Prepare templated beads (mini-scale)

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Applied Biosystems SOLiD™4 System Templated Bead Preparation Guide* (PN 4448378) and the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* (PN 4448379). For every chemical, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare the mini-scale emulsion PCR (ePCR) reaction

1. Prepare the oil phase:
   a. Use 3-mL and 1-mL syringes to dispense Emulsion Stabilizers 1 and 2 into a 50-mL conical tube:

Component	Volume
Emulsion Stabilizer 1	1.8 mL
Emulsion Stabilizer 2	400 µL
Emulsion Oil	37.8 mL
Total	40 mL

   b. Vortex thoroughly and allow the mixture to degas for at least 20 minutes.
   c. Prime a 10-mL syringe by drawing in about 2 mL of oil phase and dispensing it back into the tube.
   d. Dispense 9 mL of oil phase to an uncapped SOLiD™ ePCR Tube using the 10-mL syringe. Cap the tube.
2. Prepare the aqueous phase:
   a. Dilute ePCR Primer 1 to prepare 10 µM of working stock solution.
   b. Prepare a dilution of the library template to a final concentration of 500 pM in 1X Low TE Buffer.
   c. Prepare the aqueous phase in a Nalgene wide-mouthed jar and keep it on ice until it is ready to use.

	Library concentration		
	0.5 pM	1.0 pM	*X* pM
	Volume per reaction (µL)‡		
10X PCR Buffer	280	280	280
dNTP Mix (100 mM mix comprised of 25 mM each of dATP, dTTP, dCTP, dGTP)	392	392	392
Magnesium Chloride (1 M)	70	70	70
ePCR Primer 1 (10 µM working stock solution)	11.2	11.2	11.2
ePCR Primer 2 (500 µM)	16.8	16.8	16.8
Template (500 pM)	2.8	5.6	*X* × 5.6
Nuclease-free Water	1647.2	1644.4	1650 – (*X* × 5.6)
AmpliTaq Gold® DNA PolymeraseUP (5 U/µL)	300	300	300
Total	2720	2720	2720

‡ Volumes below are for a single IKA®-based ePCR reaction to fill a 96-well plate.

3. Prepare the SOLiD™ P1 DNA Beads:
   a. Thoroughly vortex 1 tube of SOLiD™ P1 DNA Beads, then pulse-spin.
   b. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.
   c. Resuspend the beads in 200 µL of Bead Block Solution. Vortex, then pulse-spin.
   d. Sonicate the beads using the Bead Block Declump program on the Covaris™ S2 System, then pulse-spin.
   e. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.
   f. Resuspend beads in 200 µL of 1X TEX Buffer. Vortex, then pulse-spin.
4. Create the emulsion with the ULTRA-TURRAX® Tube Drive from IKA®:
   a. Place the SOLiD™ ePCR Tube with oil phase onto the ULTRA-TURRAX® Tube Drive from IKA, then lock into position.
   b. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

c. Immediately add 80 µL of SOLiD™ P1 DNA Beads to the aqueous phase and mix by gently swirling the bottle.

d. Verify the Xstream pipettor settings and attach a 10-mL Combitip Plus:
   - Dial Setting: **Pip**
   - Speed (aspirate UP): **scale 5** (mid-range)
   - Speed (dispense DOWN): **scale 1** (slowest)
   - Total volume: **2.80 mL**

e. Draw 2.8 mL of aqueous phase and bead mixture into the 10-mL Combitip Plus using the Xstream pipettor.

f. Press the **Start** button on the ULTRA-TURRAX Tube Drive from IKA, then make sure it is set to 5 minutes. Wait for the flywheel of the tube drive to engage and reach steady speed.

g. Gently place the Combitip Plus into the center sample loading hole in the SOLiD™ ePCR Tube cap, then dispense the aqueous phase and bead mixture into the spinning oil phase. When the entire volume is dispensed, press the center blue button *twice* on the pipettor to empty all of the contents from the Combitip Plus.

h. After the ULTRA-TURRAX® Tube Drive from IKA stops, attach a 5-mL Combitip Plus (with its tip cut off at the bevel using a razor blade) to an Eppendorf Repeater Plus Pipette.

i. Gently dispense 100 µL of emulsion into each well of a 96-well PCR plate. Seal the plate with clear adhesive film.

5. Run the ePCR reaction on the GeneAmp® PCR System 9700:
   - PCR conditions:

Stage	Step	Temp (°C)	Time
Holding	Denature	95	5 min
40 cycles‡ *or* 60 cycles§	Denature	93	15 sec
	Anneal	62	30 sec
	Extend	72	75 sec
Holding	Final extension	72	7 min
Holding	—	4	∞

‡ Set *40* cycles: Fragment library or 2 × 25 bp mate-paired library.
§ Set *60* cycles: 2 × 50 bp mate-paired library.

   - Ramp speed: **9600**
   - Reaction volume: **50 µL**

6. Observe the bottom of the reaction plate for beads that have fallen out of the emulsion.

Perform emulsion break and bead wash (mini-scale)

1. Break the emulsion:

a. Place the SOLiD™ Emulsion Collection Tray on top of the ePCR 96-well plate.

b. Seal the pieces together with tape and flip the entire apparatus.

c. Centrifuge the inverted plate and reservoir at 550 × g for 2 minutes.

d. Remove the tape and 96-well plate from the collection tray.

e. In a fume hood, add 10 to 12 mL of 2-butanol to the collection tray. Pipet the emulsion up and down until the solution is homogeneous.

f. Transfer the emulsion and 2-butanol to a 50-mL conical tube.

g. Rinse the reservoir with 6 mL of 2-butanol and transfer the rinse to the tube.

h. Cap the tube, then vortex the tube.

i. Centrifuge the tube at 2,000 × g for 5 minutes.

j. Decant the 2-butanol-oil phase into a waste bottle.

k. Place the tube inverted onto paper towels, then wait for 5 minutes.

2. Wash the templated beads:

a. Add 600 µL of 1X Bead Wash Buffer to the tube of templated beads. Let the pellet soak for 2 minutes.

b. Resuspend by gently pipetting up and down and then transfer the beads to a 1.5-mL LoBind Tube.

c. Rinse the bottom of the 50-mL tube with 600 µL of 1X Bead Wash Buffer and transfer to the 1.5-mL LoBind Tube.

d. Vortex the tube and centrifuge at 21,000 × g for 1 minute. (The minimum acceptable rate is 14,000 × g. 21,000 × g is preferred.)

e. Remove the top oil phase with a pipette.

f. With a new pipette tip, remove and discard the supernatant.

g. Resuspend the beads in 150 µL of 1X Bead Wash Buffer, then vortex. Pulse-spin, then transfer the beads into a new 1.5-mL LoBind Tube.

h. Rinse the bottom of the original tube with 150 µL of 1X Bead Wash Buffer, then transfer to the 1.5-mL LoBind Tube.

i. Add 1 mL of 1X Bead Wash Buffer. Vortex, then centrifuge at 21,000 × g (minimum 14,000 × g) for 1 minute.

j. Remove the supernatant, then resuspend the beads in 200 µL of 1X TEX Buffer.

k. Place the tube in a magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.

l. Resuspend the beads in 200 µL of 1X TEX Buffer.

3. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

4. Quantitate a 1:10 dilution of beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer. Combine diluted and undiluted beads.

Enrich for the templated beads (mini-scale)

1. Prepare the Denaturing Buffer solution:
   a. Transfer 1.8 mL of Denaturing Buffer into a 15-mL conical tube.
   b. Add 200 µL of Denaturant to the Denaturing Buffer. Cap the tube, then vortex well.
2. Prepare 60% glycerol:
   a. Add 4 mL of Nuclease-free Water using a syringe to a 15-mL conical tube.
   b. Add 6 mL of glycerol (dispensing glycerol twice from a 3-mL syringe). Cap the tube, then vortex well.
3. Prepare the enrichment beads:
   a. Vortex the enrichment beads, then immediately transfer 300 µL enrichment beads to a 1.5-mL LoBind Tube.
   b. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   c. Resuspend the enrichment beads in 900 µL of 1X Bind & Wash Buffer.
   d. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then remove the supernatant.
   e. Repeat steps c and d.
   f. Resuspend the enrichment beads in 150 µL of 1X Bind & Wash Buffer.
   g. Add 1.5 µL of 1 mM Enrichment Oligo. Vortex, then pulse-spin.
   h. Rotate the tube at room temperature for 30 minutes.
   i. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes and discard the supernatant.
   j. Resuspend the enrichment beads in 900 µL of 1X TEX Buffer.
   k. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   l. Repeat steps j and k.
   m. Resuspend the enrichment beads in 75 µL of 1X Low Salt Binding Buffer.
4. Prepare the templated beads for enrichment:
   a. Place the tube of templated beads in the magnetic rack for 1 minute, then discard the supernatant.
   b. Resuspend the templated beads in 300 µL of prepared Denaturing Buffer solution and let the beads stand for 1 minute.
   c. Place the tube in the magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.
   d. Repeat steps b and c *twice*.
   e. Resuspend the templated beads in 300 µL of 1X TEX Buffer. Place the tube in a magnetic rack for at least 1 minute. Discard the supernatant after the solution clears. Repeat this step *twice*.
   f. Resuspend the templated beads in 75 µL of 1X TEX Buffer, then transfer the solution to a 0.5-mL LoBind Tube.
   g. Sonicate the templated beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin the beads.
5. Enrich for the templated beads:
   a. Transfer the prepared enrichment beads to the tube of templated beads and vortex to mix, then pulse-spin the tube.
   b. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System, then pulse-spin the beads.
   c. Incubate the bead mixture at 61 °C for 15 minutes. Vortex, then pulse-spin every 5 minutes including at the end of incubation.
   d. Immediately cool the beads on ice for 2 minutes.
   e. Add 400 µL of *newly prepared* 60% glycerol to a new 1.5-mL LoBind Tube.
   f. Gently pipet the bead mixture up and down to mix, then load the entire bead mixture volume on top of the 60% glycerol solution. *Do not* vortex the tube.
   g. Centrifuge at 21,000 × g (minimum 14,000 × g) for 3 minutes.
   h. Add 1 mL of 1X TEX Buffer to a 2.0-mL LoBind Tube.
   i. Transfer the top layer of beads to the 2.0-mL LoBind Tube. Top off the tube with additional 1X TEX Buffer.
   j. Vortex the tube of beads and centrifuge at 21,000 × g (minimum 14,000 × g) for 1 minute.
   k. If the beads are pelleted, discard the supernatant and resuspend the beads in 400 µL of 1X TEX Buffer. Go to step 6. If the beads are not pelleted, go to step l.

l. Transfer half of the bead solution to a fresh tube and add 500 µL of 1X TEX Buffer to each tube, then vortex the tube.

m. Centrifuge the beads at 21,000 × g (minimum 14,000 × g) for 1 minute, then discard the supernatant.

n. Resuspend each pellet in 200 µL of 1X TEX Buffer, then pool the beads into one tube.

6. Isolate the P2-enriched beads:

a. Centrifuge the beads at 21,000 × g (minimum 14,000 × g) for 1 minute, then discard the supernatant.

b. Resuspend the beads in 400 µL of prepared Denaturing Buffer solution, then let the beads stand for 1 minute.

c. Place the tube in a magnetic rack for at least 1 minute until the supernatant is pure white or clear. Discard the supernatant.

d. Repeat steps b and c until the supernatant is clear.

e. Resuspend the beads in 400 µL of 1X TEX Buffer.

f. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear, then discard the supernatant.

g. Repeat steps e and f.

h. Resuspend the beads in 200 µL of 1X TEX Buffer, then transfer the beads to a new 1.5-mL LoBind Tube. Rinse the tube with 200 µL 1X TEX Buffer, then add the wash to the same 1.5-mL LoBind Tube.

i. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

j. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.

k. Resuspend the beads in 400 µL of 1X TEX Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant. Repeat this step until the supernatant is clear.

l. Resuspend the beads in 400 µL of 1X TEX Buffer.

Modify the 3′ ends (mini-scale)

1. Prepare the 1X Terminal Transferase Reaction Buffer (500 µL buffer per 1 ePCR reaction):

Component	Volume
10X Terminal Transferase Buffer	55
10X Cobalt Chloride	55
Nuclease-free Water	390
Total	500

2. Add 1 µL of 50 mM Bead Linker to 49 µL of 1X Low TE Buffer to prepare a 1 mM Bead Linker solution.

3. Place the tube of P2-enriched beads in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

4. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer and transfer to a new 1.5-mL LoBind Tube. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

5. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

6. Resuspend the beads in 178 µL of 1X Terminal Transferase Reaction Buffer.

7. Add 20 µL 1 mM Bead Linker solution.

8. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System, then pulse-spin.

9. Add 2 µL Terminal Transferase (20 U/µL), then vortex the solution. Pulse-spin.

10. Rotate the tube at 37 °C for 2 hours.

11. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

12. Resuspend the beads in 400 µL of 1X TEX Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

13. Resuspend the beads in 200 µL of 1X TEX Buffer.

14. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

15. Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer.

Prepare templated beads (full-scale)

Prepare the full-scale emulsion PCR (ePCR) reaction

1. Prepare the oil phase:
   a. Use 3-mL and 1-mL syringes to dispense Emulsion Stabilizers 1 and 2 into a 50-mL conical tube:

Component	Volume
Emulsion Stabilizer 1	1.8 mL
Emulsion Stabilizer 2	400 µL
Emulsion Oil	37.8 mL
Total	40 mL

   b. Vortex thoroughly and allow the mixture to degas for at least 20 minutes.
   c. Prime a 10-mL syringe by drawing in about 2 mL of oil phase and dispensing it back into the tube.
   d. Dispense 9 mL of oil phase to an uncapped SOLiD™ ePCR Tube using the 10-mL syringe. Cap the tube.
2. Prepare the aqueous phase:
   a. Dilute ePCR Primer 1 to prepare 10 µM working stock solution.
   b. Prepare a dilution of the library template to a final concentration of 500 pM in 1X Low TE Buffer.
   c. Prepare the aqueous phase in a Nalgene wide-mouthed jar and keep on ice until ready to use:

	Library concentration		
	0.5 pM	1.0 pM	*X* pM
	Volume per reaction (µL)‡		
10X PCR Buffer	560	560	560
dNTP Mix (100 mM mix comprised of 25 mM each of dATP, dTTP, dCTP, dGTP)	784	784	784
Magnesium Chloride (1 M)	140	140	140
ePCR Primer 1 (10 µM working stock solution)	22.4	22.4	22.4
ePCR Primer 2 (500 µM)	33.6	33.6	33.6
Template (500 pM)	5.6	11.2	*X* × 11.2
Nuclease-free Water	3294.4	3288.8	3300 – (*X* × 11.2)
AmpliTaq Gold® DNA PolymeraseUP (5 U/µL)	600	600	600
Total	5440	5440	5440

‡ Volumes below are for a single IKA®-based ePCR reaction to fill a 96-well plate.

3. Prepare the SOLiD™ P1 DNA Beads:
   a. Thoroughly vortex 1 tube of SOLiD™ P1 DNA Beads, then pulse-spin.
   b. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.
   c. Resuspend the beads in 200 µL of Bead Block Solution. Vortex, then pulse-spin.
   d. Sonicate the beads using the Bead Block Declump program on the Covaris™ S2 System, then pulse-spin.
   e. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.
   f. Resuspend beads in 200 µL of 1X TEX Buffer. Vortex, then pulse-spin.
4. Create the emulsion with the ULTRA-TURRAX® Tube Drive from IKA®:
   a. Place the SOLiD™ ePCR Tube with oil phase onto the ULTRA-TURRAX Tube Drive from IKA, then lock the tube into position.
   b. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.
   c. Immediately add 160 µL of SOLiD™ P1 DNA Beads to the aqueous phase and mix by gently swirling the bottle.

d. Verify the Xstream pipettor settings and attach a 10-mL Combitip Plus:
   - Dial Setting: **Pip**
   - Speed (aspirate UP): **scale 5** (mid-range)
   - Speed (dispense DOWN): **scale 1** (slowest)
   - Total volume: **5.60 mL**

e. Draw 5.6 mL of aqueous phase and bead mixture into the 10-mL Combitip Plus using the Xstream pipettor.

f. Press the **Start** button on the ULTRA-TURRAX Tube Drive from IKA, then make sure it is set to 5 minutes. Wait for the flywheel of the tube drive to engage and reach steady speed.

g. Gently place the Combitip Plus into the center sample loading hole in the SOLiD™ ePCR Tube cap, then dispense aqueous phase and bead mixture into the spinning oil phase. When the entire volume is dispensed, press the center blue button *twice* on the pipettor to empty all contents from the Combitip Plus.

h. After the ULTRA-TURRAX Tube Drive from IKA stops, attach a 5-mL Combitip Plus (with its tip cut off at the bevel using a razor blade) to an Eppendorf Repeater Plus Pipette.

i. Gently dispense 150 µL of emulsion into each well of a 96-well PCR plate. Seal the plate with clear adhesive film.

5. Run the ePCR reaction on the GeneAmp® PCR System 9700:
   - PCR conditions:

Stage	Step	Temp (°C)	Time
Holding	Denature	95	5 min
40 cycles‡ *or* 60 cycles§	Denature	93	15 sec
	Anneal	62	30 sec
	Extend	72	75 sec
Holding	Final extension	72	7 min
Holding	—	4	∞

‡ Set *40* cycles: Fragment library or 2 × 25 bp mate-paired library.
§ Set *60* cycles: 2 × 50 bp mate-paired library.

   - Ramp speed: **9600**
   - Reaction volume: **50 µL**

6. Observe the bottom of the reaction plate for beads that have fallen out of the emulsion.

Perform emulsion break and bead wash (full-scale)

1. Break the emulsion:
   a. Place the SOLiD™ Emulsion Collection Tray on top of the ePCR 96-well plate.
   b. Seal the pieces together with tape and flip the entire apparatus.
   c. Centrifuge the inverted plate and reservoir at 550 × g for 2 minutes.
   d. Remove the tape and 96-well plate from the collection tray.
   e. In a fume hood, add 10 to 12 mL of 2-butanol to the collection tray. Pipet the emulsion up and down until the solution is homogeneous.
   f. Transfer the emulsion and 2-butanol to a 50-mL conical tube.
   g. Rinse the reservoir with 6 mL of 2-butanol and transfer the rinse to the tube.
   h. Cap the tube, then vortex the tube.
   i. Centrifuge the tube at 2,000 × g for 5 minutes.
   j. Decant the 2-butanol-oil phase into a waste bottle.
   k. Place the tube inverted onto paper towels, then wait for 5 minutes.

2. Wash the templated beads:
   a. Add 600 µL of 1X Bead Wash Buffer to the tube of templated beads. Let the pellet soak for 2 minutes.
   b. Resuspend by gently pipetting up and down and then transfer the beads to a 1.5-mL LoBind Tube.
   c. Rinse the bottom of the 50-mL tube with 600 µL of 1X Bead Wash Buffer and transfer to the 1.5-mL LoBind Tube.
   d. Vortex the tube and centrifuge at 21,000 × g for 1 minute. (The minimum acceptable rate is 14,000 × g. 21,000 × g is preferred.)
   e. Remove the top oil phase with a pipette.
   f. With a new pipette tip, remove and discard the supernatant.
   g. Resuspend the beads in 150 µL of 1X Bead Wash Buffer, then vortex. Pulse-spin, then transfer the beads into a new 1.5-mL LoBind Tube.
   h. Rinse the bottom of the original tube with 150 µL of 1X Bead Wash Buffer, then transfer to the 1.5-mL LoBind Tube.
   i. Add 1 mL of 1X Bead Wash Buffer. Vortex, then centrifuge at 21,000 × g (minimum 14,000 × g) for 1 minute.
   j. Remove the supernatant, then resuspend the beads in 200 µL of 1X TEX Buffer.

k. Place the tube in a magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.

l. Resuspend the beads in 200 µL of 1X TEX Buffer.

3. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

4. Quantitate a 1:10 dilution of beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer. Combine diluted and undiluted beads.

Enrich for the templated beads (full-scale)

1. Prepare the Denaturing Buffer solution:
   a. Transfer 1.8 mL of Denaturing Buffer into a 15-mL conical tube.
   b. Add 200 µL of Denaturant to the Denaturing Buffer. Cap the tube, then vortex well.
2. Prepare 60% glycerol:
   a. Add 4 mL of Nuclease-free Water using a syringe to a 15-mL conical tube.
   b. Add 6 mL of glycerol (dispensing glycerol twice from a 3-mL syringe). Cap the tube, then vortex well.
3. Prepare the enrichment beads:
   a. Vortex the enrichment beads, then immediately transfer 650 µL of enrichment beads to a 1.5-mL LoBind Tube.
   b. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   c. Resuspend the enrichment beads in 900 µL of 1X Bind & Wash Buffer.
   d. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then remove the supernatant.
   e. Repeat steps c and d.
   f. Resuspend the enrichment beads in 350 µL of 1X Bind & Wash Buffer.
   g. Add 3.5 µL of 1 mM Enrichment Oligo. Vortex, then pulse-spin.
   h. Rotate the tube at room temperature for 30 minutes.
   i. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes and discard the supernatant.
   j. Resuspend the enrichment beads in 900 µL of 1X TEX Buffer.
   k. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   l. Repeat steps j and k.
   m. Resuspend the beads in 150 µL of 1X Low Salt Binding Buffer.
4. Prepare the templated beads for enrichment:
   a. Place the tube of templated beads in the magnetic rack for 1 minute, then discard the supernatant.
   b. Resuspend the templated beads in 300 µL of prepared Denaturing Buffer solution and let the beads stand for 1 minute.
   c. Place the tube in the magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.
   d. Repeat steps b and c *twice*.
   e. Resuspend the templated beads in 300 µL of 1X TEX Buffer. Place the tube in a magnetic rack for at least 1 minute. Discard the supernatant after the solution clears. Repeat this step *twice*.
   f. Resuspend the templated beads in 150 µL of 1X TEX Buffer, then transfer the solution to a 0.5-mL LoBind Tube.
   g. Sonicate the templated beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin the beads.
5. Enrich for the templated beads:
   a. Transfer the prepared enrichment beads to the tube of templated beads and vortex to mix, then pulse-spin the tube.
   b. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System, then pulse-spin the beads.
   c. Incubate the bead mixture at 61 °C for 15 minutes. Vortex, then pulse-spin every 5 minutes including at the end of incubation.
   d. Immediately cool the beads on ice for 2 minutes.
   e. Add 600 µL of *newly prepared* 60% glycerol to a new 1.5-mL LoBind Tube.
   f. Gently pipet the bead mixture up and down to mix, then load the entire bead mixture volume on top of the 60% glycerol solution. *Do not* vortex the tube.
   g. Centrifuge at 21,000 × g (minimum 14,000 × g) for 3 minutes.
   h. Add 1 mL of 1X TEX Buffer to a 2.0-mL LoBind Tube.
   i. Transfer the top layer of beads to the 2.0-mL LoBind Tube. Top off the tube with additional 1X TEX Buffer.
   j. Vortex the tube of beads and centrifuge at 21,000 × g (minimum 14,000 × g) for 1 minute.
   k. If the beads are pelleted, discard the supernatant and resuspend the beads in 400 µL of 1X TEX Buffer. Go to step 6. If the beads are not pelleted, go to step l.

l. Transfer half of the bead solution to a fresh tube and add 500 µL of 1X TEX Buffer to each tube, then vortex the tube.

m. Centrifuge the beads at 21,000 × g (minimum 14,000 × g) for 1 minute, then discard the supernatant.

n. Resuspend each pellet in 200 µL of 1X TEX Buffer, then pool the beads into one tube.

6. Isolate the P2-enriched beads:

a. Centrifuge the beads at 21,000 × g (minimum 14,000 × g) for 1 minute, then discard the supernatant.

b. Resuspend the beads in 400 µL prepared Denaturing Buffer solution, then let the beads stand for 1 minute.

c. Place the tube in a magnetic rack for at least 1 minute until the supernatant is pure white or clear. Discard the supernatant.

d. Repeat steps b and c until the supernatant is clear.

e. Resuspend the beads in 400 µL of 1X TEX Buffer.

f. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear, then discard the supernatant.

g. Repeat steps e and f.

h. Resuspend the beads in 200 µL of 1X TEX Buffer, then transfer the beads to a new 1.5-mL LoBind Tube. Rinse the tube with 200 µL of 1X TEX Buffer, then add the wash to the same 1.5-mL LoBind Tube.

i. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

j. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.

k. Resuspend the beads in 400 µL of 1X TEX Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant. Repeat this step until the supernatant is clear.

l. Resuspend the beads in 400 µL of 1X TEX Buffer.

Modify the 3′ ends (full-scale)

1. Prepare the 1X Terminal Transferase Reaction Buffer (500 µL buffer per 1 ePCR reaction):

Component	Volume per reaction (µL)
10X Terminal Transferase Buffer	55
10X Cobalt Chloride	55
Nuclease-free Water	390
Total	500

2. Add 1 µL 50 mM Bead Linker to 49 µL of 1X Low TE Buffer to prepare a 1 mM Bead Linker solution.

3. Place the tube of P2-enriched beads in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

4. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer and transfer to a new 1.5-mL LoBind Tube. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

5. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

6. Resuspend the beads in 178 µL of 1X Terminal Transferase Reaction Buffer.

7. Add 20 µL of 1 mM Bead Linker solution.

8. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System, then pulse-spin.

9. Add 2 µL Terminal Transferase (20 U/µL), then vortex the solution. Pulse-spin.

10. Rotate the tube at 37 °C for 2 hours.

11. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

12. Resuspend the beads in 400 µL of 1X TEX Buffer.

13. Repeat steps 11 and 12.

14. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

15. Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer.

Prepare templated beads (macro-scale: 4 ePCR reactions)

Prepare 4 emulsion PCR (ePCR) reactions

See "Prepare the full-scale emulsion PCR (ePCR) reaction" on page 5 to prepare 4 ePCR reactions. Four ePCR reactions will provide an adequate bead yield for two wells of a 4-well slide for sequencing. Store each 96-well plate at 4 °C or proceed to "Perform emulsion break and bead wash".

Perform emulsion break and bead wash

See "Perform emulsion break and bead wash (full-scale)" on page 6 for full-scale templated bead preparation to perform the emulsion break and bead wash procedure on each of the 4 ePCR reactions. Store each tube of beads at 4 °C or proceed to "Enrich for the templated beads (4 ePCR reactions)".

Enrich for the templated beads (4 ePCR reactions)

1. Prepare the Denaturing Buffer solution:
   a. Transfer 5.4 mL of Denaturing Buffer into a 15-mL conical tube.
   b. Add 600 µL of Denaturant to the Denaturing Buffer. Cap the tube, then vortex well.
2. Prepare 60% glycerol:
   a. Add 4 mL of Nuclease-free Water using a syringe to a 15-mL conical tube.
   b. Add 6 mL of glycerol (dispensing glycerol twice from a 3-mL syringe). Cap the tube, then vortex well.
3. Prepare the enrichment beads:
   a. Vortex the enrichment beads, then immediately transfer 825 µL of enrichment beads to each of two 2.0-mL LoBind Tubes.
   b. Centrifuge the enrichment beads at 21,000 × g for 2 minutes, then discard the supernatant. (The minimum acceptable rate is 14,000 × g. 21,000 × g is preferred.)
   c. Resuspend the enrichment beads in each tube with 500 µL of 1× Bind & Wash Buffer. Transfer the bead suspension from one tube into the other tube.
   d. Combine the contents of the two tubes into a single tube, resulting in one 2.0-mL tube containing enrichment beads in 1 mL of 1× Bind & Wash Buffer.
   e. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   f. Resuspend the enrichment beads in 500 µL of 1× Bind & Wash Buffer.
   g. Add 5.0 µL of 1 mM Enrichment Oligo. Vortex, then pulse-spin.
   h. Rotate the tube at room temperature for 30 minutes.
   i. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   j. Resuspend the enrichment beads in 1 mL of 1× TEX Buffer.
   k. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   l. Repeat steps j and k.
   m. Resuspend the enrichment beads in 500 µL of 1× Low Salt Binding Buffer.
4. Prepare the templated beads for enrichment:
   a. Place a 2.0-mL LoBind Tube in a magnetic rack.
   b. Transfer the suspension of beads from the first ePCR reaction to the tube in the magnetic rack.
   c. Rinse the bottom of the first tube of templated beads with 100 µL of 1× TEX Buffer, then transfer the rinse to the tube in the magnetic rack.
   d. Wait for at least 1 minute. Discard the supernatant after the solution clears.
   e. Transfer the suspension of templated beads from the next ePCR reaction to the tube in the magnetic rack.
   f. Rinse the tube with 100 µL of 1× TEX Buffer, then transfer the rinse to the tube in the magnetic rack.
   g. Wait for at least 1 minute. Discard the supernatant after the solution clears.
   h. Repeat steps e to g until all templated beads are in the LoBind Tube in the magnetic rack.
   i. Resuspend the templated beads in 450 µL of prepared Denaturing Buffer solution, then let the beads stand for 1 minute.
   j. Place the tube in the magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.
   k. Repeat steps i and j *twice*.
   l. Resuspend the beads in 1.0 mL of 1× TEX Buffer.
   m. Place the tube in a magnetic rack or at least 1 minute. Discard the supernatant after the solution clears.
   n. Repeat steps l and m *twice*.
   o. Resuspend the beads in 500 µL of 1× TEX Buffer.

p. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

5. Enrich for the templated beads:
   a. Transfer all (500 µL) of the enrichment bead suspension to the 2.0-mL tube with the templated beads. Vortex to mix, then pulse-spin the tube.
   b. Sonicate the enrichment-templated beads using the Covalent Declump 3 program on the Covaris™ S2 System, then pulse-spin the beads.
   c. Incubate the bead mixture at 61 °C for 15 minutes. Vortex, then pulse-spin every 5 minutes, including at the end of incubation.
   d. Immediately cool the beads on ice for 2 minutes.
   e. Add 10 mL of *newly prepared* 60% glycerol to a new 50-mL conical tube.
   f. Use a 1-mL pipettor tip to gently pipet the bead mixture up and down to mix, then load the entire bead mixture volume *carefully* on top of the 60% glycerol solution. *Do not* vortex the tube.
   g. Centrifuge at 3400 × g (minimum 2284 × g) for 10 minutes. The centrifuge brake should be off and the temperature should be set to room temperature.
   h. Add 10 mL of 1X TEX Buffer to a 50-mL conical polypropylene tube.
   i. Transfer the top layer of beads to the tube with 1X TEX Buffer. Top off the tube with additional 1X TEX Buffer to the 25-mL mark.
   j. Vortex the tube of beads, then centrifuge at 3400 × g (minimum 2284 × g) for 10 minutes.
   k. If the beads are pelleted, remove and discard the supernatant, then go to step 6. If the beads are *not* pelleted, go to step l.
   l. Carefully remove as much supernatant as possible without pipetting up beads.
   m. Top off the tube with additional 1X TEX Buffer to the 25-mL mark.
   n. Repeat steps j and k.

6. Isolate the P2-enriched beads:
   a. Resuspend the beads in 900 µL of prepared Denaturing Buffer solution, then transfer the beads to a new 2.0-mL LoBind Tube. Let the beads stand for 1 minute.
   b. Rinse the 50-mL tube with 300 µL of prepared Denaturing Buffer solution, then transfer the rinse to the 2.0-mL LoBind Tube.
   c. Place the tube in a magnetic rack for at least 1 minute. Discard the supernatant after the solution becomes pure white or clear.
   d. Resuspend the beads in 1 mL of prepared Denaturing Buffer solution, then let the beads stand for 1 minute.
   e. Repeat steps c and d until the supernatant is clear.
   f. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
   g. Resuspend the beads in 1 mL of 1X TEX Buffer.
   h. Repeat steps f and g *twice*.
   i. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.
   j. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.
   k. Resuspend the beads in 1 mL of 1X TEX Buffer.
   l. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.
   m. Repeat steps k and l until the supernatant is clear.
   n. Resuspend the beads in 1 mL of 1X TEX Buffer.

Modify the 3′ ends (4 ePCR reactions)

1. If the P2-enriched beads have been stored overnight or longer, sonicate the beads using the Covalent Declump 3 program, then pulse-spin the beads.
2. Prepare the 1X Terminal Transferase Reaction Buffer (1.5 mL of buffer per 4 ePCR reactions):

Component	Volume per reaction (µL)
10X Terminal Transferase Buffer	165
10X Cobalt Chloride	165
Nuclease-free Water	1170
Total	1500

3. Add 2 µL of 50 mM Bead Linker to 98 µL of 1X Low TE Buffer to prepare a 1 mM Bead Linker solution.
4. Place the tube of P2-enriched beads in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
5. Resuspend the beads in 300 µL of 1X Terminal Transferase Reaction Buffer, then transfer the beads to a new 2.0-mL LoBind Tube.
6. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
7. Resuspend the beads in 300 µL of 1X Terminal Transferase Reaction Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
8. Resuspend the beads in 712 µL of 1X Terminal Transferase Reaction Buffer.
9. Add 80 µL of 1 mM Bead Linker solution.
10. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System, then pulse-spin.

11. Add 8 µL of Terminal Transferase (20 U/µL), then vortex the solution. Pulse-spin.
12. Seal the tube with Parafilm, then rotate the tube at 37 °C for 2 hours.
13. Pulse-spin the tube.
14. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
15. Resuspend the beads in 400 µL of 1X TEX Buffer.
16. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
17. Resuspend the beads in 400 µL of 1X TEX Buffer.
18. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.
19. Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer.

Prepare templated beads (macro-scale: 8 ePCR reactions)

Prepare 8 emulsion PCR (ePCR) reactions

See "Prepare the full-scale emulsion PCR (ePCR) reaction" on page 5 to prepare 8 emulsion PCR reactions. Eight ePCR reactions will provide an adequate bead yield for one full slide for sequencing. Store each 96-well plate at 4 °C or proceed to "Perform emulsion break and bead wash".

Perform emulsion break and bead wash

See "Perform emulsion break and bead wash (full-scale)" on page 6 for full-scale templated bead preparation to perform the emulsion break and bead wash procedure on each of the 8 emulsions. Store each tube of beads at 4 °C or proceed to "Enrich for the templated beads (8 ePCR reactions)".

Enrich for the templated beads (8 ePCR reactions)

1. Prepare the Denaturing Buffer solution:
   a. Transfer 8.1 mL of Denaturing Buffer into a 15-mL conical tube.
   b. Add 900 µL of Denaturant to the Denaturing Buffer. Cap the tube, then vortex well.
2. Prepare 60% glycerol:
   a. Add 8 mL of Nuclease-free Water using a syringe to a 15-mL conical tube.
   b. Add 12 mL of glycerol (dispensing glycerol four times from a 3-mL syringe). Cap the tube, then vortex well.
3. Prepare the enrichment beads:
   a. Vortex the enrichment beads, then immediately transfer 825 µL of enrichment beads to each of four 2.0-mL LoBind Tubes.
   b. Centrifuge the enrichment beads at 21,000 × g for 2 minutes, then discard the supernatant. (The minimum acceptable rate is 14,000 × g. 21,000 × g is preferred.)
   c. Resuspend the enrichment beads in each tube with 500 µL of 1× Bind & Wash Buffer per tube.
   d. Transfer the bead suspension from two of the tubes into the other two tubes to reduce the total number of tubes to two. Each tube contains bead suspension in 1 mL of 1× Bind & Wash Buffer.
   e. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   f. Resuspend the enrichment beads in 500 µL of 1× Bind & Wash Buffer per tube.
   g. Add 5.0 µL 1 mM Enrichment Oligo ***per tube***. Vortex, then pulse-spin the tubes.
   h. Rotate the tubes at room temperature for 30 minutes.
   i. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   j. Resuspend the enrichment beads in 1 mL of 1× TEX Buffer per tube.
   k. Centrifuge the enrichment beads at 21,000 × g (minimum 14,000 × g) for 2 minutes, then discard the supernatant.
   l. Repeat steps j and k.
   m. Resuspend the enrichment beads in each of the two tubes with 500 µL of 1× Low Salt Binding Buffer per tube.
4. Prepare the templated beads for enrichment:
   a. Place a 2.0-mL LoBind Tube in a magnetic rack.
   b. Transfer the suspension of beads from the first ePCR reaction to the tube in the magnetic rack.
   c. Rinse the bottom of the first tube of templated beads with 100 µL of 1× TEX Buffer, then transfer the rinse to the tube in the magnetic rack.
   d. Wait for at least 1 minute. Discard the supernatant after the solution clears.
   e. Transfer the suspension of templated beads from the next ePCR reaction to the tube in the magnetic rack.
   f. Rinse the tube with 100 µL of 1× TEX Buffer, then transfer the rinse to the tube in the magnetic rack.
   g. Wait for at least 1 minute. Discard the supernatant after the solution clears.
   h. Repeat steps e to g until all templated beads from the 4 tubes are in the LoBind Tube in the magnetic rack.
   i. Repeat steps a to h for the remaining 4 tubes.
   j. Resuspend the templated beads in each tube with 450 µL of prepared Denaturing Buffer solution, then let the beads stand for 1 minute.
   k. Place the tubes in the magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.
   l. Repeat steps j and k *twice*.
   m. Resuspend the beads in each tube with 1.0 mL 1× TEX Buffer.

n. Place the tubes in a magnetic rack for at least 1 minute. Discard the supernatant after the solution clears.

o. Repeat steps m and n *twice*.

p. Resuspend the beads in each tube with 500 µL of 1× TEX Buffer.

q. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

5. Enrich for the templated beads:

a. Transfer one tube of enrichment beads (500 µL) into one 2.0-mL tube of templated beads.

b. Immediately sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System, then pulse-spin the beads.

c. Repeat steps a and b for the second tube of enrichment and templated beads.

d. Incubate each tube of the bead mixture at 61 °C for 15 minutes. Vortex, then pulse-spin every 5 minutes, including at the end of incubation.

e. Immediately cool the beads on ice for 2 minutes.

f. Add 10 mL of *newly prepared* 60% glycerol to each of 2 new 50-mL conical tube.

g. Use a 1-mL pipettor tip to gently pipet the bead mixture from one of the tubes up and down to mix, then load the entire bead mixture volume on top of one of the 60% glycerol solutions. *Do not* vortex the tube.

h. Centrifuge at 3400 × g (minimum 2284 × g) for 10 minutes. The centrifuge brake should be off and the temperature should be set to room temperature.

i. Add 20 mL of 1× TEX Buffer to a 50-mL conical polypropylene tube.

j. Transfer the top layer of beads to the tube with 1× TEX Buffer. Top off the tube with additional 1× TEX Buffer to the 35-mL mark.

k. Vortex the tube of beads, then centrifuge at 3400 × g (minimum 2284 × g) for 10 minutes.

l. If the beads are pelleted, remove and discard the supernatant, then go to step 6. If the beads are *not* pelleted, go to step m.

m. Carefully remove as much supernatant as possible without pipetting up beads.

n. Top off the tube with additional 1× TEX Buffer to the 25-mL mark, then vortex the tube.

o. Repeat steps j and k.

6. Isolate the P2-enriched beads:

a. Resuspend the beads in 900 µL of prepared Denaturing Buffer solution, then transfer the beads into a 2.0-mL LoBind Tube. Let the beads stand for 1 minute.

b. Rinse the 50-mL tube with 300 µL of prepared Denaturing Buffer solution, then transfer the rinse to the 2.0-mL LoBind Tube.

c. Place the tube in a magnetic rack for at least 1 minute. Discard the supernatant after the solution becomes pure white or clear.

d. Resuspend the beads in 1 mL of prepared Denaturing Buffer solution, then let the beads stand for 1 minute.

e. Repeat steps c and d until the supernatant is clear.

f. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

g. Resuspend the beads in 1 mL of 1× TEX Buffer.

h. Repeat steps f and g *twice*.

i. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.

j. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.

k. Resuspend the beads in 1 mL of 1× TEX Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant.

l. Repeat steps j and k until the supernatant is clear.

m. Resuspend the beads in 1 mL of 1× TEX Buffer.

Modify the 3′ends (8 ePCR reactions)

1. If the P2-enriched beads have been stored overnight or longer, sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System.

2. Prepare the 1× Terminal Transferase Reaction Buffer (2.4 mL of buffer per 8 ePCR reactions):

Component	Volume per reaction (µL)
10× Terminal Transferase Buffer	264
10× Cobalt Chloride	264
Nuclease-free Water	1872
Total	2400

3. Add 4 µL of 50 mM Bead Linker to 196 µL of 1× Low TE Buffer to prepare a 1 mM Bead Linker solution.

4. Place the tube of P2-enriched beads in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

5. Resuspend the beads in 300 µL of 1× Terminal Transferase Reaction Buffer, then transfer the beads to a 2.0-mL LoBind Tube.

6. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

7. Resuspend the beads in 300 µL of 1X Terminal Transferase Reaction Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
8. Resuspend the beads in 1424 µL of 1X Terminal Transferase Reaction Buffer.
9. Add 160 µL of 1 mM Bead Linker solution.
10. Transfer 792 µL of bead solution to a new 2.0-mL LoBind Tube.
11. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System, then pulse-spin.
12. Add 8 µL of Terminal Transferase (20 U/µL) to each tube, then vortex the solution. Pulse-spin.
13. Seal each tube with Parafilm, then rotate the tubes at 37 °C for 2 hours.
14. Pulse-spin the tubes, then pool the beads in one LoBind Tube.
15. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
16. Resuspend the beads in 400 µL of 1X TEX Buffer.
17. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
18. Resuspend the beads in 400 µL of 1X TEX Buffer.
19. Sonicate the beads using the Covalent Declump 1 program on the Covaris™ S2 System, then pulse-spin.
20. Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer.
21.

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