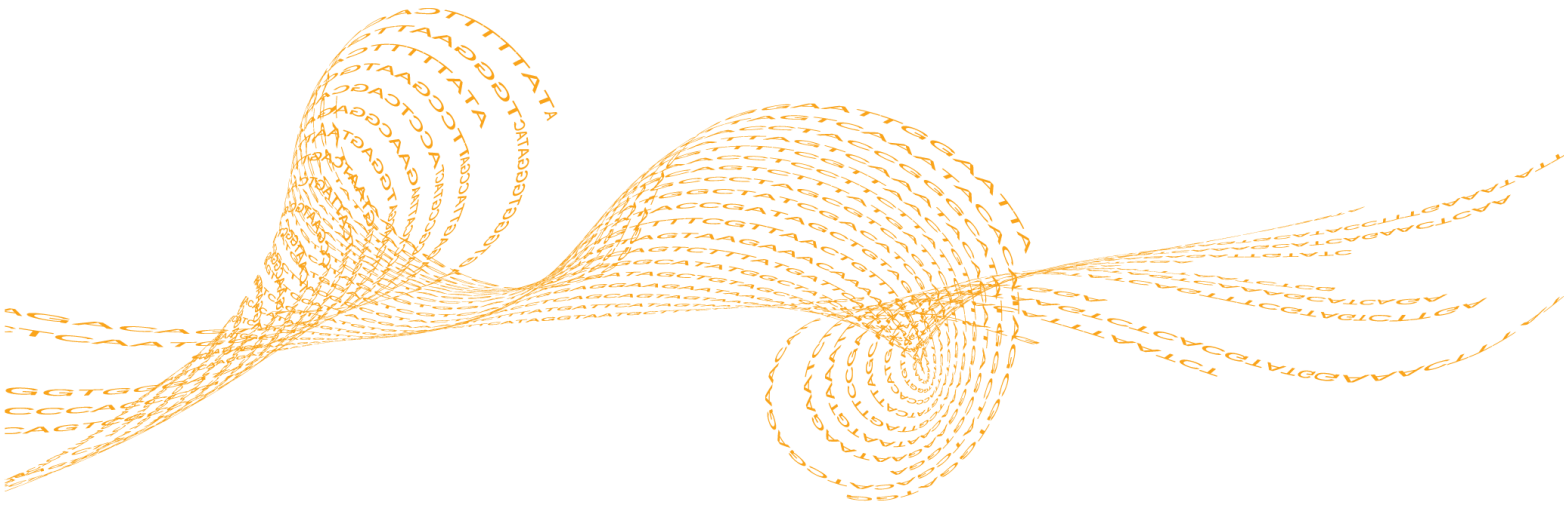
TruSeq Exome Library Prep Kit

Protocol Guide

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



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Fragment DNA

Preparation

1 Turn on and set up the Covaris instrument according to manufacturer guidelines.

2 [Plate] Calibrate the microplate shaker with a stroboscope and set it to 1200 rpm.

Procedure

1 Quantify gDNA using a fluorometric-based method.

2 Mix 5 ml RSB and 10 µl EDTA.

3 Normalize 100 ng gDNA with shearing buffer premix to 50 µl, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

4 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

5 Transfer 50 µl DNA to Covaris tubes or plate wells.

6 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

7 Fragment the DNA using the following Covaris settings.

Covaris Setting	M220	S2	S220	E220	LE220
Duty Factor (%)	20	10	10	10	30
Intensity	—	5	—	—	—
Peak Power (W)	50	—	175	175	450
Cycles/Burst	200	200	200	200	200
Duration (seconds)	375	280	280	280	360/rack; 420/tube
Temperature (°C)	20	7	7	7	7
Water Level	—	12	12	6	6
Intensifier	—	—	—	Yes	—

8 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

9 Transfer 50 µl supernatant to a new midi plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

10 Add 100 µl SPB, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

11 Incubate at room temperature for 5 minutes.

12 Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.

13 Place on a magnetic stand and wait until the liquid is clear (~8 minutes).

14 Remove and discard all supernatant.

15 Wash 2 times with 200 µl 80% EtOH.

16 Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.

17 Incubate on the magnetic stand for 30 seconds.

18 Use a 20 µl pipette to remove residual EtOH.

19 Air-dry on the magnetic stand until dry (~5 minutes).

20 Add 62.5 µl RSB.

21 Remove from the magnetic stand, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 2 minutes.
   - [Tube] Pipette up and down.

22 Incubate at room temperature for 2 minutes.

23 Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.

24 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

25 Transfer 60 µl supernatant to a new Hard-Shell PCR plate or to a new 8-tube strip.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Repair Ends and Select Library Size

Preparation

1. [Plate] Preheat the microheating system to 30°C.
2. [Tube] Save the following ERP program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 30°C for 30 minutes
   - Hold at 4°C
   - Each tube contains 100 µl.

Procedure

1. Add 40 µl ERP3, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 2 minutes.
   - [Tube] Pipette up and down.
2. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
3. Incubate as follows.
   - [Plate] Place on the 30°C microheating system with the lid closed for 30 minutes, and then place on ice.
   - [Tube] Place on the thermal cycler and run the ERP program.
4. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
5. Add 90 µl SPB, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 2 minutes.
   - [Tube] Pipette up and down.
6. Incubate at room temperature for 5 minutes.
7. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
8. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
9. Transfer 185 µl supernatant to a new midi plate or to a new 1.5 ml microcentrifuge tube.
10. Add 125 µl SPB, and then mix thoroughly as follows.
    - [Plate] Shake at 1200 rpm for 2 minutes.
    - [Tube] Pipette up and down.
11. Incubate at room temperature for 5 minutes.
12. Centrifuge as follows.
    - [Plate] Centrifuge at 280 × g for 1 minute.
    - [Tube] Centrifuge briefly.

13 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

14 Remove and discard all supernatant.

15 Wash 2 times with 200 µl 80% EtOH.

16 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

17 Incubate on the magnetic stand for 30 seconds.

18 Use a 20 µl pipette to remove residual EtOH.

19 Air-dry on the magnetic stand until dry (~5 minutes).

20 Add 20 µl RSB.

21 Remove from the magnetic stand, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

22 Incubate at room temperature for 2 minutes.

23 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

24 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

25 Transfer 17.5 µl supernatant to a new Hard-Shell PCR plate or to a new 8-tube strip.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Adenylate 3′ Ends

Preparation

1. [Plate] Preheat 2 microheating systems, one to 37°C and another to 70°C.
2. [Tube] Save the following ATAIL70 program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 37°C for 30 minutes
   - 70°C for 5 minutes
   - 4°C for 5 minutes
   - Hold at 4°C
   - Each tube contains 30 µl.

Procedure

1. Add 12.5 µl ATL2, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 2 minutes.
   - [Tube] Pipette up and down.
2. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
3. [Plate] Incubate as follows.
   a. Place on the 37°C microheating system with the lid closed for 30 minutes.
   b. Move to the 70°C microheating system with the lid closed for 5 minutes.
   c. Place on ice for 5 minutes.
4. [Tube] Place on the thermal cycler and run the ATAIL70 program.
5. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.

Ligate Adapters

Preparation

1. [Plate] Preheat a microheating system to 30°C.
2. [Tube] Save the following LIG program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 30°C for 10 minutes
   - Hold at 4°C
   - Each tube contains 37.5 µl.

Procedure

1. Add the following reagents in the order listed.
   - RSB (2.5 µl)
   - LIG2 (2.5 µl)
   - DNA adapters (2.5 µl)
2. Mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 2 minutes.
   - [Tube] Pipette up and down.
3. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
4. Incubate as follows.
   - [Plate] Place on the 30°C microheating system with the lid closed for 10 minutes, and then place on ice.
   - [Tube] Place on the thermal cycler and run the LIG program.
5. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
6. Add 5 µl STL, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 2 minutes.
   - [Tube] Pipette up and down.
7. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
8. Perform steps 9 through 24 using the **Round 1** volumes.
9. Add SPB.

	Round 1	Round 2
SPB	42.5 µl	50 µl

10. Mix thoroughly as follows.
    - [Plate] Shake at 1200 rpm for 2 minutes.
    - [Tube] Pipette up and down.

11 Incubate at room temperature for 5 minutes.

12 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

13 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

14 Remove and discard all supernatant.

15 Wash 2 times with 200 µl 80% EtOH.

16 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

17 Incubate on the magnetic stand for 30 seconds.

18 Use a 20 µl pipette to remove residual EtOH.

19 Air-dry on the magnetic stand until dry (~5 minutes).

20 Add RSB.

	Round 1	Round 2
RSB	52.5 µl	27.5 µl

21 Mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

22 Incubate at room temperature for 2 minutes.

23 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

24 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

25 Transfer 50 µl supernatant to a new midi plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

26 Repeat steps 9 through 24 with the new plate or tube using the **Round 2** volumes.

27 Transfer 25 µl supernatant to a new Hard-Shell PCR plate or to a new 8-tube strip.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Enrich DNA Fragments

Preparation

1. Save the following PCRNano program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 95°C for 3 minutes
   - 8 cycles of:
     - 98°C for 20 seconds
     - [Plate] 60°C for 20 seconds
     - [Tube] 60°C for 15 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 4°C
   - Each well or tube contains 50 µl.

Procedure

1. Place the plate or tube on ice and add 5 µl PPC.
2. Add 20 µl EPM, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 20 seconds.
   - [Tube] Pipette up and down.
3. Centrifuge briefly.
4. Place on the thermal cycler and run the PCRNano program.
5. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
6. Add 35 µl SPB.
7. Mix thoroughly, as follows.
   - [Plate] Shake at 1200 rpm for 2 minutes.
   - [Tube] Pipette up and down.
8. Incubate at room temperature for 5 minutes.
9. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
10. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
11. Transfer 82 µl supernatant to a new Hard-Shell PCR plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.
12. Add 82 µl SPB, and then mix thoroughly as follows.
    - [Plate] Shake at 1200 rpm for 2 minutes.
    - [Tube] Pipette up and down.
13. Incubate at room temperature for 5 minutes.
14. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

15 Remove and discard all supernatant.

16 Wash 2 times with 200 µl 80% EtOH.

17 Centrifuge briefly.

18 Incubate on the magnetic stand for 30 seconds.

19 Use a 20 µl pipette to remove residual EtOH.

20 Air-dry on the magnetic stand until dry (~5 minutes).

21 Add 17.5 µl RSB, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 2 minutes.
- [Tube] Pipette up and down.

22 Incubate at room temperature for 2 minutes.

23 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

24 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

25 Transfer 15 µl supernatant to a new Hard-Shell PCR plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Validate Libraries

Quantify Libraries

1. Quantify the libraries using the Qubit dsDNA HS Assay Kit.
   a. Use 1 µl as the loading volume.
   b. Use the dsDNA and high sensitivity settings.
   c. Record STD1 and STD2 readings.
   d. Measure the library concentration in duplicate and use the average.

Check Library Quality

1. Check the library size distribution:
   - If using a High Sensitivity DNA chip:
     - Dilute the DNA library 1:10 with RSB.
     - Run 1 µl diluted DNA library.
   - If using a DNA 1000 chip, run 1 µl undiluted DNA library.

Hybridize Probes

Preparation

1 Save the TE HYB program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 95°C for 10 minutes
- 18 cycles of 1 minute each, starting at 94°C, then decreasing 2°C per cycle
- 58°C for 90 minutes
- Hold at 58°C
- Each tube contains 100 µl.

Pool Libraries

1 Combine the following amount of each DNA library, making sure that each library has a unique index.

Plexity	Each Library	Total Pool
3-plex	250 ng	750 ng
6-plex	200 ng	1200 ng
9-plex	150 ng	1350 ng
12-plex	100 ng	1200 ng

- If the total volume is > 40 µl, concentrate the pooled sample to 40 µl.
- If the total volume is < 40 µl, increase the volume to 40 µl with RSB.

Procedure

1 Add the following reagents in the order listed to a new 8-tube strip. Pipette to mix.
- DNA library pool (40 µl)
- CT3 (50 µl)
- CEX (10 µl)

2 Centrifuge briefly.

3 Place on the thermal cycler and run the TE HYB program.

4 Keep at the 58°C holding temperature for at least 90 minutes and up to 24 hours.

Capture Hybridized Probes

Preparation

1 Preheat a heat block to 50°C.

Procedure

1 Add 250 µl SMB to a new 1.5 ml microcentrifuge tube.

2 Immediately transfer the total sample volume (~100 µl) from the thermal cycler to the 1.5 ml microcentrifuge tube containing SMB. Pipette to mix.

3 Incubate at room temperature for 25 minutes.

4 Centrifuge briefly.

5 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

6 Remove and discard all supernatant.

7 Remove from the magnetic stand.

8 Add 200 µl SWS. Pipette to mix.

9 Place on the 50°C heat block for 30 minutes.

10 Immediately place on a magnetic stand and wait until the liquid is clear (~2 minutes).

11 Remove and discard all supernatant.

12 Remove from the magnetic stand.

13 Repeat steps 8–12 for a total of 2 washes.

14 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex.

15 Add 23 µl elution premix. Pipette to mix.

16 Incubate at room temperature for 2 minutes.

17 Centrifuge briefly.

18 Place on a magnetic stand and wait until the liquid is clear (~5 minutes).

19 Transfer 21 µl supernatant to a new 8-tube strip.

20 Add 4 µl ET2. Pipette to mix.

21 Centrifuge briefly.

Perform Second Hybridization

Procedure

1. Add the following reagents in the order listed to the 8-tube strip. Pipette to mix.
   - DNA library pool (25 µl)
   - RSB (15 µl)
   - CT3 (50 µl)
   - CEX (10 µl)
2. Centrifuge briefly.
3. Place on the thermal cycler and run the TE HYB program.
4. Keep at the 58°C holding temperature for at least 14.5 hours and up to 24 hours.

Perform Second Capture

Preparation

1 Preheat a heat block to 50°C.

Procedure

1 Add 250 µl SMB to a new 1.5 ml microcentrifuge tube.

2 Immediately transfer the total sample volume (~100 µl) from the thermal cycler to the 1.5 ml microcentrifuge tube containing SMB. Pipette to mix.

3 Incubate at room temperature for 25 minutes.

4 Centrifuge briefly.

5 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

6 Remove and discard all supernatant.

7 Remove from the magnetic stand.

8 Add 200 µl SWS. Pipette to mix.

9 Place on the 50°C heat block for 30 minutes.

10 Immediately place on a magnetic stand and wait until the liquid is clear (~2 minutes).

11 Remove and discard all supernatant.

12 Remove from the magnetic stand.

13 Repeat steps 8–12 for a total of 2 washes.

14 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex.

15 Add 23 µl elution premix. Pipette to mix.

16 Incubate at room temperature for 2 minutes.

17 Centrifuge briefly.

18 Place on a magnetic stand and wait until the liquid is clear (~5 minutes).

19 Transfer 21 µl supernatant to a new Hard-Shell PCR plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

20 Add 4 µl ET2, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 1 minute.
- [Tube] Pipette up and down.

21 Centrifuge as follows.
- [Plate] Centrifuge at 280 × g for 1 minute.
- [Tube] Centrifuge briefly.

Clean Up Captured Library

Procedure

1. Add 45 µl SPB. Pipette to mix.
2. Incubate at room temperature for 5 minutes.
3. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
4. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
5. Remove and discard all supernatant.
6. Wash 2 times with 200 µl 80% EtOH.
7. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
8. Incubate on the magnetic stand for 30 seconds.
9. Use a 20 µl pipette to remove residual EtOH.
10. Air-dry on the magnetic stand until dry (~5 minutes).
11. Add 27.5 µl RSB. Pipette to mix.
12. Incubate at room temperature for 2 minutes.
13. Centrifuge as follows.
    - [Plate] Centrifuge at 280 × g for 1 minute.
    - [Tube] Centrifuge briefly.
14. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
15. Transfer 25 µl supernatant to a new Hard-Shell PCR plate or to a new 8-tube strip.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Amplify Enriched Library

Preparation

1. Save the following AMP8 program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 98°C for 30 seconds
   - 8 cycles of:
     - 98°C for 10 seconds
     - [Plate] 60°C for 35 seconds
     - [Tube] 60°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 4°C
   - Each well or tube contains 50 µl.

Procedure

1. Add 5 µl PPC.
2. Add 20 µl NEM. Pipette to mix.
3. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
4. Place on the thermal cycler and run the AMP8 program.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Amplified Enriched Library

Procedure

1. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
2. Add 45 µl SPB. Pipette to mix.
3. Incubate at room temperature for 5 minutes.
4. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
5. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
6. Remove and discard all supernatant.
7. Wash 2 times with 200 µl 80% EtOH.
8. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
9. Incubate on the magnetic stand for 30 seconds.
10. Use a 20 µl pipette to remove residual EtOH.
11. Air-dry on the magnetic stand until dry (~5 minutes).
12. Add 22 µl RSB. Pipette to mix.
13. Incubate at room temperature for 2 minutes.
14. Centrifuge as follows.
    - [Plate] Centrifuge at 280 × g for 1 minute.
    - [Tube] Centrifuge briefly.
15. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
16. Transfer 20 µl supernatant to a new Hard-Shell PCR plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Validate Enriched Libraries

Quantify Libraries

1 Quantify the postenriched library using the Qubit dsDNA HS Assay Kit.

   a Use 1 µl as the loading volume.
   b Use the dsDNA and high sensitivity settings.
   c Record STD1 and STD2 readings.
   d Measure the library concentration in duplicate and use the average of the 2 measurements.

Assess Quality

1 Run 1 µl of post enriched library using a High Sensitivity DNA chip.

Acronyms

Acronym	Definition
ATL2	A Tailing Mix
CEX	Coding Exome Oligos
CT3	Capture Target Buffer 3
DAP	DNA Adapter Plate
EE1	Enrichment Elution Buffer 1
EPM	Enhanced PCR Mix
ERP	End Repair Mix
ET2	Elute Target Buffer 2
HP3	2N NaOH
LIG	Ligation Mix
NEM	Enrichment Amplification Mix
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
STL	Stop Ligation Buffer
SWS	Streptavidin Wash Solution

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

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