix thoroughly

Synthesize Second Strand cDNA - Clean Up CDP
Step 74.

Incubate the tubes at RT for 2 min

Synthesize Second Strand cDNA - Clean Up CDP
Step 75.

Place the tubes on the magnetic stand at RT for 5 min

Synthesize Second Strand cDNA - Clean Up CDP
Step 76.

Transfer 60 µL supernatant (ds cDNA) to a new set of tubes

NOTES
Alyssa Alsante 28 Jun 2017

This is a safe stopping point for storage at -20 degrees C

Perform End Repair - Make IMP
Step 77.

Add End Repair Mix (ERP) to each tube

AMOUNT
40 µl Additional info:

Perform End Repair - Make IMP
Step 78.

Adjust the pipette to 100 µL and gently pipette the entire volume up and down 10 times to mix thoroughly

Perform End Repair - Incubate 1 IMP
Step 79.

Pre-heat the thermal cycler to 30ºC with the End Repair program

NOTES
Alyssa Alsante 28 Jun 2017

Program details can be found in the guidelines

Perform End Repair - Incubate 1 IMP
Step 80.

Incubate the tubes at 30ºC for 30 min

Perform End Repair - Incubate 1 IMP
Step 81.

Remove the tubes from the thermal cycler

Perform End Repair - Clean Up IMP
Step 82.

Vortex the AMPure XP beads until they are well dispersed

Perform End Repair - Clean Up IMP
Step 83.

Add 160 µL beads to each tube containing 100 µL of sample

Perform End Repair - Clean Up IMP

Step 84.

Adjust the pipette to 200 µL and gently pipette up and down 10 times to mix thoroughly

Perform End Repair - Clean Up IMP
Step 85.

Incubate the tubes at RT for 15 min

Perform End Repair - Clean Up IMP
Step 86.

Place the tubes on the magnetic stand at RT for 5 min or until liquid appears clear

Perform End Repair - Clean Up IMP
Step 87.

Remove and discard 127.5 µL of the supernatant from each tube

Perform End Repair - Clean Up IMP
Step 88.

Repeat step 87 once

Perform End Repair - Clean Up IMP
Step 89.

With the tubes remaining on the magnetic stand, add 80% freshly prepared EtOH to each tube without disturbing the beads

AMOUNT
200 µl Additional info:

Perform End Repair - Clean Up IMP
Step 90.

Incubate the tubes at RT for 30 sec

Perform End Repair - Clean Up IMP
Step 91.

Remove and discard the supernatant from each tube without disturbing the beads

Perform End Repair - Clean Up IMP
Step 92.

Repeat steps 89-91 once, for a total of two 80% EtOH washes

Perform End Repair - Clean Up IMP
Step 93.

Let the tubes stand at RT for 15 min to dry

Perform End Repair - Clean Up IMP
Step 94.

Remove the tubes from the magnetic stand

Perform End Repair - Clean Up IMP
Step 95.

Resuspend the dried pellet in each tube in Resuspension Buffer (RSB)

AMOUNT
18.5 µl Additional info:

Perform End Repair - Clean Up IMP
Step 96.

Gently pipette the entire volume up and down 10 times to mix thoroughly

Perform End Repair - Clean Up IMP
Step 97.

Incubate the tubes at RT for 2 min

Perform End Repair - Clean Up IMP
Step 98.

Place the tubes on the magnetic stand at RT for 5 min, or until the liquid appears clear.

Perform End Repair - Clean Up IMP
Step 99.

Transfer 17.5 µL of the clear supernatant to a new set of tubes

NOTES
Alyssa Alsante 28 Jun 2017

This is a safe stopping point for storage at -20 degrees C

Adenylate 3' Ends - Add ATL
Step 100.

Add A-Trailing Mix (ATL) to each tube

AMOUNT
12.5 µl Additional info:

Adenylate 3' Ends - Add ATL
Step 101.

Adjust the pipette to 30 µL and gently pipette the entire volume up and down 10 times to mix thoroughly

Adenylate 3' Ends - Incubate 1 ATL
Step 102.

Pre-heat the thermal cycler to 37ºC with the A Tail program

NOTES
Alyssa Alsante 28 Jun 2017

Program details can be found in the guidelines

Adenylate 3' Ends - Incubate 1 ATL
Step 103.

Incubate the tubes at 37ºC for 30 min

Adenylate 3' Ends - Incubate 1 ATL
Step 104.

Immediately remove the tubes from the thermal cycler, and proceed immediately to Ligate Adapters

Ligate Adapters - Add LIG
Step 105.

Add Resuspension Buffer (RSB) to each tube

AMOUNT
2.5 µl Additional info:

Ligate Adapters - Add LIG
Step 106.

Immediately before use, remove the Ligation Mix (LIG) tube from -20ºC storage.

Ligate Adapters - Add LIG
Step 107.

Add Ligation Mix (LIG) to each tube

AMOUNT
2.5 µl Additional info:

NOTES
Alyssa Alsante 28 Jun 2017

Return the LIG back to -20 C immediately after use

Ligate Adapters - Add LIG
Step 108.

Add appropriate RNA Adapter Index to each tube

AMOUNT
2.5 µl Additional info:

Ligate Adapters - Add LIG
Step 109.

Adjust the pipette to 37.5 µL and gently pipette the entire volume up and down 10 times to mix thoroughly

Ligate Adapters - Incubate 2 ALP
Step 110.

Pre-heat the thermal cycler to 30ºC with the Ligate Adapters program

NOTES
Alyssa Alsante 28 Jun 2017

Program details can be found in the guidelines

Ligate Adapters - Incubate 2 ALP
Step 111.

Incubate the tubes at 30ºC for 10 min

Ligate Adapters - Incubate 2 ALP
Step 112.

Remove the tubes from the thermal cycler

Ligate Adapters - Add STL
Step 113.

Add Stop Ligation Buffer (STL) to each tube

AMOUNT
5 µl Additional info:

NOTES
Alyssa Alsante 28 Jun 2017

This step inactivates ligation

Ligate Adapters - Add STL
Step 114.

Adjust the pipette to 42.5 µL and pipette the entire volume up and down 10 times to mix thoroughly

Ligate Adapters - Clean Up ALP
Step 115.

Vortex the AMPure XP beads until they are well dispersed

Ligate Adapters - Clean Up ALP

Step 116.

Add beads to each tube

AMOUNT

42 µl Additional info:

Ligate Adapters - Clean Up ALP

Step 117.

Adjust the pipette to 85 µL, then gently pipette the entire volume up and down 10 times to mix thoroughly.

Ligate Adapters - Clean Up ALP

Step 118.

Incubate the tubes at RT for 15 min

Ligate Adapters - Clean Up ALP

Step 119.

Place the tubes on the magnetic stand at RT for 5 min or until the liquid appears clear

Ligate Adapters - Clean Up ALP

Step 120.

Remove and discard 79.5 µL of the supernatant from each tube

Ligate Adapters - Clean Up ALP

Step 121.

With the tubes remaining on the magnetic stand, add freshly prepared 80% EtOH to each tube without disturbing the beads.

AMOUNT

200 µl Additional info:

Ligate Adapters - Clean Up ALP

Step 122.

Incubate the tubes at RT for 30 sec

Ligate Adapters - Clean Up ALP

Step 123.

Remove and discard the supernatant from each tube without disturbing the beads

Ligate Adapters - Clean Up ALP

Step 124.

Repeat steps 121-123 for a total of two 80% EtOH washes

Ligate Adapters - Clean Up ALP

Step 125.

Let the tubes stand at RT for 15 min to dry

Ligate Adapters - Clean Up ALP

Step 126.

Remove the tubes from the magnetic stand

Ligate Adapters - Clean Up ALP

Step 127.

Resuspend the dried pellet in each tube in Resuspension Buffer (RSB)

AMOUNT

51 µl Additional info:

Ligate Adapters - Clean Up ALP

Step 128.

Gently pipette the entire volume up and down 10 times to mix thoroughly

Ligate Adapters - Clean Up ALP

Step 129.

Incubate the tubes at RT for 2 min

Ligate Adapters - Clean Up ALP

Step 130.

Place the tubes on the magnetic stand at RT for 5 min or until the liquid appears clear

Ligate Adapters - Clean Up ALP

Step 131.

Transfer 50 µL of the clear supernatant to a new set of tubes

Ligate Adapters - Clean Up ALP

Step 132.

Vortex the AMPure XP beads until they are well dispersed

Ligate Adapters - Clean Up ALP

Step 133.

Add beads to each tube for a second clean up

AMOUNT

50 µl Additional info:

Ligate Adapters - Clean Up ALP

Step 134.

Adjust the pipette to 100 µL, then gently pipette the entire volume up and down 10 times to mix thoroughly.

Ligate Adapters - Clean Up ALP

Step 135.

Incubate the tubes at RT for 15 min

Ligate Adapters - Clean Up ALP

Step 136.

Place the tubes on the magnetic stand at RT for 5 min or until the liquid appears clear

Ligate Adapters - Clean Up ALP

Step 137.

Remove and discard 95 µL of the supernatant from each tube

Ligate Adapters - Clean Up ALP

Step 138.

With the tubes remaining on the magnetic stand, add freshly prepared 80% EtOH to each tube without disturbing the beads.

AMOUNT

200 µl Additional info:

Ligate Adapters - Clean Up ALP

Step 139.

Incubate the tubes at RT for 30 sec

Ligate Adapters - Clean Up ALP

Step 140.

Remove and discard the supernatant from each tube without disturbing the beads

Ligate Adapters - Clean Up ALP

Step 141.

Repeat steps 138-140 for a total of two 80% EtOH washes

Ligate Adapters - Clean Up ALP

Step 142.

Let the tubes stand at RT for 15 min to dry

Ligate Adapters - Clean Up ALP
Step 143.

Remove the tubes from the magnetic stand

Ligate Adapters - Clean Up ALP
Step 144.

Resuspend the dried pellet in each tube in RSB

AMOUNT
21 µl Additional info:

Ligate Adapters - Clean Up ALP
Step 145.

Gently pipette the entire volume up and down 10 times to mix thoroughly

Ligate Adapters - Clean Up ALP
Step 146.

Incubate the tubes at RT for 2 min

Ligate Adapters - Clean Up ALP
Step 147.

Place the tubes on the magnetic stand at RT for 5 min or until the liquid appears clear

Ligate Adapters - Clean Up ALP
Step 148.

Transfer 20 µL of the clear supernatant to a new set of tubes

NOTES
Alyssa Alsante 03 Jul 2017

This is a safe stopping point for storage at -20 C

Enrich DNA Fragments - Make PCR
Step 149.

Add thawed PCR Primer Cocktail (PPC) to each tube

AMOUNT
5 µl Additional info:

Enrich DNA Fragments - Make PCR
Step 150.

Add thawed PCR Master Mix (PMM) to each tube

AMOUNT
25 µl Additional info:

Enrich DNA Fragments - Make PCR
Step 151.

Adjust the pipette to 50 µL then gently pipette the entire volume up and down 10 times to mix thoroughly

Enrich DNA Fragments - Amp PCR
Step 152.

Pre-heat the thermal cycler lid to 100ºC

Enrich DNA Fragments - Amp PCR
Step 153.

Run the PCR program

NOTES
Alyssa Alsante 03 Jul 2017

Program details can be found in the guidelines

Enrich DNA Fragments - Clean Up PCR
Step 154.

Vortex the AMPure XP beads until they are well dispersed

Enrich DNA Fragments - Clean Up PCR
Step 155.

Add beads to each tube

AMOUNT
50 µl Additional info:

Enrich DNA Fragments - Clean Up PCR
Step 156.

Adjust the pipette to 100 µL, then gently pipette the entire volume up and down 10 times to mix thoroughly.

Enrich DNA Fragments - Clean Up PCR
Step 157.

Incubate the tubes at RT for 15 min

Enrich DNA Fragments - Clean Up PCR

Step 158.

Place the tubes on the magnetic stand at RT for 5 min or until the liquid appears clear

Enrich DNA Fragments - Clean Up PCR
Step 159.

Remove and discard 95 µL of the supernatant from each tube

Enrich DNA Fragments - Clean Up PCR
Step 160.

With the tubes remaining on the magnetic stand, add 80% freshly prepared EtOH to each tube without disturbing the beads

AMOUNT
200 µl Additional info:

Enrich DNA Fragments - Clean Up PCR
Step 161.

Incubate the tubes at RT for 30 sec

Enrich DNA Fragments - Clean Up PCR
Step 162.

Remove and discard the supernatant from each tube without disturbing the beads

Enrich DNA Fragments - Clean Up PCR
Step 163.

Repeat steps 160-162 for a total of two 80% EtOH washes

Enrich DNA Fragments - Clean Up PCR
Step 164.

Let the tubes stand at RT for 15 min to dry

Enrich DNA Fragments - Clean Up PCR
Step 165.

Remove the tubes from the magnetic stand

Enrich DNA Fragments - Clean Up PCR
Step 166.

Resuspend the dried pellet in each tube in Resuspension Buffer (RSB)

AMOUNT
51 µl Additional info:

Enrich DNA Fragments - Clean Up PCR

Step 167.

Gently pipette the entire volume up and down 10 times to mix thoroughly

Enrich DNA Fragments - Clean Up PCR
Step 168.

Incubate the tubes at RT for 2 min

Enrich DNA Fragments - Clean Up PCR
Step 169.

Place the tubes on the magnetic stand at RT for 5 min or until the liquid appears clear

Enrich DNA Fragments - Clean Up PCR
Step 170.

Transfer 50 µL of the clear supernatant to a new set of tubes

Enrich DNA Fragments - Clean Up PCR
Step 171.

Repeat steps 154-170 once. During the repeated steps, modify step 166 to add 31 ul Resuspension Buffer (RSB) and step 170 to transfer 30 ul to separate 1.5 ml Eppendorf tubes.

Enrich DNA Fragments - Clean Up PCR
Step 172.

Take one aliquot for Qubit and one aliquot for qPCR in 1.5 mL tubes

AMOUNT
1 µl Additional info:

Enrich DNA Fragments - Clean Up PCR
Step 173.

Freeze cleaned PCR and both aliquot tubes at -20ºC

Quantitate Library with Qubit dsDNA HS Assay
Step 174.

Make Qubit working solution in a 1.5 mL Eppendorf tube by mixing 7 µL Qubit dsDNA HS reagent (the fluorescent label) with 1393 µL Qubit dsDNA HS buffer, and mix well.

AMOUNT
1.4 ml Additional info: enough for 5 libraries and 2 standards

Quantitate Library with Qubit dsDNA HS Assay
Step 175.

Prepare standard 1 and standard 2 by mixing 10 µL of each standard with 190 µL working solution in seperate Qubit assay tubes

Quantitate Library with Qubit dsDNA HS Assay

Step 176.

Add 1 µL of each library to 199 µL working solution in seperate Qubit assay tubes (1:200 dilution)

Quantitate Library with Qubit dsDNA HS Assay

Step 177.

Mix all tubes by vortexing for 2-3 sec

Quantitate Library with Qubit dsDNA HS Assay

Step 178.

Incubate tubes at RT for 2 min

Quantitate Library with Qubit dsDNA HS Assay

Step 179.

Read standards 1 and 2 on the Qubit 2.0 Fluorometer

Quantitate Library with Qubit dsDNA HS Assay

Step 180.

Read all library samples on the fluorometer and record their concentration (in ng/mL)

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 181.

Dilute 1 µL aliquots of each library in seperate tubes to 300 pg/µL

NOTES

Alyssa Alsante 03 Jul 2017

Determine the appropriate dilution to achieve this concentration by dividing the Qubit concentration by 1.5. For example, if the Qubit concentration is 152 ng/mL, then the library should be diluted at 1:101 (=152/1.5) for 300 pg/uL.

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 182.

Allow the gel-dye mix to equilibriate to RT for 30 min before use

NOTES

Alyssa Alsante 03 Jul 2017

If gel-dye mix is not already prepared, allow the HS DNA dye concentrate (blue) and the HS DNA gel matrix (red) to equilibrate to RT for 30 minutes, add 15 uL of HS DNA dye concentrate to a HS DNA gel matrix vial, vortex well, spin down and transfer to a spin filter. Centrifuge at 2240 xg +/- 20% for 10 minutes and discard the filter.

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 183.

Clean the Bioanalyzer electrodes by pipetting 350 µL nuclease-free water into a cleaning chip and placing it into the machine

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 184.

Ensure that the chip priming station syringe clip is at the lowest position, that the base plate is in position C, and that the plunger is positioned at 1 mL.

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 185.

Pipette gel-dye mix in the well at the third row and last column

AMOUNT

9 µl Additional info:

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 186.

Pop bubbles with a 30 gauge needle

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 187.

Put the chip on the priming station and close it firmly until it locks into position

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 188.

Press the plunger until it is held by the clip, wait exactly 1 minute, and release the clip.

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 189.

Wait for at least 5 sec and slowly pull back the plunger to the 1 mL position

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 190.

Open the priming station and remove the chip

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 191.

Pipette gel dye mix in the remaining 3 wells of the last column

AMOUNT

9 µl Additional info:

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 192.

Pipette marker (green) in all sample and ladder wells

AMOUNT

5 µl Additional info:

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 193.

Pipette HS DNA ladder (yellow) in the well marked with a ladder (third column, last row)

AMOUNT

1 µl Additional info:

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 194.

Pipette diluted 300 pg/µL library aliquot into wells 1-11

AMOUNT

1 µl Additional info:

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 195.

Add marker (green) into each unused well

AMOUNT

1 µl Additional info:

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 196.

Move to the chip vortexer and vortex for 1 min at 2400 rpm

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 197.

While vortexing, remove the cleaning chip and leave the Bioanalyzer open to dry the electrodes

Validate Libraries with Agilent Bioanalyzer 2100 High Sensitivity DNA Chip

Step 198.

Run the chip in the Agilent 2100 Bioanalyzer within 5 min (to avoid evaporation).