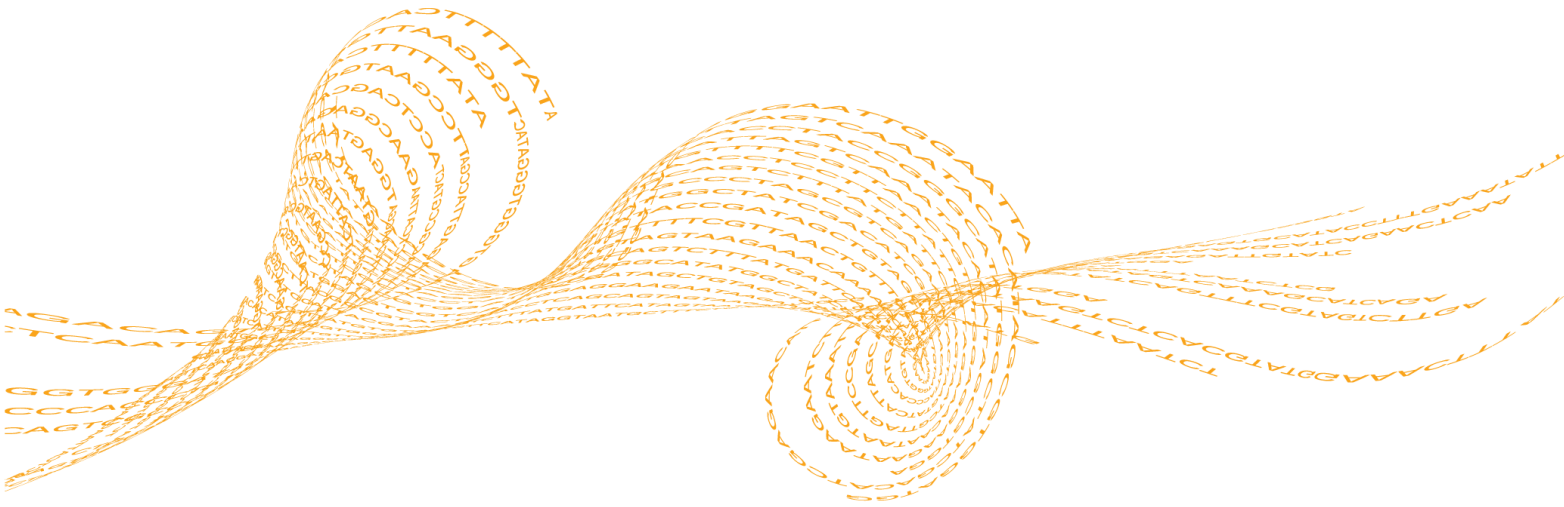
TruSeq Rapid Exome Library Prep

Protocol Guide

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



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Tagment Genomic DNA

Preparation

1. Save the following TAG58 program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 58°C for 10 minutes
   - Hold at 10°C
   - Each well or tube contains 50 µl.
2. Save the following TAG60 program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 60°C for 5 minutes
   - Hold at 10°C
   - Each well or tube contains 65 µl.

Procedure

1. Quantify gDNA using a fluorometric method.
2. Dilute gDNA in Tris-HCl 10 mM, pH 8.5 to a final volume of 10 µl at 5 ng/µl.
3. Add the following items in the order listed to a new Hard-Shell PCR plate or to a new 0.2 ml thin-wall PCR tube or 8-tube strip.
   - TD (25 µl)
   - Normalized gDNA (10 µl)
   - TDE2 (15 µl)
4. Mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 1 minute.
   - [Tube] Pipette up and down.
5. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
6. Place on the preprogrammed thermal cycler and run the TAG58 program.
7. Add 15 µl ST2, and then pipette to mix
8. Place on the preprogrammed thermal cycler and run the TAG60 program.

Clean Up Tagmented DNA

Procedure

1. Transfer all supernatant to a new midi plate or to a new 1.5 ml microcentrifuge tube.
2. Add 52 µl SPB, and then mix thoroughly as follows.
   - [Plate] Pipette up and down 10 times.
   - [Tube] Pipette up and down.
3. Incubate at room temperature for 5 minutes.
4. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
5. Transfer 98 µl supernatant to a new midi plate or to a new 1.5 ml microcentrifuge tube.
6. Add 137 µl SPB, and then mix thoroughly as follows.
   - [Plate] Pipette up and down 10 times.
   - [Tube] Pipette up and down.
7. Incubate at room temperature for 5 minutes.
8. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
9. Remove and discard all supernatant.
10. Wash 2 times with 200 µl 80% EtOH.
11. Using a 20 µl pipette, remove residual 80% EtOH.
12. Air-dry on the magnetic stand for 5 minutes.
13. Add 22.5 µl RSB, and then mix thoroughly as follows.
    - [Plate] Shake at 1800 rpm for 1 minute.
    - [Tube] Pipette up and down.
14. Remove from the magnetic stand.
15. Incubate at room temperature for 2 minutes.
16. Centrifuge as follows.
    - [Plate] Centrifuge at 280 × g for 1 minute.
    - [Tube] Centrifuge briefly.
17. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
18. Transfer 20 µl supernatant to a new Hard-Shell PCR plate or to a new 8-tube strip.

Amplify Tagmented DNA

Preparation

1. Save the following LAM AMP program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 72°C for 3 minutes
   - 98°C for 30 seconds
   - 10 cycles of:
     - 98°C for 10 seconds
     - 60°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 10°C
   - Each well or tube contains 50 µl.

Procedure

1. [Plate] Arrange Index 1 (i7) adapters in columns 1–12 of the TruSeq Index Plate Fixture.
2. [Plate] Arrange Index 2 (i5) adapters in rows A–H of the TruSeq Index Plate Fixture.
3. [Plate] Place the plate on the TruSeq Index Plate Fixture.
4. Add 5 µl of each Index 1 (i7) adapter as follows.
   - [Plate] Using a multichannel pipette, add to each column.
   - [Tube] Add a different index to each tube.
5. Replace the cap on each i7 adapter tube with a new orange cap.
6. Add 5 µl of each Index 2 (i5) adapter as follows.
   - [Plate] Using a multichannel pipette, add to each column.
   - [Tube] Add a different index to each tube.
7. Replace the cap on each i5 adapter tube with a new white cap.
8. Add 20 µl LAM, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 1 minute.
   - [Tube] Pipette up and down.
9. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
10. Place on the thermal cycler and run the LAM AMP 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 DNA

Procedure

1. Centrifuge at 280 × g for 1 minute.
2. Transfer 50 µl total volume to a new midi plate or to a new 1.5 ml microcentrifuge tube.
3. Add 90 µl SPB, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 1 minute.
   - [Tube] Pipette up and down.
4. Incubate at room temperature for 5 minutes.
5. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
6. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
7. Remove and discard all supernatant.
8. Wash 2 times with 200 µl 80% EtOH.
9. Using a 20 µl pipette, remove residual 80% EtOH.
10. Air-dry on the magnetic stand for 5 minutes.
11. Add 17 µl RSB, and then mix thoroughly as follows.
    - [Plate] Shake at 1800 rpm for 1 minute.
    - [Tube] Pipette up and down.
12. Remove from the magnetic stand.
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 minutes).
16. Transfer 15 µl supernatant to a new Hard-Shell PCR plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.
17. Quantify the library using a fluorometric method.

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 14 days.

Hybridize Probes

Preparation

1. Save the TRE HYB program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 95°C for 10 minutes
   - 58°C for 30 minutes
   - Each well or tube contains 10 µl.

Pool Libraries

1. Combine 500 ng of each DNA library, making sure that each library has a unique index.
   - If the total volume is > 30 µl, concentrate the pooled sample to 30 µl.
   - If the total volume is < 30 µl, increase the volume to 30 µl with RSB.

Procedure

1. Add the following reagents in the order listed to a new midi plate or to a new 1.5 ml microcentrifuge tube.
   - DNA library sample or pool (30 µl)
   - BLR (10 µl)
   - CEX (10 µl)
2. Mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 1 minute.
   - [Tube] Pipette up and down.
3. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
4. Add 125 µl SPB, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 1 minute.
   - [Tube] Pipette up and down.
5. Incubate at room temperature for 10 minutes.
6. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
7. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
8. Remove and discard all supernatant.
9. Wash 2 times with 200 µl 80% EtOH.
10. Using a 20 µl pipette, remove residual 80% EtOH.
11. Air-dry on the magnetic stand for 10 minutes.

12 Add 7.7 µl EHB1, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 1 minute.
   - [Tube] Pipette up and down.

13 Remove from the magnetic stand.

14 Incubate at room temperature for 2 minutes.

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

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

17 Transfer 7.5 µl supernatant to a new Hard-Shell PCR plate or to a new thin-wall PCR tube or 8-tube strip.

18 Add 2.5 µl EHB2, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 1 minute.
   - [Tube] Pipette up and down.

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

20 Place on the thermal cycler and run the TRE HYB program.

Capture Hybridized Probes

Preparation

1. [Plate] Preheat a microheating system with midi plate insert to 50°C.
2. [Tube] Preheat a heat block to 50°C.

Procedure

1. Centrifuge at 280 × g for 1 minute.
2. Transfer all (~10 µl) to a new midi plate or to a new 1.5 ml microcentrifuge tube.
3. Add 250 µl SMB, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 5 minutes.
   - [Tube] Pipette up and down.
4. Incubate at room temperature for 25 minutes.
5. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
6. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
7. Remove and discard all supernatant.
8. Remove from the magnetic stand.
9. Add 200 µl EEW, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 4 minutes. Pipette to resuspend the bead pellet further.
   - [Tube] Pipette up and down.
10. Incubate as follows.
    - [Plate] Place on the 50°C microheating system with the lid closed for 30 minutes.
    - [Tube] Place on the 50°C heat block for 30 minutes.
11. Immediately place on a magnetic stand and wait until the liquid is clear (~2 minutes).
12. Remove and discard all supernatant.
13. Remove from the magnetic stand.
14. Repeat steps 9–13 for a total of 2 washes.
15. Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex.
16. Add 23 µl elution premix, and then mix thoroughly as follows.
    - [Plate] Shake at 1800 rpm for 2 minutes.
    - [Tube] Pipette up and down.
17. Incubate at room temperature for 2 minutes.
18. Centrifuge as follows.
    - [Plate] Centrifuge at 280 × g for 1 minute.
    - [Tube] Centrifuge briefly.

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

20 Transfer 21 µl supernatant to a new midi plate or to a new 1.5 ml microcentrifuge tube.

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

22 Add 5 µl RSB, and then mix thoroughly as follows.
- [Plate] Shake at 1200 rpm for 1 minute.
- [Tube] Pipette up and down.

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

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.

Perform Second Hybridization

Procedure

1. Add the following reagents in the order listed.
   - BLR (10 µl)
   - CEX (10 µl)
2. Mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 1 minute.
   - [Tube] Pipette up and down.
3. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
4. Add 125 µl SPB, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 1 minute.
   - [Tube] Pipette up and down.
5. Incubate at room temperature for 10 minutes.
6. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
7. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
8. Remove and discard all supernatant.
9. Wash 2 times with 200 µl 80% EtOH.
10. Using a 20 µl pipette, remove residual 80% EtOH.
11. Air-dry on the magnetic stand for 10 minutes.
12. Add 7.7 µl EHB1, and then mix thoroughly as follows.
    - [Plate] Shake at 1800 rpm for 1 minute.
    - [Tube] Pipette up and down.
13. Remove from the magnetic stand.
14. Incubate at room temperature for 2 minutes.
15. Centrifuge as follows.
    - [Plate] Centrifuge at 280 × g for 1 minute.
    - [Tube] Centrifuge briefly.
16. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
17. Transfer 7.5 µl supernatant to a new Hard-Shell PCR plate or to a new 8-tube strip.
18. Add 2.5 µl EHB2, and then mix thoroughly as follows.
    - [Plate] Shake at 1800 rpm for 1 minute.
    - [Tube] Pipette up and down.
19. Centrifuge as follows.
    - [Plate] Centrifuge at 280 × g for 1 minute.
    - [Tube] Centrifuge briefly.

20 Place on the thermal cycler and run the TRE HYB program.

Perform Second Capture

Preparation

1. [Plate] Preheat a microheating system with midi plate insert to 50°C.
2. [Tube] Preheat a heat block to 50°C.

Procedure

1. Centrifuge at 280 × g for 1 minute.
2. Transfer 10 µl supernatant to a new midi plate or to a new 1.5 ml microcentrifuge tube.
3. Add 250 µl SMB, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 5 minutes.
   - [Tube] Pipette up and down.
4. Incubate at room temperature for 25 minutes.
5. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
6. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
7. Remove and discard all supernatant.
8. Remove from the magnetic stand.
9. Add 200 µl EEW, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 4 minutes. Pipette to resuspend the bead pellet further.
   - [Tube] Pipette up and down.
10. Incubate as follows.
    - [Plate] Place on the 50°C microheating system with the lid closed for 30 minutes.
    - [Tube] Place on the 50°C heat block for 30 minutes.
11. Immediately place on a magnetic stand and wait until the liquid is clear (~2 minutes).
12. Remove and discard all supernatant.
13. Remove from the magnetic stand.
14. Repeat steps 9–13 for a total of 2 washes.
15. Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex.
16. Add 23 µl elution premix, and then mix thoroughly as follows.
    - [Plate] Shake at 1800 rpm for 2 minutes.
    - [Tube] Pipette up and down.
17. Incubate at room temperature for 2 minutes.

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

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

20 Transfer 21 µl supernatant to a new midi plate or to a new 1.5 ml microcentrifuge tube or 8-tube strip.

21 Add 4 µl ET2, and then mix thoroughly as follows.
- [Plate] Shake at 1800 rpm for 1 minute.
- [Tube] Pipette up and down.

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

Clean Up Captured Library

Procedure

1. Add 45 µl SPB, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 1 minute.
   - [Tube] Pipette up and down.
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. Use a 20 µl pipette to remove residual EtOH.
8. Air-dry on the magnetic stand until dry (~5 minutes).
9. Add 27.5 µl RSB, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 1 minute.
   - [Tube] Pipette up and down.
10. Remove from the magnetic stand.
11. Incubate at room temperature for 2 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. 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 AMP10 program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 98°C for 30 seconds
   - 10 cycles of:
     - 98°C for 10 seconds
     - 60°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 10°C
   - Each well or tube contains 50 µl.

Procedure

1. Add 5 µl PPC.
2. Add 20 µl EAM, and then mix thoroughly as follows.
   - [Plate] Shake at 1200 rpm for 1 minute.
   - [Tube] Pipette up and down.
3. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
4. Place on the thermal cycler and run the AMP10 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. Transfer 50 µl to a new midi plate or to a new 1.5 ml microcentrifuge tube.
3. Add 50 µl SPB, and then mix thoroughly as follows.
   - [Plate] Shake at 1800 rpm for 1 minute.
   - [Tube] Pipette up and down.
4. Incubate at room temperature for 5 minutes.
5. Centrifuge as follows.
   - [Plate] Centrifuge at 280 × g for 1 minute.
   - [Tube] Centrifuge briefly.
6. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
7. Remove and discard all supernatant.
8. Wash 2 times with 200 µl 80% EtOH.
9. Use a 20 µl pipette to remove residual EtOH.
10. Air-dry on the magnetic stand until dry (~5 minutes).
11. Add 32 µl RSB, and then mix thoroughly as follows.
    - [Plate] Shake at 1800 rpm for 1 minute.
    - [Tube] Pipette up and down.
12. Remove from the magnetic stand.
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 30 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 BR Assay Kit.

Assess Quality

1 If the library concentration is higher than the supported quantitative range for the High Sensitivity DNA chip, dilute the library 1:10 with RSB.

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

Acronyms

Acronym	Definition
BLR	Blocker
CEX	Coding Exome Oligos
EAM	Enrichment Amplification Mix
EE1	Enrichment Elution Buffer 1
EEW	Enhanced Enrichment Wash Solution
EHB1	Enrichment Hybridization Buffer 1
EHB2	Enrichment Hybridization Buffer 2
ET2	Elute Target Buffer 2
HP3	2N NaOH
LAM	Library Amplification Mix
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
ST2	Stop Tagment Buffer 2
TD	Tagment DNA Buffer
TDE2	Tagment DNA Enzyme 2

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	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

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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