TruSeq RNA kit protocol (with one-third reaction volumes and some minor changes)

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Abstract

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Protocol

Step 1.
If not performing Poly A select mRNA, proceed directly to step 24.

Step 2.
Mix 500 ng total RNA and dH2O to a final volume of 16.67 uL.

Step 3.
Vortex RNA purification Beads and add 16.67 uL to RNA sample.

Step 4.
Mix by pipetting up and down until beads are in a homogenous suspension.

Step 5.
Incubate in thermocycler:

65 °C	5 min
4 °C	hold

Step 6.
When thermocycler reaches 4 °C remove sample and place on bench at room temperature for 5 min.

DURATION
00:05:00

Step 7.
Place sample in magnetic rack for 5 min.

DURATION
00:05:00

Step 8.
Remove and discard all the supernatant.

Step 9.
Remove sample from rack.

Step 10.
Add 66.7 uL of Bead Washing Buffer and pipet up and down until beads are in a homogenous suspension.

Step 11.
Place the sample back in the magnetic rack for 5 min.

DURATION
00:05:00

Step 12.
Remove and discard all of the supernatant.

Step 13.
Add 16.67 uL of Elution Buffer and pipet up and down until beads are in a homogenous suspension.

Step 14.
Incubate in thermocycler:

80 °C	2 min
25 °C	hold

Step 15.
Remove sample from thermocycler when it reaches 25 °C and keep at room temp.

Step 16.
Add 16.67 uL of Bead Binding Buffer and pipet up and down until beads are in a homogenous suspension.

Step 17.
Incubate at room temperature for 5 min.

DURATION
00:05:00

Step 18.
Place sample in magnetic separator for 5 min.

DURATION
00:05:00

Step 19.
Remove and discard all supernatant.

Step 20.
Remove sample from rack.

Step 21.
Add 66.7 uL of Bead Washing Buffer and pipet up and down until beads are in a homogenous suspension.

Step 22.
Place sample in magnetic separator for 5 min.

DURATION
00:05:00

Step 23.
Remove and discard all supernatant.

Step 24.
Add 6.5 uL Elute, Prime, Fragment Mix and pipet up and down until beads are in a homogenous suspension.

If proceeding directly from step 1, add total RNA to 6.5 uL of Elute, Prime, and Fragment mix, and bring up the total volume to no more than 10 uL with dH2O.

Step 25.
Incubate in thermocycler:

94 °C	8 min
4 °C	hold

If not performing Poly A select mRNA, proceed directly to step 28.

Step 26.
Place sample in a magnetic rack for 5 min.

DURATION
00:05:00

Step 27.
Transfer 5.67 uL of the supernatant to a new 0.2 mL PCR tube.

Step 28.
Add 2.67 uL of First Strand Master Mix / Super Script II mix to sample.

Step 29.

Incubate in thermocycler:

25 °C	10 min
42 °C	50 min
70 °C	15 min
4 °C	hold

Step 30.
Add 8.33 uL of Second Strand Master Mix to sample.

Step 31.
Incubate in thermocycler at 16 °C for 1 hour.

Step 32.
Remove sample from thermocycler and let warm to room temperature.

Step 33.
Add 30 uL of well-mixed AMPure XP beads and mix by pipetting up and down until beads are in a homogenous suspension.

Step 34.

Incubate at room temperature for 15 min.

DURATION
00:15:00

Step 35.
Place on magnetic rack for 5 min.

DURATION
00:05:00

Step 36.
Remove and discard 45 uL of the supernatant.

Step 37.
Keep sample in magnetic rack and add 200 uL of 80% ethanol.

Step 38.
Incubate for 30 seconds. Remove and discard all supernatant.

DURATION
00:00:30

Step 39.
Repeat steps 37 and 38 once more for a total of two washes.

Step 40.
Add 22 uL Resuspension Buffer and pipet up and down until beads are in a homogenous suspension.

Step 41.
Incubate at room temperature for 5 min.

DURATION
00:05:00

Step 42.
Place in magnetic rack for 5 min.

DURATION
00:05:00

Step 43.
Transfer 20 uL of the supernatant to a new 0.2 mL PCR tube.

Step 44.
Add 13.3 uL of End Repair Mix to sample.

Step 45.
Incubate at 30 °C for 30 min.

DURATION
00:30:00

Step 46.
Add 53.5 uL of well-mixed AMPure XP Beads and mix by pipetting up and down until beads are in a homogenous suspension.

Step 47.

Incubate at room temperature for 15 min.

DURATION
00:15:00

Step 48.
Place on magnetic rack for 5 min.

DURATION
00:05:00

Step 49.
Remove and discard 81.6 uL of the supernatant.

Step 50.
Keep sample in magnetic rack and add 200 uL of 80% ethanol.

Step 51.
Incubate for 30 seconds. Remove and discard all supernatant.

DURATION
00:00:30

Step 52.
Repeat steps 50 and 51 once more for a total of two washes.

Step 53.
Add 7.83 uL Resuspension buffer and mix by pipetting up and down until beads are in a homogenous suspension.

Step 54.
Incubate at room temperature for 5 min.

DURATION
00:05:00

Step 55.
Place in magnetic rack for 5 min.

DURATION
00:05:00

Step 56.
Transfer 5.83 uL of the supernatant to a new 0.2 mL PCR tube.

Step 57.
Add 4.17 uL A-Tailing Mix to sample.

Step 58.
Incubate at 37 °C for 30 min.

DURATION
00:30:00

Step 59.
Add
0.83 uL DNA Ligase Mix
0.83 uL Resuspension Buffer

0.83 uL RNA Adapter Index
and mix by pipet or flicking, and spin down.

Step 60.
Incubate at 30 °C for 10 min.

DURATION
00:10:00

Step 61.
Add 1.67 uL Stop Ligase Mix.

Step 62.
Add 14 uL well-mixed AMPure XP Beads and mix by pipetting up and down until beads are in a homogenous suspension.

Step 63.
Incubate at room temperature for 15 min.

DURATION
00:15:00

Step 64.
Place on magnetic rack for at least 5 min.

DURATION
00:05:00

Step 65.
Remove and discard 23.16 uL of the supernatant.

Step 66.
Keep sample in magnetic rack and add 200 uL of 80% ethanol.

Step 67.
Incubate for 30 seconds. Remove and discard all supernatant.

DURATION
00:00:30

Step 68.
Repeat steps 66 and 67 one more time.

Step 69.
Add 18.67 uL Resuspension Buffer and pipet up and down until beads are in a homogenous suspension.

Step 70.
Incubate at room temperature for 5 min.

DURATION
00:05:00

Step 71.
Place in magnetic rack for 5 min.

DURATION
00:05:00

Step 72.
Transfer 16.67 uL of the supernatant to a new 0.2 mL PCR tube.

Step 73.
Add 16.67 uL of well-mixed AMPure XP beads. Mix by pipetting up and down until the beads are in a homogenous suspension.

Step 74.
Incubate at room temperature for 15 min.

DURATION
00:15:00

Step 75.
Place on magnetic rack for at least 5 min.

DURATION
00:05:00

Step 76.
Remove and discard 28.34 uL of the supernatant.

Step 77.
Keep sample in magnetic rack and add 200 uL of 80% ethanol.

Step 78.
Incubate for 30 seconds. Remove and discard all supernatant.

DURATION
00:00:30

Step 79.
Repeat steps 77 and 78 one more time.

Step 80.
Add 9.67 uL Resuspension Buffer and pipet up and down 10 times.

Step 81.
Incubate at room temperature for 5 min.

DURATION
00:05:00

Step 82.
Place in magnetic rack for 5 min.

DURATION
00:05:00

Step 83.
Transfer 7.67 uL of the supernatant to a new 0.2 mL PCR tube.

Step 84.
Use 1 uL to determine number of cycles to perform in following PCR amplification.

Step 85.
Mix

6.67 uL Adapter ligated DNA from step 83
1.67 uL PCR Primer Cocktail
8.33 uL PCR Master Mix

Step 86.
Amplify with the following PCR procotol

98 °C - 30 seconds
5 - 18 cycles 98 °C - 10 seconds 60 °C - 30 seconds 72 °C - 30 seconds
72 °C - 5

hold at 4 °C

Step 87.
Add 16.67 uL of well-mixed AMPure XP beads.

Step 88.
Incubate at room temperature for 15 min.

DURATION
00:15:00

Step 89.
Place on magnetic rack for at least 5 min.

DURATION
00:05:00

Step 90.
Remove and discard 28.3 uL of the supernatant.

Step 91.
Keep sample in magnetic rack and add 200 uL 80% ethanol.

Step 92.
Incubate 30 seconds. Remove and discard all supernatant.

DURATION
00:00:30

Step 93.
Repeat steps 91 and 92 one more time.

Step 94.
Let the beads dry at room temperature for 2 min.

DURATION
00:02:00

Step 95.
Add 12 uL Resuspension Buffer and pipet up and down 10 times.

Step 96.
Incubate at room temperature for 2 min.

DURATION
00:02:00

Step 97.
Place in magnetic rack for 5 min.

DURATION
00:05:00

Step 98.
Transfer 10 uL of the supernatant to a new 1.5 mL PCR tube.

Step 99.
Use 1 uL for qPCR quantiation (http://ethanomics.wordpress.com/ngs-qpcr-library-quantitation-protocol/) and run 1 uL on the Bioanalyzer.