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Applied Biosystems SOLiD™ 3 Plus System

Templated Bead Preparation Guide

October 2009

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Part Number 4442695 Rev. A
10/2009

Contents

Preface

Safety information

Note: For important instrument safety information, refer to the *Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Guide* (PN 4442357). For general safety information, see this Preface and Appendix H, "Safety" on page 127. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

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WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, results in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "MSDSs" on page 130.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

How to use this guide

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

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Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

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1 Introduction

Templated bead preparation is performed after library construction [refer to the *Applied Biosystems SOLiD™ 3 Plus System Library Preparation Guide* (PN 4442697)]. To prepare templated beads, each library template is clonally amplified on SOLiD™ P1 DNA Beads by emulsion PCR (ePCR). After ePCR and enrichment of the templated beads, the templated beads are deposited onto a slide. The templates are sequenced on the SOLiD 3 Plus System.

Workflows

If you are preparing an ePCR reaction of a new library, you will obtain better sequencing results for a particular scale of templated bead preparation by titrating the library concentration to find the optimal library concentration for ePCR (see Figure 1 on page 2). To find the optimal library concentration, you perform two separate ePCR reactions at library concentrations of 0.5 pM and 1.0 pM. Next, you perform a workflow analysis (WFA) run on the SOLiD 3 Plus System to evaluate ePCR performance for each library concentration [refer to the *Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Guide* (PN 4442357)]. You then use the optimal library concentration to prepare templated beads at the *same scale* of templated bead preparation as you used to determine the optimal library concentration. You can determine the optimal library concentration for macro-scale templated bead preparation with the full-scale templated bead preparation.

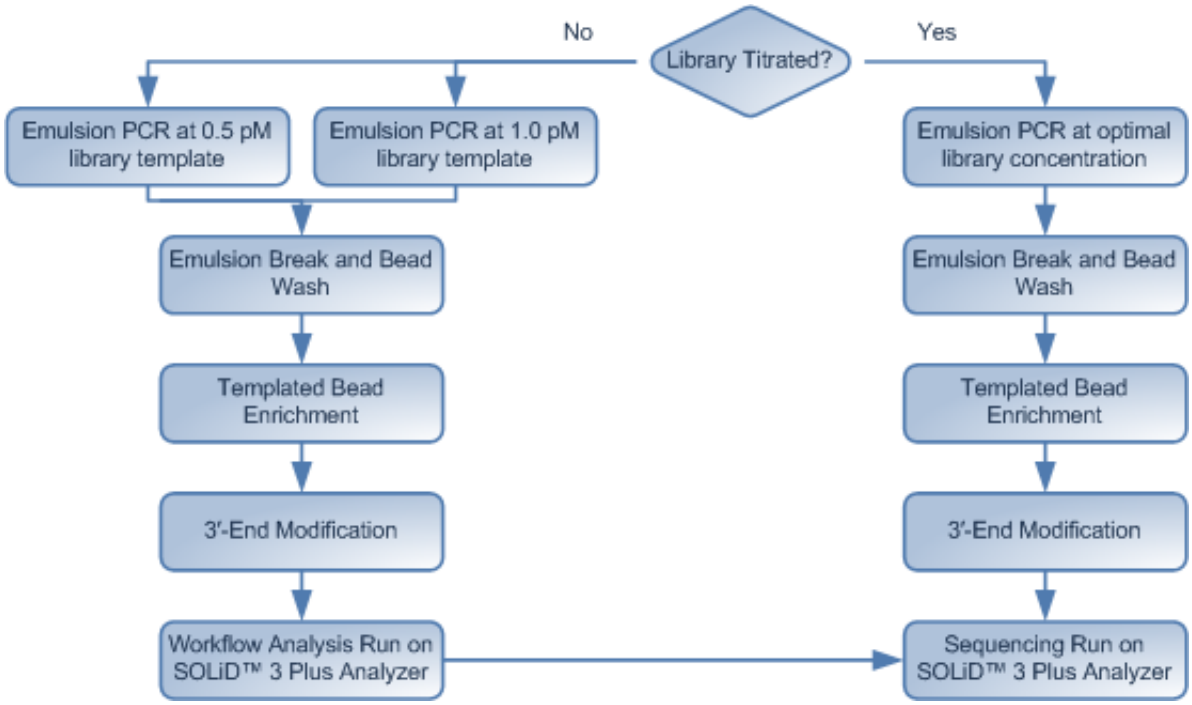


Figure 1 Workflow to prepare templated beads for SOLiD™ System sequencing using workflow analysis.

An alternative to determining the optimal library concentration and performing a WFA run is quantitative PCR (qPCR). qPCR is a method to accurately measure library concentration. You can set up an emulsion PCR reaction according to the qPCR results because the molar optimal library concentration correlates with ePCR performance (see Figure 2). For details on qPCR, see "Calculation of the Emulsion PCR Library Concentration" on page 87.

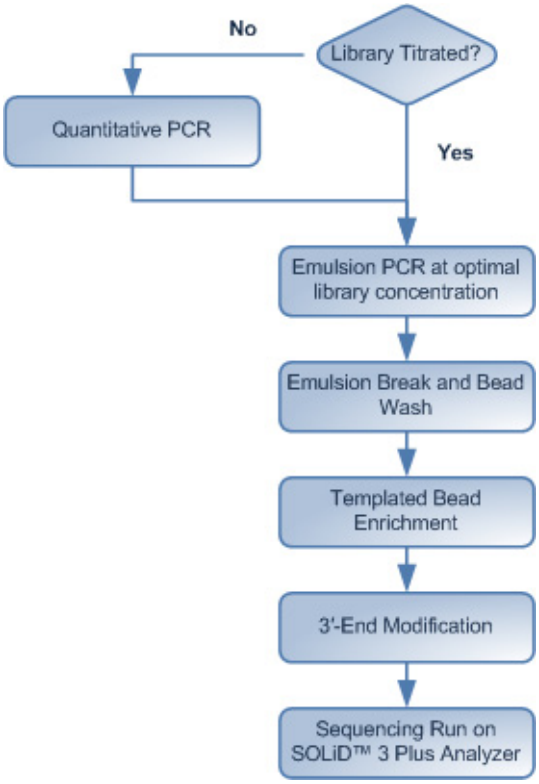


Figure 2 Workflow to prepare templated beads for SOLiD™ System sequencing with quantitative PCR.

Scales of preparation

You can prepare templated beads according to the amount of library that you want to amplify (see Table 1):

Table 1 Three ways to prepare templated beads according to the scale of preparation

Scale of preparation	Features	Go to...
Mini	• **Yield**: 75 to 150 million templated beads • **ePCR reaction**: 1 ePCR reaction seeded with 800 million SOLiD™ P1 DNA Beads	Section 2.1, Prepare templated beads (mini-scale) on page 12.
Full	• **Yield**: 150 to 300 million templated beads • **ePCR reaction**: 1 ePCR reaction seeded with 1.6 billion SOLiD™ P1 DNA Beads	Section 2.2, Prepare templated beads (full-scale) on page 33.
Macro	• **Yield**: 600 million to 1.2 billion templated beads • **ePCR reaction**: 4 ePCR reactions, each seeded with 1.6 billion SOLiD™ P1 DNA Beads	Section 2.3, Prepare templated beads (macro-scale) on page 55.
	• **Yield**: 1.2 billion to 2.4 billion templated beads • **ePCR reaction**: 8 ePCR reactions, each seeded with 1.6 billion SOLiD™ P1 DNA Beads	

Choose the scale of templated bead preparation based on the number of templated beads required for the slide (see Table 2). Vary the targeted bead density for deposition based on your desired output, sample, and experimental conditions:

Table 2 Number of templated beads needed according to slide configuration

Slide configuration	Templated Bead Quantity Requirements‡ (millions per spot)
1-well	520
4-well	96
8-well	41

‡ Assuming targeted bead deposition density of 220,000 beads per panel.

Decide which slide configuration is appropriate based on your desired output. Estimate expected output based on the number of beads, using the relationship shown below. Note that your actual output depends on your sample and the experimental conditions.

Expected output = (Number of templated beads) × (read length) × (% mappable beads)

Examples

1. For a fragment library with a 50-bp read length on 1 spot of an 8-well slide deposited at 220,000 beads/panel and assuming 50% matching:

   Expected output = (41 million beads) × (50 bp) × 50% = 1.025 GB

2. For a mate-paired library with a 25-bp read length on a 1-well slide deposited at 220,000 beads/panel and assuming 60% matching:

   Expected output = (520 million beads) × (2 × 25 bp) × 60% = 15.6 GB

2 Prepare Templated Beads

This chapter covers:

Overview

This chapter describes how to clonally amplify short-fragment DNA populations onto SOLiD™ P1 DNA Beads using an emulsion method. Emulsions are made up of an oil phase containing emulsifiers and an aqueous phase, which includes PCR components (template, primers, DNA polymerase, and SOLiD P1 DNA Beads; see Figure 3).

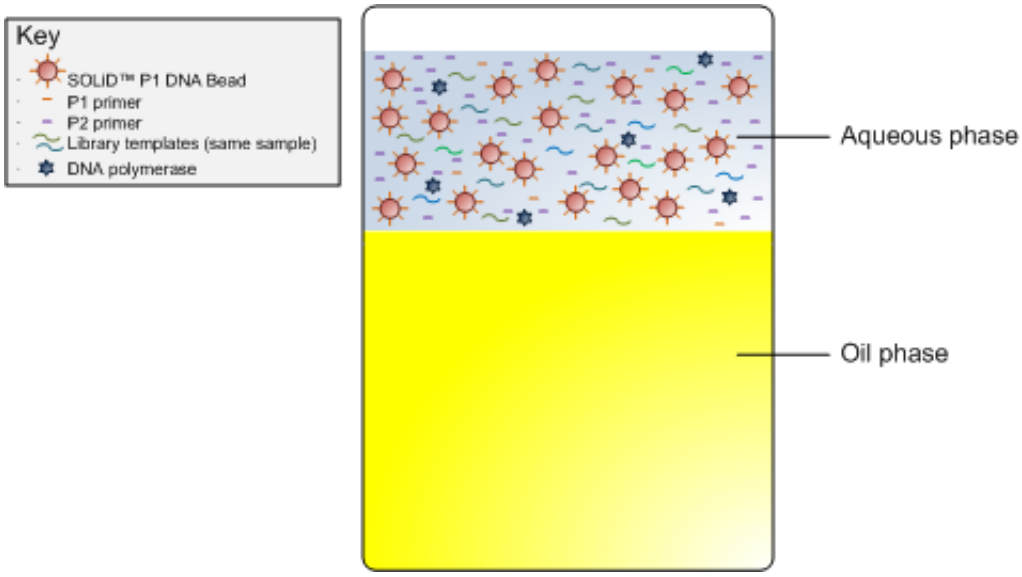


Figure 3 Aqueous phase and oil phase prior to the emulsification.

An emulsion is created using the ULTRA-TURRAX® Tube Drive from IKA®. An emulsion is made up of droplets of aqueous phase, or *micro-reactors*, in which the clonal amplification takes place. Micro-reactors containing a single SOLiD P1 DNA Bead and a single template, called *monoclonal micro-reactors*, are desired. However, Poisson bead distribution and Poisson template distribution allow for other types of reactors, including: *polyclonal micro-reactors* (contain multiple templates); *non-clonal micro-reactors* (contain no template); *multi-bead micro-reactors*; and micro-reactors with combinations of these characteristics (see Figure 4 on page 9).

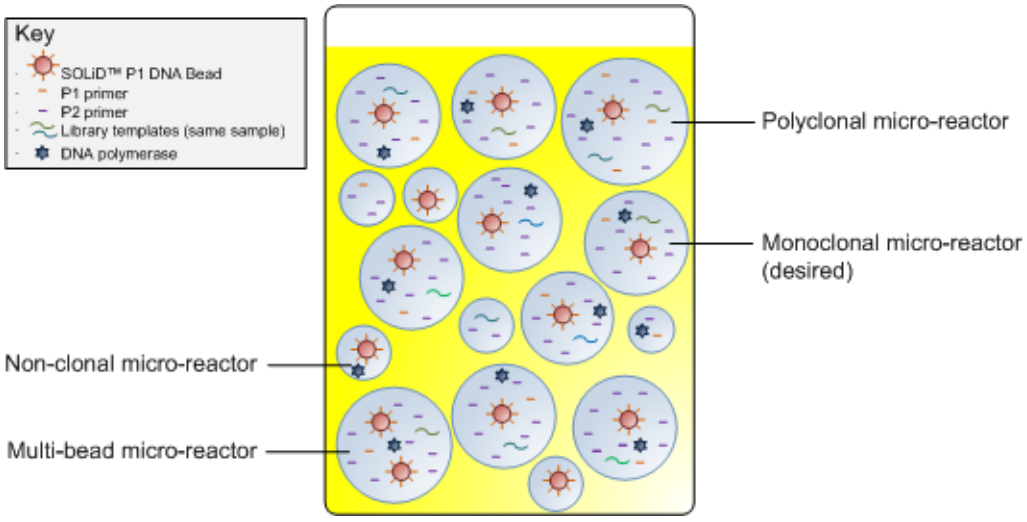


Figure 4 Emulsion before amplification (ePCR).

The emulsion is placed on a thermal cycler and run at standard PCR conditions. During emulsion PCR (ePCR), 30,000 or more copies of template are amplified onto each SOLiD P1 DNA Bead with the P1 Adaptor attached to the bead. In monoclonal and polyclonal micro-reactors, monoclonal and polyclonal templated beads are formed, respectively. In nonclonal micro-reactors, the SOLiD P1 DNA Bead cannot amplify. Multi-bead micro-reactors lead to suboptimal amplification (see Figure 5).

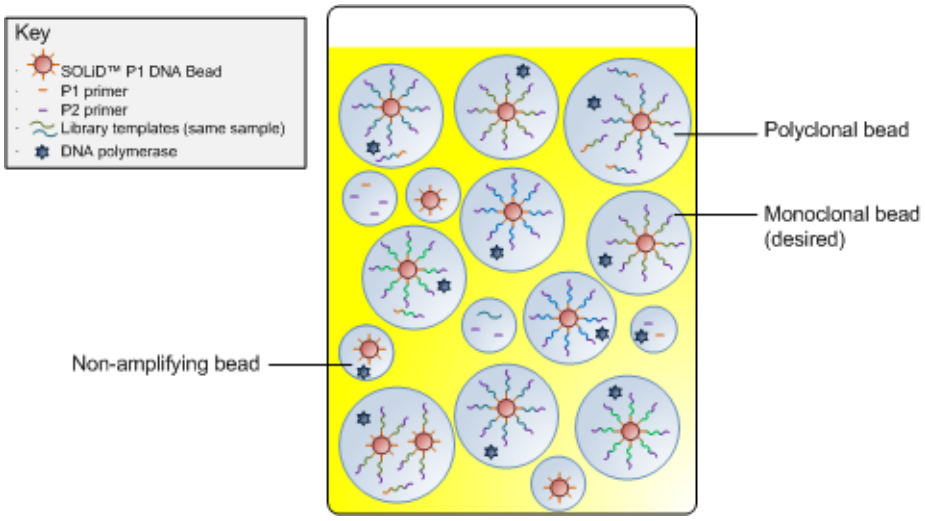


Figure 5 Emulsion after amplification (ePCR).

After emulsion PCR is complete, the micro-reactors in the emulsion are broken with 2-butanol, and the templated beads and nonamplifying beads are washed to clear away the oil and emulsifiers (see Figure 6 on page 10).

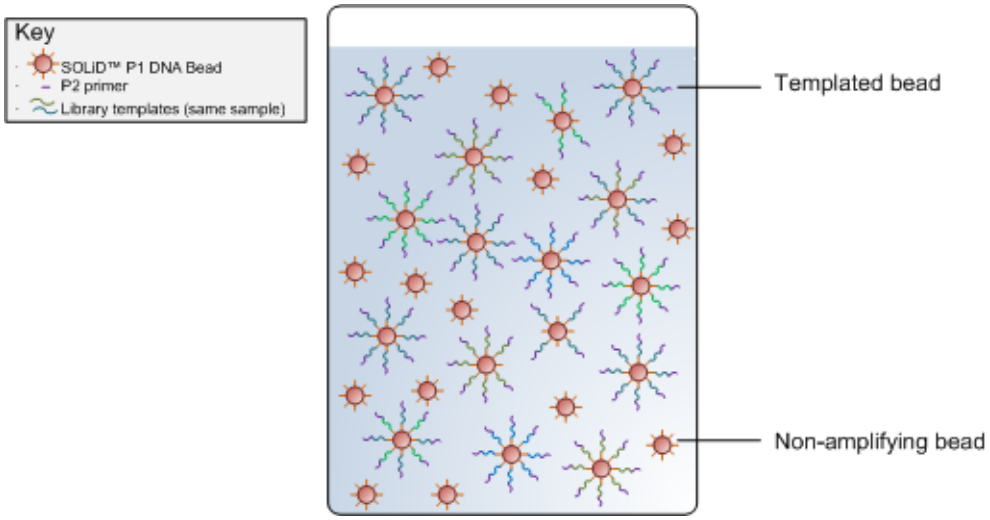


Figure 6 Templated and non-amplifying beads after emulsion break and bead wash.

Enrichment is required to isolate templated beads from non-amplifying or poorly amplifying beads. In an enrichment step, polystyrene beads with a single-stranded P2 Adaptor attached are used to capture templated beads. The mixture of enrichment beads, enrichment bead-templated bead complexes, and non-amplifying beads is centrifuged on a 60% glycerol cushion. The enrichment step results in a layer of enrichment beads (with or without templated beads attached) at the top and a layer of non-amplifying beads at the bottom. The layer of enrichment beads is extracted and denatured to dissociate the templated beads from the enrichment beads (see Figure 7).

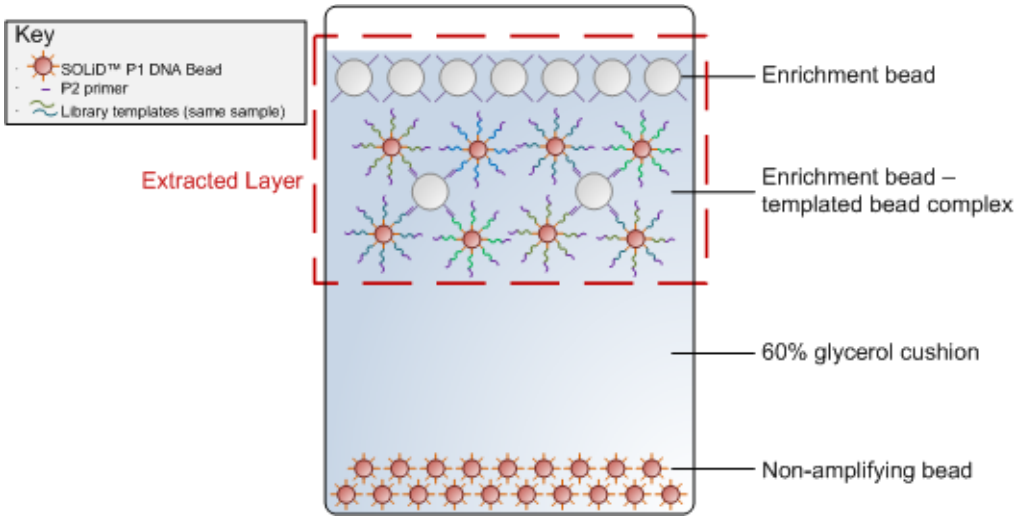


Figure 7 Enrichment beads and SOLiD™ P1 DNA beads after centrifugation with 60% glycerol.

In order to prepare the P2-enriched beads for deposition, a dUTP is added to the 3′-end of the P2 templates using a terminal transferase reaction (see Figure 8 on page 11).

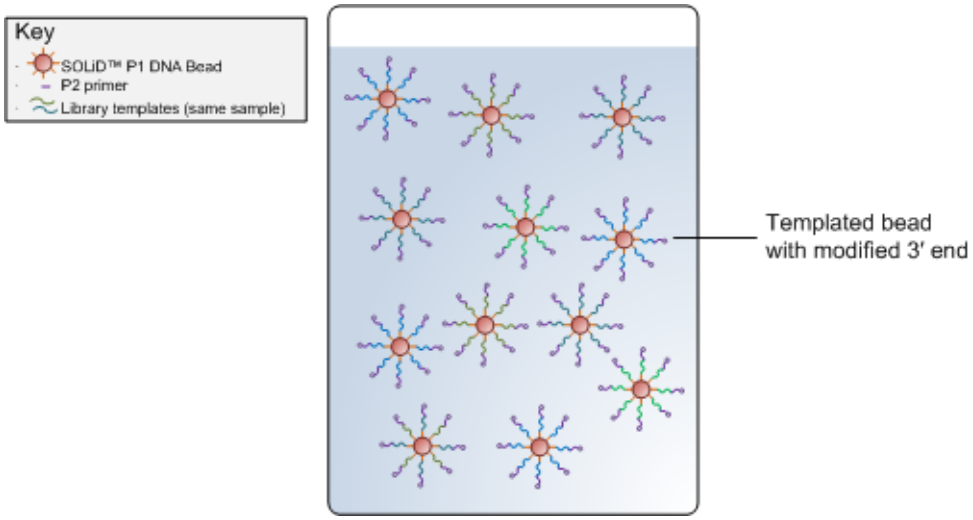


Figure 8 Templated beads after 3′-end modification.

This chapter is organized into three sections:

- Section 2.1 on page 12 describes how to generate 75 to 150 million templated beads using the *mini*-scale templated bead preparation method.
- Section 2.2 on page 33 describes how to generate 150 to 300 million templated beads using the *full*-scale templated bead preparation method.
- Section 2.3 on page 55 describes how to generate 600 million to 2.4 billion templated beads using the *macro*-scale templated bead preparation method.

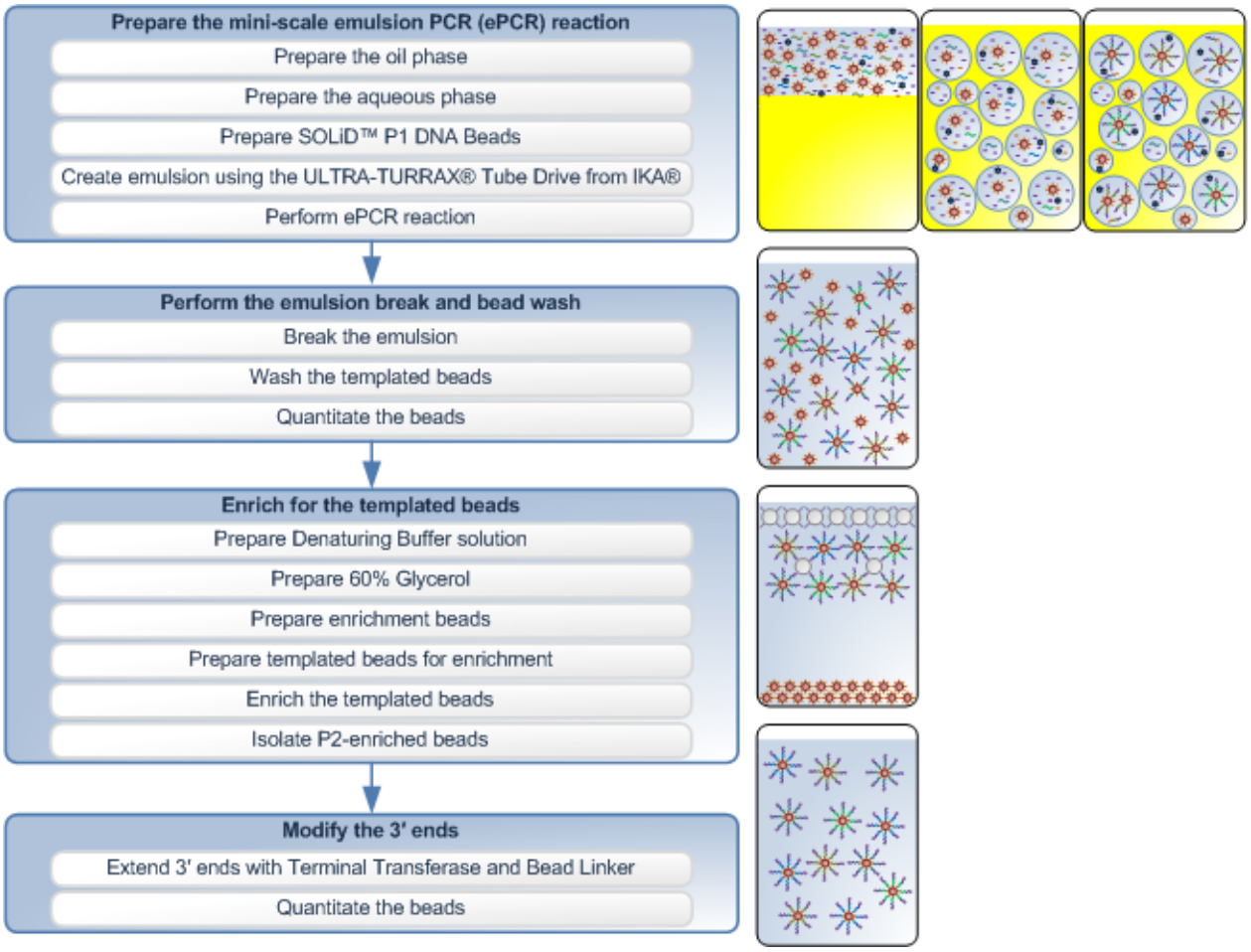
Section 2.1 Prepare templated beads (mini-scale)

Materials and equipment required

See Appendix A on page 77 for a list of equipment, kits, and consumables necessary for this procedure.

Workflow

See the descriptions of the steps below the workflow.



Prepare the mini-scale emulsion PCR (ePCR) reaction

The oil phase and aqueous phase of the emulsion are prepared separately, then emulsified using the ULTRA-TURRAX™ Tube Drive from IKA®. Each emulsion is seeded with 800 million SOLiD™ P1 DNA Beads, then transferred into a single, 96-well plate for cycling. Different library template lengths require different numbers of cycles for thermal cycling.

Perform the emulsion break and bead wash

The emulsion break uses 2-butanol to purify emulsified templated beads from the oil phase following amplification. The beads are washed to remove any residual 2-butanol, oil, and aqueous phase containing PCR reagents. There are two methods available to break the emulsion. In the *standard* method, a multi-channel pipettor is used to add and mix 2-butanol into the emulsion in each well of the 96-well plate. The pipettor is then used to transfer the solution into a 50-mL reservoir. In the *alternative* method, the SOLiD™ Emulsion Collection Tray is placed over the 96-well plate, then the plate is centrifuged. Centrifuging the plate forces the emulsion from each well to a single reservoir. After centrifugation, 2-butanol is added to the reservoir. For both methods, the broken emulsion is transferred to a 50-mL tube for further processing.

Enrich for the templated beads

The templated bead enrichment procedure isolates beads with full-length extension products following ePCR. Beads with full-length extension products are isolated by oligo hybridization using the sequence of the P2 primer. Both monoclonal and polyclonal beads are enriched. The procedure is designed to enrich for templated beads derived from one ePCR reaction yielding 75 to 150 million templated beads.

Modify the 3′ ends

The P2-enriched beads are extended with a Bead Linker by Terminal Transferase.

Tips

General

- Syringes are required to accurately measure viscous reagents. Aspirate the volume very slowly from the reagent bottle to ensure that no air bubbles are trapped within the syringe. The best practice is to draw some reagent into the syringe, dispense the entire reagent back to the reagent bottle, then draw the correct volume of reagent. The volume should be measured to the point where the plunger contacts the side of the syringe.
- Perform all steps requiring 0.5-mL, 1.5-mL, and 2.0-mL tubes with Eppendorf LoBind tubes.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols. Applied Biosystems recommends the Eppendorf 5417R tabletop microcentrifuge.

SOLiD™ P1 DNA Beads

- Do not freeze SOLiD™ P1 DNA Beads or templated beads. Store the SOLiD™ P1 DNA Beads at 4 °C in 1✕ TEX Buffer.
- If beads remain in the original tube after transfer, you can use a small additional volume of the appropriate buffer to recover the remaining beads. Do not exceed a total volume of 1.3 mL for a 1.5-mL LoBind tube.

Covaris™ S2 System

- The procedures are optimized for the Covaris™ S2 System. The Covaris S2 System must be specially adapted to prepare beads for the Applied Biosystems SOLiD™ 3 Plus System. Do not use the Covaris S1 sonicator or an unadapted Covaris S2 System for bead preparation. For more information, contact an Applied Biosystems SOLiD™ System applications specialist.
- Ensure that the Covaris™ S2 System is degassed, that no bubbles are present in the system, and that the instrument and tube are properly aligned for appropriate sonication of beads.
- To ensure optimal sonication by the Covaris™ S2 System, use the appropriate adaptor with the Covaris S2 System. For sample volumes ≤ 200 µL, use a 0.5-mL LoBind tube and 0.65-mL tube adaptor. For sample volumes between 200 µL and 600 µL, use a 1.5-mL LoBind tube and 1.5-mL tube adaptor. For sample volumes between 600 µL and 1.2 mL, use a 2.0-mL LoBind tube and the same adaptor as used for the 1.5-mL tubes. Place the tube collar at the indicator line of the adaptor.

Prepare the mini-scale emulsion PCR (ePCR) reaction

Prepare the oil phase

1. Use a *3-mL syringe* to dispense 1.8 mL of Emulsion Stabilizer 1 into the 50-mL conical tube.
2. Use a *1-mL syringe* to dispense 400 µL of Emulsion Stabilizer 2 very slowly into the 50-mL tube.
3. Pour the Emulsion Oil (approximately 37.8 mL) into the tube that has the Emulsion Stabilizer 1 and Emulsion Stabilizer 2 so that the final volume is 40 mL.
4. Cap the 50-mL tube, then vortex the mixture until all Emulsion Stabilizer 1 and Emulsion Stabilizer 2 are incorporated into the Emulsion oil.
5. Allow the mixture to degas for a minimum of 20 minutes while you prepare the aqueous phase (see "Prepare the aqueous phase"). To degas, place the mixture in a conical tube rack and slightly unscrew the conical tube cap.
6. Prime a 10-mL syringe by drawing in about 2 mL of oil phase then dispensing it back into the tube.
7. Take off the cap of a new SOLiD™ ePCR Tube. Use the primed 10-mL syringe to dispense 9 mL of oil phase into the SOLiD ePCR Tube, then cap the tube.

STOPPING POINT. The oil phase may be stored at 4 °C for up to 2 months. Before using the stored oil phase, thoroughly vortex and degas the solution for 20 minutes.

Prepare the aqueous phase

1. Dilute ePCR Primer 1 to prepare a 10-µM working stock solution. For each ePCR reaction, add 2 µL of ePCR Primer 1 to 18 µL of 1✕ Low TE Buffer. Mix well.
2. Using only 1✕ Low TE Buffer and LoBind tubes, prepare a dilution of the library template to a final concentration of 500 pM. Use Table 3 on page 16 to convert the mass/volume concentration to molar concentration for each library (for calculation details, see "Library Concentration Conversion" on page 109). Dilute only enough template for the desired number of emulsions. If needed, perform a serial dilution of the library to accurately obtain the desired library concentration. For example, perform a 5✕ dilution from 50 nM to 10 nM, then perform a 20✕ dilution from 10 nM to 500 pM.

Table 3 Concentration conversions by library type

Library Type	Molar Concentration (pM)	Mass/Volume Concentration
Fragment Library	500	60 pg/µL
Mate-Paired Library (2 × 25 bp)	500	50 pg/µL
Mate-Paired Library (2 × 50 bp)	500	96 pg/µL

IMPORTANT! Do not freeze-thaw dilutions of the library more than 3 to 4 times. Stock solutions and dilutions of libraries should be stored at – 20 °C at a concentration of 5 ng/µL or greater.

3. Choose the appropriate library concentration, then prepare the aqueous phase by combining the following reagents in a Nalgene wide-mouth jar according to the table below (see Table 4) For workflow analysis, prepare aqueous phase for library concentrations of 0.5 pM and 1.0 pM.

Table 4 Prepare the aqueous phase

		Library concentration		
Component	**Final concentration‡**	**0.5 pM**	**1.0 pM**	***X* pM**
		Volume per reaction (µL)§		
10× PCR Buffer	1×	280	280	280
dNTP Mix (100 mM mix comprised of 25 mM each dATP, dTTP, dCTP, dGTP)	14 mM (3.5 mM of each dNTP)	392	392	392
Magnesium Chloride (1 M)	25 mM	70	70	70
ePCR Primer 1 (10 µM working stock solution)	40 nM	11.2	11.2	11.2
ePCR Primer 2 (500 µM)	3 µM	16.8	16.8	16.8
Template (500 pM)	0.5 pM or 1.0 pM	2.8	5.6	$X \times 5.6$
Nuclease-free water	N/A	1647.2	1644.4	$1650 - (X \times 5.6)$
AmpliTaq Gold® DNA Polymerase, UP (5 U/µL)	0.54 U/µL	300	300	300
Total	N/A	2720	2720	2720

‡ The final concentration is based on a total volume of 2800 µL, which includes 2720 µL of liquid components and 80 µL of beads.
§ Volumes are for a single IKA®-based ePCR reaction to fill a 96-well plate.

4. Keep the aqueous phase on ice until ready to use.

Prepare the SOLiD™ P1 DNA Beads

1. Thoroughly vortex one tube of SOLiD™ P1 DNA Beads. Invert the tube at least once during vortexing to ensure that any beads stuck to the cap are washed down, then pulse-spin the tube.
2. Place the tube of beads in the magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
3. Resuspend the beads in 200 µL of Bead Block Solution. Vortex the solution to ensure that all beads are suspended, then pulse-spin the tube.

   IMPORTANT! Keep the Bead Block Solution at 4 °C until ready for use.

4. Sonicate the beads using the Bead Block Declump program on the Covaris S2 System (for program conditions, see "Bead Block Declump" on page 119), then pulse-spin the tube.
5. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
6. Resuspend the beads in 200 µL of 1× TEX Buffer and vortex to ensure that all beads are suspended, then pulse-spin the beads.

Create the emulsion with the ULTRA-TURRAX® Tube Drive from IKA®

1. Place the SOLiD™ ePCR Tube containing 9 mL of oil phase onto the ULTRA-TURRAX®device, then twist the tube to lock it into position (see Figure 9).

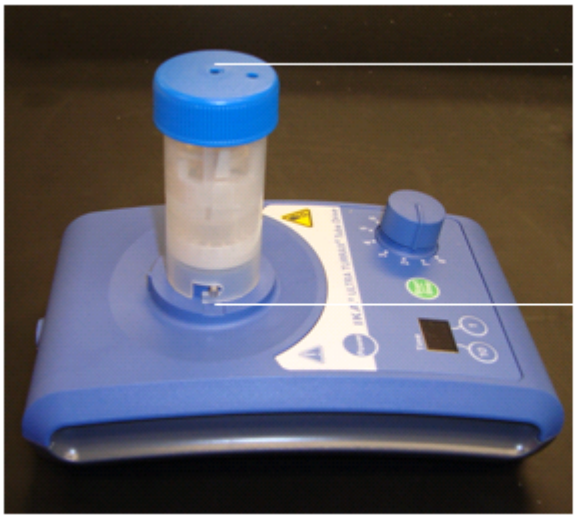


Figure 9 SOLiD™ ePCR Tube on the ULTRA-TURRAX® Tube Drive from IKA®.

2. Sonicate the SOLiD™ P1 DNA Beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin.

3. Immediately add 80 µL of the SOLiD™ P1 DNA Beads to the aqueous phase, then mix by gently swirling the bottle to ensure that the beads are uniformly dispersed (see Figure 10).

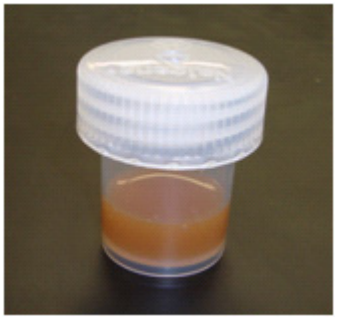


Figure 10 SOLiD™ P1 DNA Beads mixed in aqueous phase.

4. Verify that the Xstream pipettor is set up for mini-scale emulsions (see Figure 11):
   - Dial Setting: **Pip**
   - Speed (aspirate UP): **scale 5** (mid-range)
   - Speed (dispense DOWN): **scale 1** (slowest)
   - Total volume: **2.80 mL**

   If necessary, reprogram the Xstream pipettor (see "Program the Eppendorf Repeater® Xstream Pipettor" on page 94).

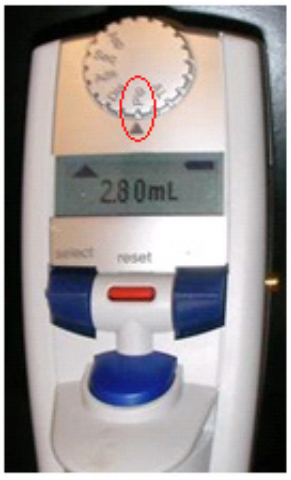


Figure 11 Xstream pipettor settings.

5. Attach a 10-mL Combitip Plus tip onto the Xstream pipettor.

6. Fill the 10-mL Combitip Plus tip with the entire 2.80 mL of aqueous phase and bead mixture with the Xstream pipettor (see Figure 12 on page 19).

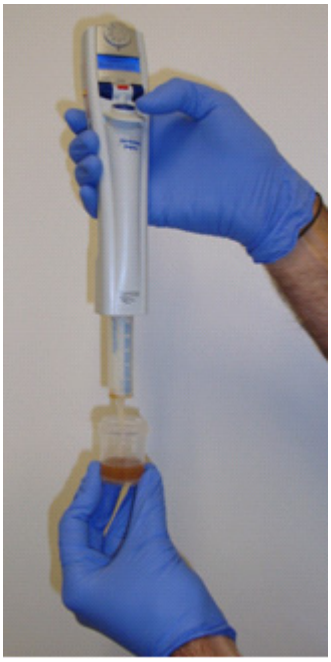


Figure 12 Filling the 10-mL Combitip Plus tip with the aqueous phase and bead mixture using the Xstream pipettor.

7. Verify the time on the ULTRA-TURRAX® Tube Drive from IKA® is set to **5** minutes, then press the **Start** button.

8. Wait for the instrument's fly wheel to engage and to reach proper speed, then gently place the Combitip Plus tip into the center sample loading hole in the ULTRA-TURRAX® cap (see Figure 13 on page 20).

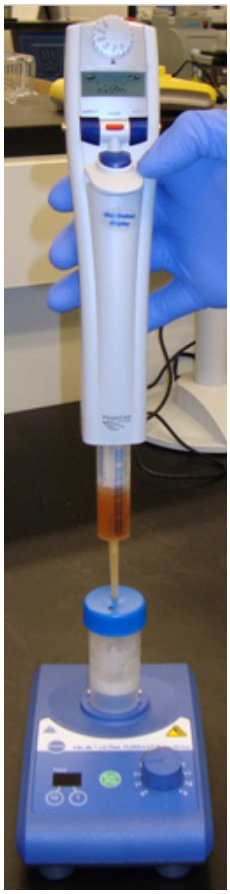


Figure 13 Correct placement of Combitip Plus into sample port in SOLiD™ ePCR Tube cap.

9. 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 contents from the Combitip Plus tip.

10. Remove a 5-mL Combitip Plus tip from its packaging, then cut off its end at the bevel with a razor blade (see Figure 14).

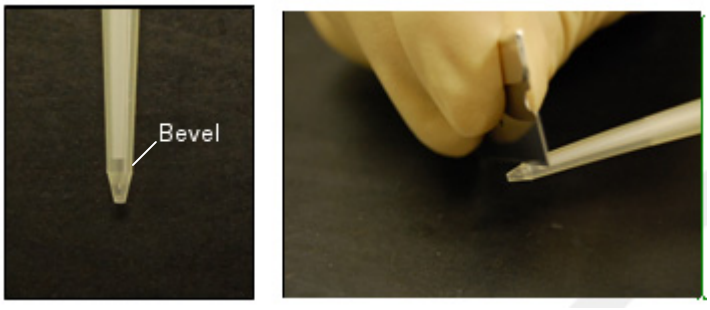


Figure 14 Cutting the Combitip Plus tip for emulsion dispersion.

11. Attach the cut Combitip Plus tip onto an Eppendorf Repeater® Plus Pipette.

12. Gently dispense 100 µL of emulsion into each well of a 96-well PCR plate, then seal the plate with clear adhesive film (see Figure 15).

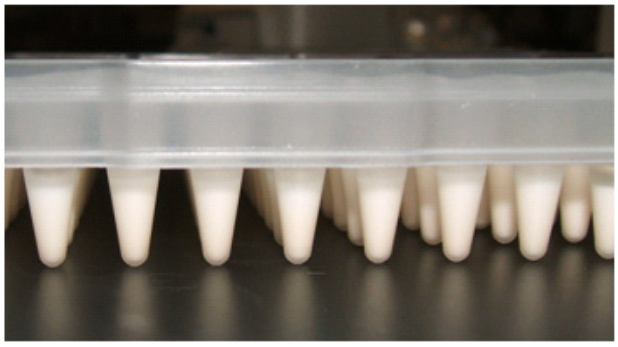


Figure 15 Emulsion transferred to a 96-well plate.

Perform the ePCR reaction and inspect the emulsion

1. Set up the ePCR conditions on the GeneAmp® PCR System 9700:
   - ePCR thermal cycling program:

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**

2. Place the 96-well plate in a GeneAmp® PCR System 9700, then start the run.

3. After the ePCR Program finishes, inspect the bottom of the reaction plate for beads that fell out of the emulsion. Beads appear as amber-colored specks at the bottom of a well. A small number of beads may fall out of emulsion and appear as small brown flecks at the bottom of a well. Applied Biosystems does not recommend any further processing of emulsions that have more than 3 wells of broken emulsion, where aqueous phase appears at the bottom of a well (see Figure 16 on page 22).

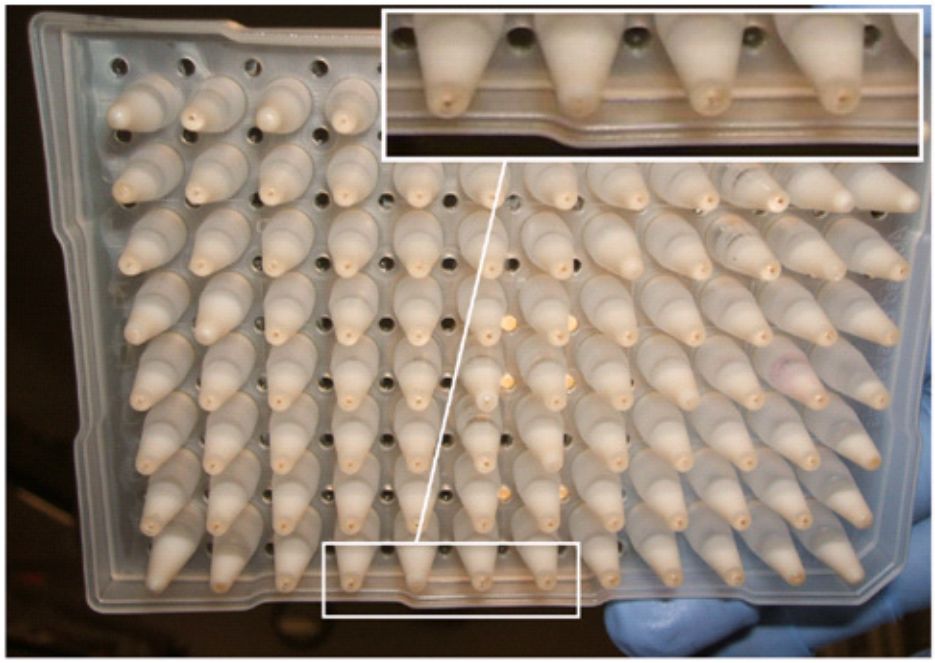


Figure 16 Broken emulsions.

STOPPING POINT. Store the 96-well plate at 4 °C, or proceed to "Perform emulsion break and bead wash" on page 23.

Perform emulsion break and bead wash

Break the emulsion

Note: An alternative method to break the emulsion can be found in "Break the emulsion with the SOLiD™ Emulsion Collection Tray" on page 95.

1. In a fume hood, fill a clean, labeled 50-mL reservoir with 2-butanol.
2. Using a multi-channel pipettor, transfer 100 µL of 2-butanol to each well of the 96-well plate containing the emulsion. Carefully pipette up and down 4 times to mix the 2-butanol into the emulsion.
3. Transfer all rows of the emulsion mix into a clean 50-mL reservoir. To obtain high bead yields, check the plate to ensure that all beads are transferred from the wells to the reservoir. If beads remain in the plate, rinse the wells with additional 2-butanol, then transfer the rinse to the reservoir.
4. Transfer all the emulsion and 2-butanol into a 50-mL conical polypropylene tube.
5. Rinse the reservoir with additional 2-butanol to ensure that all residual beads are recovered. Use this rinse volume to fill the conical tube to 30 mL, then discard the excess rinse volume.
6. Cap the tube and vortex to mix the solution.
7. Pellet the templated beads by centrifuging at 2000 × g for 5 minutes. Consult the manual specific to your centrifuge or rotor to convert g-forces to rpm.
8. Gently decant the 2-butanol-oil phase into a waste bottle. Keep the tube inverted, then place it onto paper towels to drain residual 2-butanol-oil. Wait 5 minutes to ensure that all the oil is removed.

   IMPORTANT! If the pellet begins to slide out, stop decanting, then remove the 2-butanol using a pipette.

Wash the templated beads

1. Place the 50-mL tube upright in a rack, then add 600 µL of 1X Bead Wash Buffer. Let the pellet soak in 1X Bead Wash Buffer for 2 minutes.
2. Resuspend the pellet by gently pipetting up and down, then transfer the beads from the 50-mL tube to a 1.5-mL LoBind tube.
3. Rinse the *bottom* of the 50-mL tube with an additional 600 µL of 1X Bead Wash Buffer, then transfer the wash to the 1.5-mL LoBind tube.
4. Vortex the 1.5-mL LoBind tube, then centrifuge the tube at 21,000 × g (minimum 14,000 × g) for 1 minute.
5. Remove the top oil phase with a pipette. Remove as much of the oil at the meniscus as possible.
6. With a new pipette tip, carefully remove and discard the supernatant.
7. Resuspend the pellet by adding 150 µL of 1X Bead Wash Buffer to the tube, then vortex the tube. Pulse-spin the tube, then transfer the mixture to a new 1.5-mL LoBind tube.
8. Rinse the *bottom* of the original tube with an additional 150 µL of 1X Bead Wash Buffer, then transfer the wash to the new tube.
9. Add 1 mL of 1X Bead Wash Buffer to the new tube, the vortex the tube.
10. Centrifuge the tube at 21,000 × g (minimum 14,000 × g) for 1 minute, then remove and discard the supernatant.
11. Resuspend the beads in 200 µL of 1X TEX Buffer.
12. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
13. Resuspend the beads in 200 µL of 1X TEX Buffer.

STOPPING POINT. Store the templated beads at 4 °C in 1X TEX Buffer, or proceed to "Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer" or "Quantitate the beads using a hemocytometer" on page 106.

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

1. If necessary, generate a standard curve (see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).
2. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin the beads.

3. Make a 1-mL dilution of beads in 1X TEX Buffer (1:10 dilution recommended) in a 1.5-mL LoBind tube.

4. Use the SOLiD™ Bead Concentration Chart (Applied Biosystems PN 4415131) to estimate the bead concentration of the beads (see Figure 17).

5. Adjust the volume of beads so that the color of the bead solution matches a color in the optimal range (750,000 beads/µL to 1.25 million beads/µL; see Figure 18).

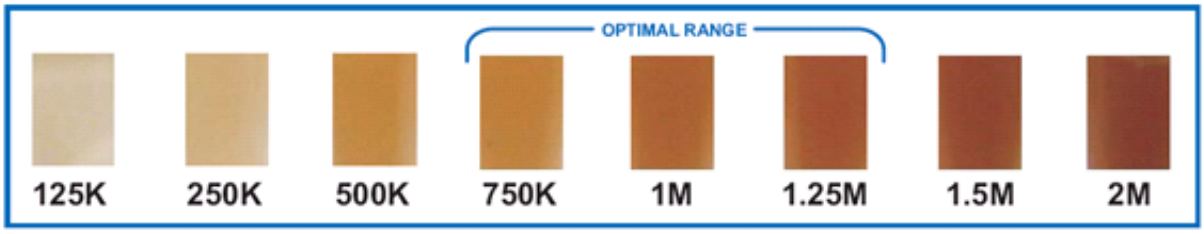


Figure 17 The SOLiD™ Bead Concentration Chart. For best results, use the SOLiD™ Bead Concentration Chart (PN 4415131), supplied separately.

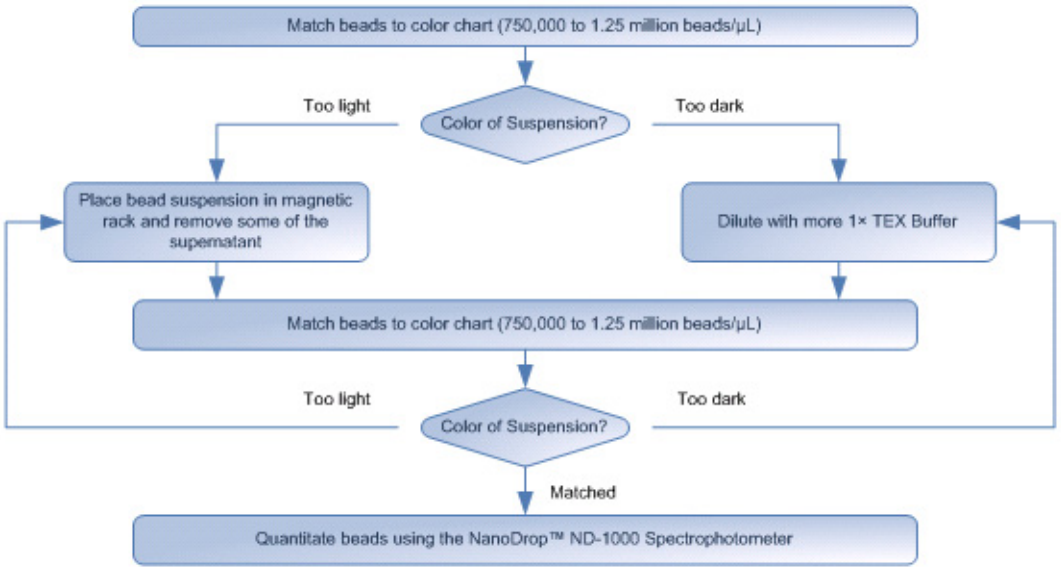


Figure 18 The SOLiD™ Bead Concentration Chart workflow.

6. When the bead concentration is within accurate range, quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer. Take 3 readings, then average them. Calculate the bead concentration using the appropriate standard curve (for more details, see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

7. Combine all diluted and undiluted beads.

STOPPING POINT. Store the beads at 4 °C in 1X TEX Buffer, or proceed to "Enrich for the templated beads" on page 26.

Enrich for the templated beads

Prepare the Denaturing Buffer solution

1. For each ePCR reaction, transfer 1.8 mL of Denaturing Buffer to a 15-mL conical tube.
2. Add 200 µL of Denaturant to the 1.8 mL of Denaturing Buffer, then cap the tube and vortex.

IMPORTANT! Prepare the prepared Denaturing Buffer solution fresh weekly.

Prepare 60% glycerol

1. With a 10-mL syringe, add 4 mL of nuclease-free water to a 15-mL conical tube.
2. With a 3-mL syringe, add 6 mL of glycerol to the nuclease-free water by dispensing 3 mL of glycerol twice with the syringe. Fill and dispense the glycerol slowly to ensure that the total volume of glycerol is dispensed.
3. Cap the tube, then vortex to mix the solution well.

IMPORTANT! Prepare the 60% glycerol fresh weekly.

Prepare the enrichment beads

1. Vortex the enrichment beads and immediately transfer 300 µL of the enrichment beads to a 1.5-mL LoBind tube.
2. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
3. Resuspend the enrichment beads in 900 µL of 1✕ Bind & Wash Buffer.
4. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
5. Repeat steps 3 and 4.
6. Resuspend the enrichment beads in 150 µL of 1✕ Bind & Wash Buffer.
7. Add 1.5 µL of 1 mM Enrichment Oligo to the tube of enrichment beads, then vortex and pulse-spin the tube.
8. Rotate the tube at room temperature for 30 minutes.
9. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
10. Resuspend the beads in 900 µL of 1✕ TEX Buffer.

11. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.

12. Repeat steps 10 and 11.

13. Resuspend the enrichment beads in 75 µL of 1✕ Low Salt Binding Buffer.

STOPPING POINT. Store the prepared enrichment beads at 4 °C in 1✕ Low Salt Binding Buffer, or proceed to "Prepare the templated beads for enrichment". Prepared enrichment beads should be used within one week of preparation

Prepare the templated beads for enrichment

1. Place the tube in the magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

2. Resuspend the templated beads in 300 µL of prepared Denaturing Buffer solution, then let the suspension stand for 1 minute.

3. Place the tube in the magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

4. Repeat steps 2 and 3.

5. Resuspend the templated beads in 300 µL of 1✕ TEX Buffer.

6. Place the tube in the magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

7. Repeat steps 5 and 6.

8. Resuspend the templated beads in 75 µL of 1✕ TEX Buffer, then transfer the templated bead suspension to a new 0.5-mL LoBind tube.

9. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119).

Enrich the templated beads

1. Transfer the prepared enrichment beads to the tube of templated beads, then vortex and pulse-spin the bead mixture.
2. Sonicate the enrichment-templated bead mixture using the Covalent Declump 3 program on the Covaris S2 System (for program conditions, see "Covalent Declump 3" on page 119), then pulse-spin the beads.
3. Incubate the bead mixture at 61 °C for 15 minutes. During the incubation, vortex and pulse-spin the bead mixture every 5 minutes including at the end of the incubation.
4. Immediately cool the beads on ice for 2 minutes.
5. Add 400 µL of *freshly prepared* 60% glycerol to a new 1.5-mL LoBind tube.
6. Gently pipette the bead mixture up and down to mix, then load the entire volume *carefully* on top of the 60% glycerol solution. Do *not* vortex the tube
7. Centrifuge the tubes for 3 minutes at 21,000 × g (minimum 14,000 × g).
8. Add 1 mL of 1X TEX Buffer to a new 2.0-mL LoBind tube.
9. Transfer the top layer of beads into the tube with 1X TEX Buffer. Aspirate as little glycerol as possible to collect all of the beads at the top layer without touching the un-templated beads at the bottom of the tube. When you dispense the top layer of beads into the 1X TEX Buffer, dispense the beads into the bottom of the tube. Aspirate a small amount of 1X TEX buffer to clean the pipette tip.
10. Top off the tube with additional 1X TEX Buffer to the 2.0-mL mark, then vortex.
11. Centrifuge the tube for 1 minute at 21,000 × g (minimum 14,000 × g).

Note: Verify that the beads are pelleted. Excess glycerol carried over to the 1X TEX Buffer creates a matrix that impedes pelleting of beads.

Proceed according to the table below (see Table 5):

Table 5 Steps for pelleted or unpelleted beads

If the beads are…	Then perform steps…
Pelleted	12 *and* 13
Not pelleted	14 *to* 16

12. Remove the supernatant. Add 400 µL of 1X TEX Buffer to the tube of beads and vortex.
13. Proceed to "Isolate the P2-enriched beads" on page 29.
14. Transfer half of the tube volume to a new 2.0-mL LoBind tube, then add an additional 500 µL of 1X TEX Buffer to each tube. Vortex each tube.

15. Centrifuge the tubes for 1 minute at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.

16. Add 200 µL of 1X TEX Buffer to each tube, resuspend the beads, then pool the beads into one tube.

Isolate the P2-enriched beads

1. Centrifuge the tube for 1 minute at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.

   IMPORTANT! *Never* magnet the P2-enriched beads before adding prepared Denaturing Buffer solution to the beads. If you do, the templated beads linked to the enrichment beads are lost when the supernatant is removed.

2. Resuspend the pellet with 400 µL of prepared Denaturing Buffer solution, then let the solution stand for 1 minute.

3. Place the tube in a magnetic rack for at least 1 minute until the supernatant is pure white or clear, then remove and discard the supernatant.

4. Repeat steps 2 and 3 until the supernatant is clear (all white enrichment beads have been removed).

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

6. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear. Remove and discard the supernatant.

7. Repeat steps 5 and 6.

8. Resuspend the beads in 200 µL of 1X TEX Buffer. Vortex, pulse-spin, then transfer the bead solution to a 1.5-mL LoBind tube.

9. Rinse the 2.0-mL tube with 200 µL of 1X TEX Buffer and transfer the rinse to the 1.5-mL LoBind tube.

10. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119). Pulse-spin the beads.

11. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

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

13. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

14. If the supernatant appears cloudy due to residual enrichment beads, repeat steps 12 and 13 until the supernatant is clear.

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

STOPPING POINT. Store the prepared enrichment beads at 4 °C in 1X TEX Buffer, or proceed to "Modify the 3′ ends".

Modify the 3′ ends

Extend the 3′ ends with Terminal Transferase and Bead Linker

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 (for program conditions, see "Covalent Declump 3" on page 119). Pulse-spin the beads.
2. For each ePCR reaction, prepare 500 µL of 1X Terminal Transferase Reaction Buffer (see Table 6):

Table 6 Prepare Terminal Transferase Reaction Buffer

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

Note: The 1X Terminal Transferase Reaction Buffer should be clear. If the solution becomes colored, discard then prepare fresh buffer using a new lot of material.

3. Add 1 µL of 50 mM Bead Linker to 49 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. After the supernatant clears, remove and discard the supernatant.
5. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer, then transfer the beads to a new 1.5-mL LoBind tube.
6. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
7. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer.
8. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
9. Resuspend the beads in 178 µL of 1X Terminal Transferase Reaction Buffer.
10. Add 20 µL of 1 mM Bead Linker solution.

11. Sonicate the beads using the Covalent Declump 3 program on the Covaris S2 System (for program conditions, see "Covalent Declump 3" on page 119). Pulse-spin the beads.

12. Add 2 µL of Terminal Transferase (20 U/µL) and vortex. Pulse-spin the beads.

13. Place the tube on a rotator and rotate for 2 hours at 37 °C.

14. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

15. Resuspend the beads in 400 µL of 1× TEX Buffer.

16. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

17. Resuspend the beads in 200 µL of 1× TEX Buffer.

STOPPING POINT. Store the templated beads at 4 °C in 1× TEX Buffer, or proceed to "Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer"or "Quantitate the beads using a hemocytometer" on page 106.

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

1. If necessary, generate a standard curve (see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

2. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin the beads.

3. Adjust the volume of beads so that the color of the bead solution matches a color in the optimal range (750,000 beads/µL to 1.25 million beads/µL; see Figure 19 and Figure 20 on page 32).

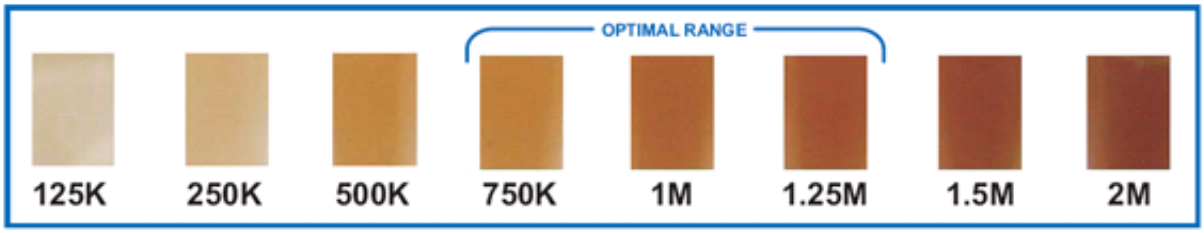


Figure 19 The SOLiD™ Bead Concentration Chart. For best results, use the SOliD™ Bead Concentration Chart (PN 4415131), supplied separately.

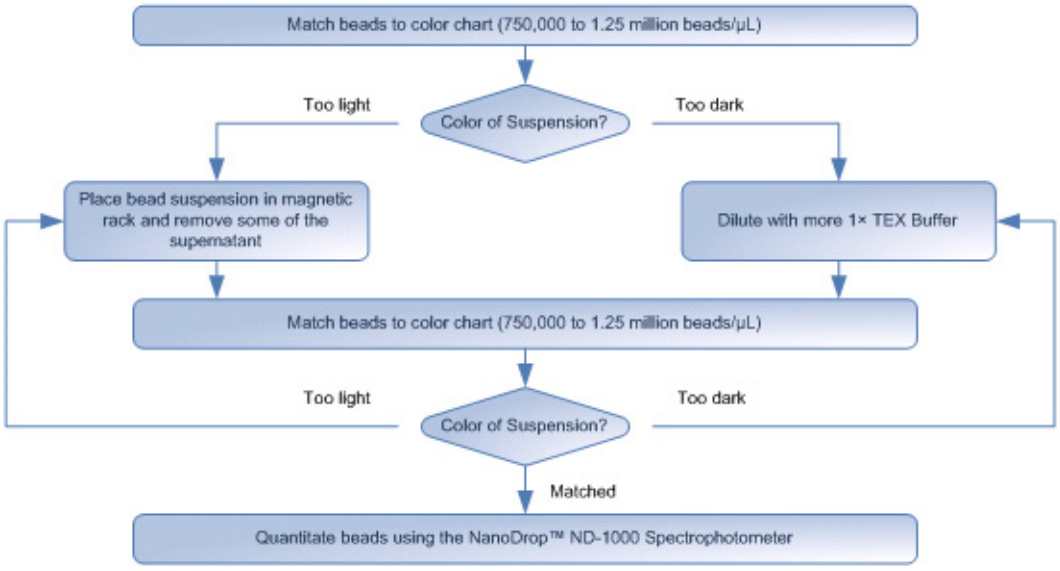


Figure 20 The SOLiD™ Bead Concentration Chart workflow.

4. When the bead concentration is within accurate range, quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer. Take 3 readings, then average them. Calculate the bead concentration using the appropriate standard curve (for more details, see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

STOPPING POINT. Store the templated beads at 4 °C in 1X TEX Buffer, or proceed to bead deposition and sequencing [refer to the *Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Guide* (PN 4442357)].

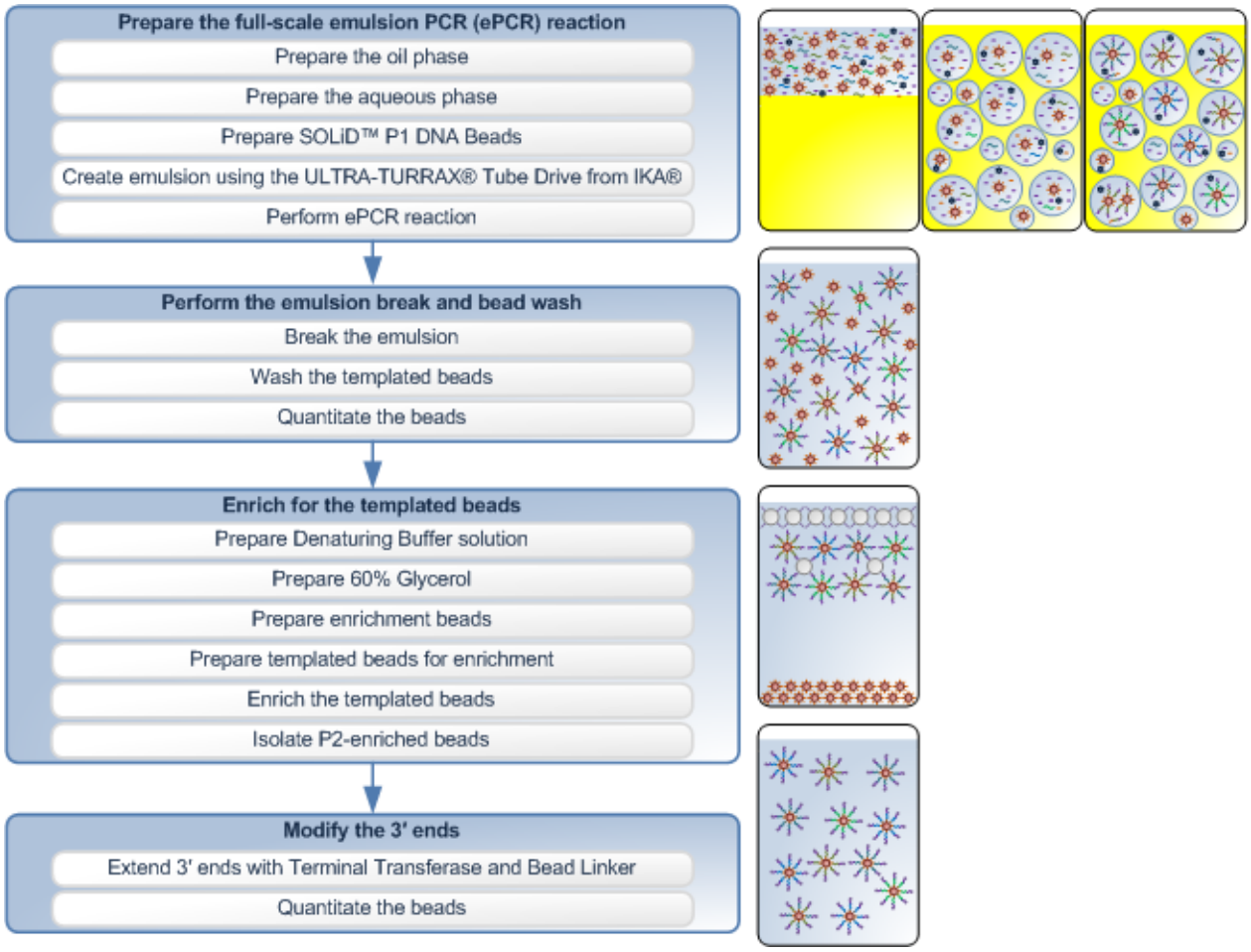
Section 2.2 Prepare templated beads (full-scale)

Materials and equipment required

See Appendix A on page 77 for a list of equipment, kits, and consumables necessary for this procedure.

Workflow

See the descriptions of the steps below the workflow.



Prepare the full-scale emulsion PCR (ePCR) reaction

The oil phase and aqueous phase of the emulsion are prepared separately, then emulsified using the ULTRA-TURRAX™ Tube Drive from IKA®. Each emulsion is seeded with 1.6 billion SOLiD™ P1 DNA Beads, then transferred into a single, 96-well plate for cycling. Different library template lengths require different numbers of cycles for thermal cycling.

Perform the emulsion break and bead wash

The emulsion break uses 2-butanol to purify emulsified templated beads from the oil phase following amplification. The beads are washed to remove residual 2-butanol, oil, and aqueous phase containing PCR reagents. There are two methods available to break the emulsion. In the *standard* method, a multi-channel pipettor is used to add and mix 2-butanol into the emulsion in each well of the 96-well plate. The pipettor is then used to transfer the solution into a 50-mL reservoir. In the *alternative* method, the SOLiD™ Emulsion Collection Tray is placed over the 96-well plate, then the plate is centrifuged. Centrifuging the plate forces the emulsion from each well to a single reservoir. After centrifugation, 2-butanol is added to the reservoir. For both methods, the broken emulsion is transferred to a 50-mL tube for further processing.

Enrich for the templated beads

The templated bead enrichment procedure isolates beads with full-length extension products following ePCR. Beads with full-length extension products are isolated by oligo hybridization using the sequence of the P2 primer. Both monoclonal and polyclonal beads are enriched. The procedure is designed to enrich for templated beads derived from one full-scale ePCR reaction yielding 150 to 300 million templated beads.

Modify the 3′ ends

The P2-enriched beads are extended with a Bead Linker by Terminal Transferase.

Tips

General

- Syringes are required to accurately measure viscous reagents. Aspirate the volume very slowly from the reagent bottle to ensure that no air bubbles are trapped within the syringe. The best practice is to draw some reagent into the syringe, dispense the entire reagent back to the reagent bottle, then draw the correct volume of reagent. The volume should be measured to the point where the plunger contacts the side of the syringe.
- Perform all steps requiring 0.5-mL, 1.5-mL, and 2.0-mL tubes with Eppendorf LoBind tubes.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols. Applied Biosystems recommends the Eppendorf 5417R tabletop microcentrifuge.

SOLiD™ P1 DNA Beads

- Do not freeze SOLiD™ P1 DNA Beads or templated beads. Store the SOLiD™ P1 DNA Beads at 4 °C in 1✕ TEX Buffer.
- If beads remain in the original tube after transfer, you can use a small additional volume of the appropriate buffer to recover the remaining beads. Do not exceed a total volume of 1.3 mL for a 1.5-mL LoBind tube.

Covaris™ S2 System

- The procedures are optimized for the Covaris™ S2 System. The Covaris S2 System must be specially adapted to prepare beads for the Applied Biosystems SOLiD™ 3 Plus System. Do not use the Covaris S1 sonicator or an unadapted Covaris S2 System for bead preparation. For more information, contact an Applied Biosystems SOLiD™ System applications specialist.
- Ensure that the Covaris™ S2 System is degassed, that no bubbles are present in the system, and that the instrument and tube are properly aligned for appropriate sonication of beads.
- To ensure optimal sonication by the Covaris™ S2 System, use the appropriate adaptor with the Covaris S2 System. For sample volumes ≤ 200 µL, use a 0.5-mL LoBind tube and 0.65-mL tube adaptor. For sample volumes between 200 µL and 600 µL, use a 1.5-mL LoBind tube and 1.5-mL tube adaptor. For sample volumes between 600 µL and 1.2 mL, use a 2.0-mL LoBind tube and the same adaptor as used for the 1.5-mL tubes. Place the tube collar at the indicator line of the adaptor.

Prepare the full-scale emulsion PCR (ePCR) reaction

Prepare the oil phase

1. Use a *3-mL syringe* to dispense 1.8 mL of Emulsion Stabilizer 1 into the 50-mL conical tube.
2. Use a *1-mL syringe* to dispense 400 µL of Emulsion Stabilizer 2 very slowly into the 50-mL tube.
3. Pour the Emulsion Oil (approximately 37.8 mL) into the tube that has the Emulsion Stabilizer 1 and Emulsion Stabilizer 2 so that the final volume is 40 mL.
4. Cap the 50-mL tube, then vortex until all Emulsion Stabilizer 1 and Emulsion Stabilizer 2 are incorporated into the Emulsion oil.
5. Allow the mixture to degas for a minimum of 20 minutes while you prepare the aqueous phase (see "Prepare the aqueous phase"). To degas, place the mixture in a conical tube rack and slightly unscrew the conical tube cap.
6. Prime a 10-mL syringe by drawing in about 2 mL of oil phase then dispensing it back into the tube.
7. Take off the cap of a new SOLiD™ ePCR Tube. Use the primed 10-mL syringe to dispense 9 mL of oil phase into the SOLiD ePCR Tube, then cap the tube.

STOPPING POINT. The oil phase may be stored at 4 °C for up to 2 months. Before using the stored oil phase, thoroughly vortex and degas the solution for 20 minutes.

Prepare the aqueous phase

1. Dilute ePCR Primer 1 to prepare a 10-µM working stock solution. For each ePCR reaction, add 4 µL of ePCR Primer 1 to 36 µL of 1✕ Low TE buffer. Mix well.
2. Using only 1✕ Low TE Buffer and LoBind tubes, prepare a dilution of the library template to a final concentration of 500 pM. Use Table 7 on page 37 to convert the mass/volume concentration to molar concentration for each library (for calculation details, see "Library Concentration Conversion" on page 109). Dilute only enough template for the desired number of emulsions. If needed, perform a serial dilution of the library to accurately obtain the desired library concentration. For example, perform a 5✕ dilution from 50 nM to 10 nM, then perform a 20✕ dilution from 10 nM to 500 pM.

Table 7 Concentration conversions by library type

Library Type	Molar Concentration (pM)	Mass/Volume Concentration
Fragment Library	500	60 pg/µL
Mate-Paired Library (2 × 25 bp)	500	50 pg/µL
Mate-Paired Library (2 × 50 bp)	500	96 pg/µL

IMPORTANT! Do not freeze-thaw dilutions of the library more than 3 to 4 times. Stock solutions and dilutions of libraries should be stored at – 20 °C at a concentration of 5 ng/µL or greater.

3. Choose the appropriate library concentration, then prepare the aqueous phase by combining the following reagents in a Nalgene wide-mouth jar according to the table below (see Table 8) For workflow analysis, prepare aqueous phase for library concentrations of 0.5 pM and 1.0 pM.

Table 8 Prepare the aqueous phase

		Library concentration		
Component	**Final concentration‡**	**0.5 pM**	**1.0 pM**	***X* pM**
		Volume per reaction (µL)§		
10× PCR Buffer	1×	560	560	560
dNTP Mix (100 mM mix comprised of 25 mM each dATP, dTTP, dCTP, dGTP)	14 mM (3.5 mM of each dNTP)	784	784	784
Magnesium Chloride (1 M)	25 mM	140	140	140
ePCR Primer 1 (10 µM working stock solution)	40 nM	22.4	22.4	22.4
ePCR Primer 2 (500 µM)	3 µM	33.6	33.6	33.6
Template (500 pM)	0.5 pM or 1.0 pM	5.6	11.2	$X \times 11.2$
Nuclease-free water	—	3294.4	3288.8	$3300 - (X \times 11.2)$
AmpliTaq Gold® DNA Polymerase, UP (5 U/µL)	0.54 U/µL	600	600	600
Total	—	5440	5440	5440

‡ The final concentration is based on a total volume of 5600 µL, which includes 5440 µL of liquid components and 160 µL of beads.
§ Volumes below are for a single IKA®-based ePCR reaction to fill a 96-well plate.

4. Keep the aqueous phase on ice until ready to use.

Prepare the SOLiD™ P1 DNA Beads

1. Thoroughly vortex one tube of SOLiD™ P1 DNA Beads. Invert the tube at least once during vortexing to ensure that any beads stuck to the cap are washed down, then pulse-spin the tube.
2. Place the tube of beads in the magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
3. Resuspend the beads in 200 µL of Bead Block Solution. Vortex the solution to ensure that all beads are suspended, then pulse-spin the tube.

   IMPORTANT! Keep the Bead Block Solution at 4 °C until ready for use.

4. Sonicate the beads using the Bead Block Declump program on the Covaris S2 System (for program conditions, see "Bead Block Declump" on page 119), then pulse-spin the tube.
5. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
6. Resuspend the beads in 200 µL of 1X TEX Buffer and vortex to ensure that all beads are suspended, then pulse-spin the beads.

Create the emulsion with the ULTRA-TURRAX® Tube Drive from IKA®

1. Place the SOLiD™ ePCR Tube containing 9 mL of oil phase onto the ULTRA-TURRAX®device, then twist the tube to lock it into position (see Figure 21).

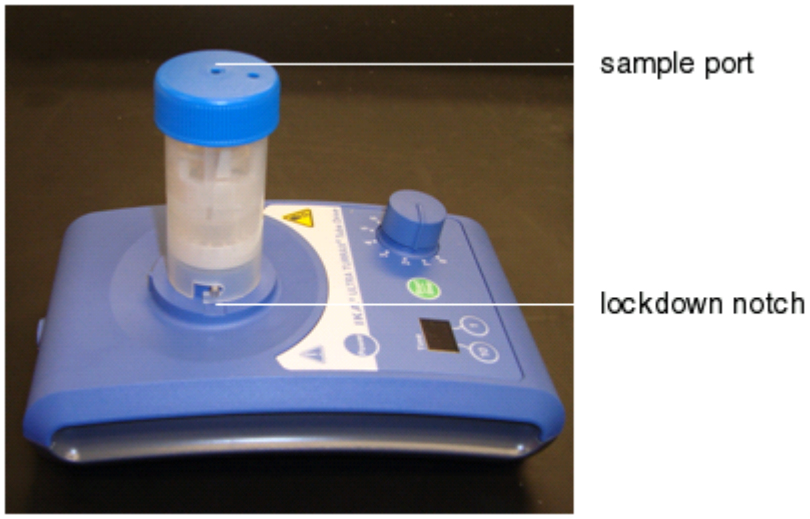


Figure 21 SOLiD™ ePCR Tube on the ULTRA-TURRAX® Tube Drive from IKA®.

2. Sonicate the SOLiD™ P1 DNA beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin the beads.

3. Immediately add 160 µL of the SOLiD™ P1 DNA Beads to the aqueous phase, then mix by gently swirling the bottle to ensure that the beads are uniformly dispersed (see Figure 22).

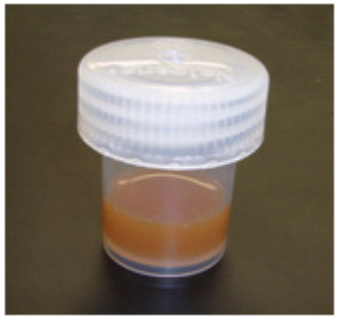


Figure 22 SOLiD™ P1 DNA Beads mixed in aqueous phase.

4. Verify that the Xstream pipettor is set up for full-scale emulsions (see Figure 23):
   - Dial Setting: **Pip**
   - Speed (aspirate UP): **scale 5** (mid-range)
   - Speed (dispense DOWN): **scale 1** (slowest)
   - Total volume: **5.60 mL**

   If necessary, reprogram the Xstream pipettor (see "Program the Eppendorf Repeater® Xstream Pipettor" on page 94).

Figure 23 Xstream pipettor settings.

5. Attach a 10-mL Combitip Plus tip onto the Xstream pipettor.

6. Fill the 10-mL Combitip Plus tip with the entire 5.60 mL of aqueous phase and bead mixture with the Xstream pipettor (see Figure 24 on page 40).

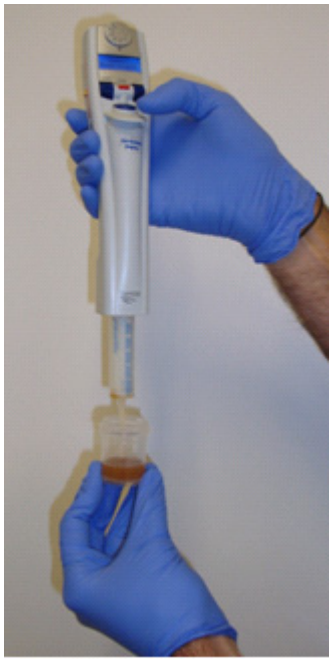


Figure 24 Filling the 10-mL Combitip Plus tip with the aqueous phase and bead mixture using the Xstream pipettor.

7. Verify the time on the he ULTRA-TURRAX® Tube Drive from IKA® is set to **5** minutes, then press the **Start** button.

8. Wait for the instrument's fly wheel to engage and to reach proper speed, then gently place the Combitip Plus tip into the center sample loading hole in the ULTRA-TURRAX® cap (see Figure 25 on page 41).

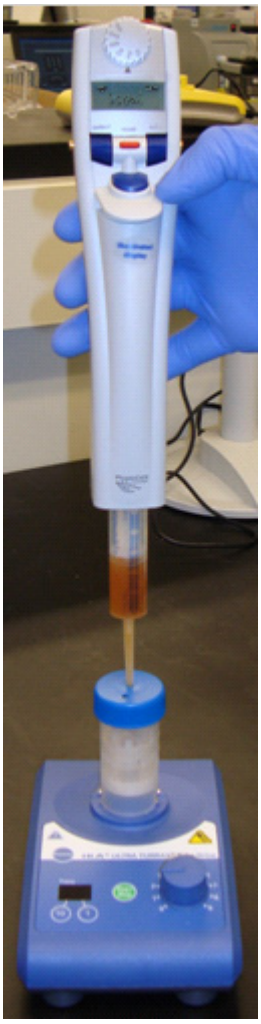


Figure 25 Correct placement of Combitip Plus into sample port in SOLiD™ ePCR Tube cap.

9. 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 contents from the Combitip Plus tip.

10. Remove a 5-mL Combitip Plus tip from its packaging, then cut off its end at the bevel with a razor blade (see Figure 26 on page 42).

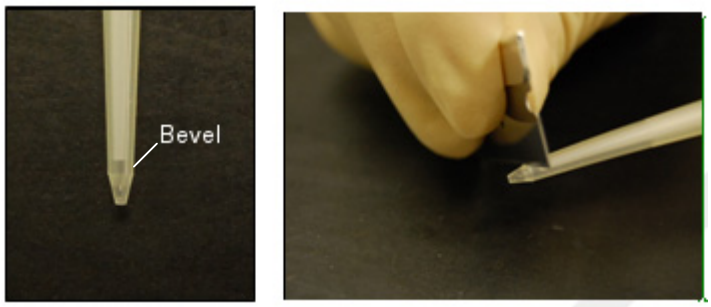


Figure 26 Cutting the Combitip Plus tip for emulsion dispersion.

11. Attach the cut Combitip Plus tip onto an Eppendorf Repeater® Plus Pipette.

12. Gently dispense 150 µL of emulsion into each well of a 96-well PCR plate, then seal the plate with clear adhesive film (see Figure 27).

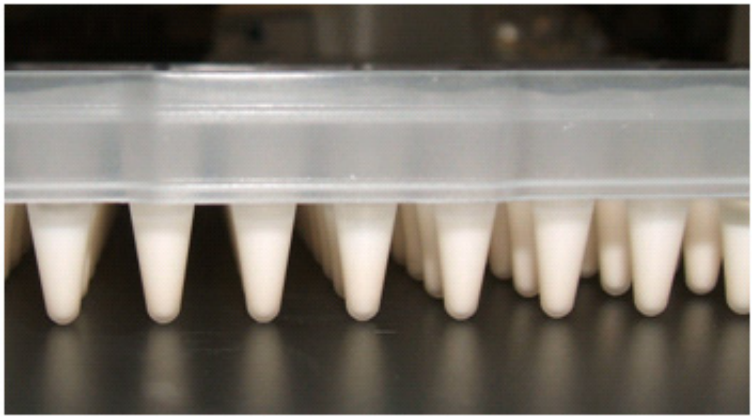


Figure 27 Emulsion transferred to a 96-well plate.

Perform the ePCR and inspect the emulsion

1. Set up the ePCR conditions on the GeneAmp® PCR System 9700:
   - ePCR thermal cycling program:

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**

2. Place the 96-well plate in a GeneAmp® PCR System 9700, then start the run.

3. After the ePCR Program finishes, inspect the bottom of the reaction plate for beads that fell out of the emulsion. Beads appear as amber-colored specks at the bottom of a well. A small number of beads may fall out of emulsion and appear as small brown flecks at the bottom of a well. Applied Biosystems does not recommend any further processing of emulsions that have more than 3 wells of broken emulsion, where aqueous phase appears at the bottom of a well (see Figure 28).

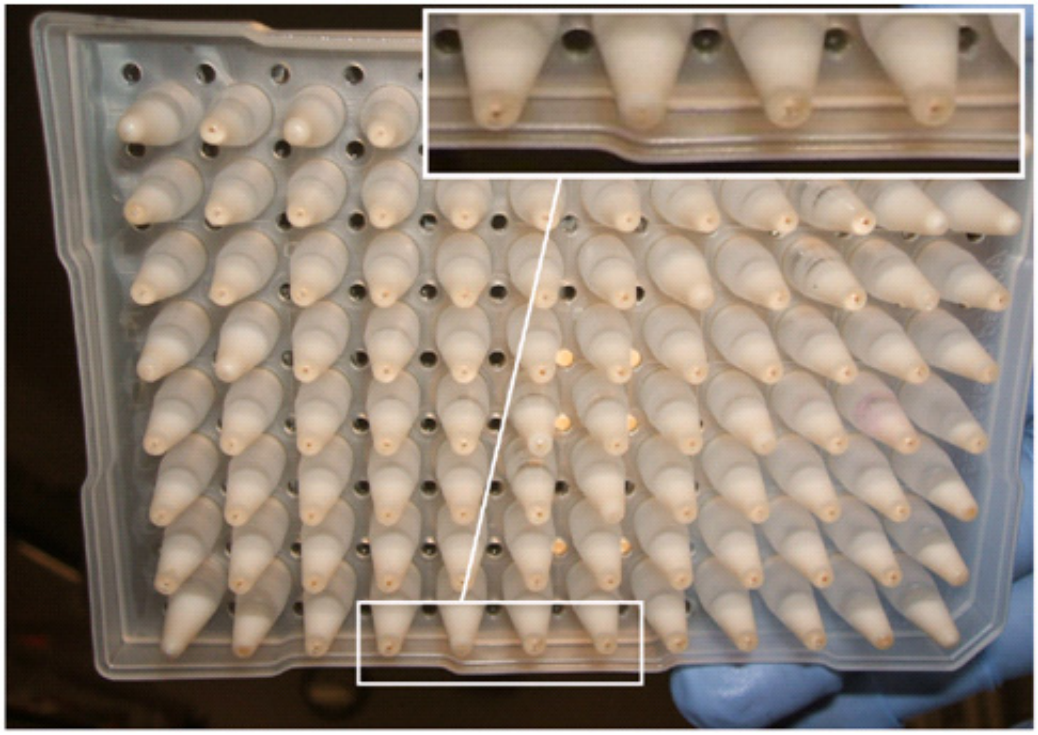


Figure 28 Broken emulsions.

STOPPING POINT. Store the 96-well plate at 4 °C, or proceed to "Perform emulsion break and bead wash" on page 44.

Perform emulsion break and bead wash

Break the emulsion

Note: An alternative method to break the emulsion can be found in "Break the emulsion with the SOLiD™ Emulsion Collection Tray" on page 95.

1. In a fume hood, fill a clean, labeled 50-mL reservoir with 2-butanol.
2. Using a multi-channel pipettor, transfer 100 µL of 2-butanol to each well of the 96-well plate containing the emulsion. Carefully pipette up and down 4 times to mix the 2-butanol into the emulsion.
3. Transfer all rows of the emulsion mix into a clean 50-mL reservoir. To obtain high bead yields, check the plate to ensure that all beads are transferred from the wells to the reservoir. If beads remain in the plate, rinse the wells with additional 2-butanol, then transfer the rinse to the reservoir.
4. Transfer all the emulsion and 2-butanol into a 50-mL conical polypropylene tube.
5. Rinse the reservoir with additional 2-butanol to ensure that all residual beads are recovered. Use this rinse volume to fill the conical tube to 30 mL, then discard the excess rinse volume.
6. Cap the tube and vortex to mix the solution.
7. Pellet the templated beads by centrifuging at 2000 × g for 5 minutes. Consult the manual specific to your centrifuge or rotor to convert g-forces to rpm.
8. Gently decant the 2-butanol-oil phase into a waste bottle, Keep the tube inverted, then place it onto paper towels to drain residual 2-butanol-oil. Wait 5 minutes to ensure that all the oil is removed.

   IMPORTANT! If the pellet begins to slide out, stop decanting, then remove the 2-butanol using a pipette.

Wash the templated beads

1. Place the 50-mL tube upright in a rack, then add 600 µL of 1X Bead Wash Buffer. Let the pellet soak in 1X Bead Wash Buffer for 2 minutes.
2. Resuspend the pellet by gently pipetting up and down, then transfer the beads from the 50-mL tube to a 1.5-mL LoBind tube.
3. Rinse the *bottom* of the 50-mL tube with an additional 600 µL of 1X Bead Wash Buffer, then transfer the wash to the 1.5-mL LoBind tube.
4. Vortex the 1.5-mL LoBind tube, then centrifuge the tube at 21,000 × g (minimum 14,000 × g) for 1 minute.
5. Remove the top oil phase with a pipette. Remove as much of the oil at the meniscus as possible.
6. With a new pipette tip, carefully remove and discard the supernatant.
7. Resuspend the pellet by adding 150 µL of 1X Bead Wash Buffer to the tube, then vortex the tube. Pulse-spin the tube, then transfer the mixture to a new 1.5-mL LoBind tube.
8. Rinse the *bottom* of the original tube with an additional 150 µL of 1X Bead Wash Buffer, then transfer the wash to the new tube.
9. Add 1 mL of 1X Bead Wash Buffer to the new tube, then vortex the tube.
10. Centrifuge the tube at 21,000 × g (minimum 14,000 × g) for 1 minute, then remove and discard the supernatant.
11. Resuspend the beads in 200 µL of 1X TEX Buffer.
12. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
13. Resuspend the beads in 200 µL of 1X TEX Buffer.

STOPPING POINT. Store the templated beads at 4 °C in 1X TEX Buffer, or proceed to "Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer" or to "Quantitate the beads using a hemocytometer" on page 106.

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

1. If necessary, generate a standard curve (see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).
2. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin the beads.

3. Make a 1-mL dilution of beads in 1✕ TEX Buffer (1:10 dilution recommended) in a 1.5-mL LoBind tube.

4. Use the SOLiD™ Bead Concentration Chart (Applied Biosystems PN 4415131) to estimate the bead concentration of the beads (see Figure 29).

5. Adjust the volume of beads so that the color of the bead solution matches a color in the optimal range (750,000 beads/µL to 1.25 million beads/µL; see Figure 29 and Figure 30).

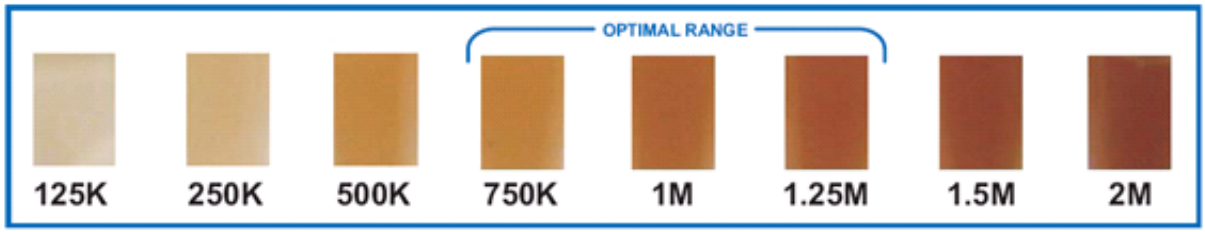


Figure 29 The SOLiD™ Bead Concentration Chart. For best results, use the SOLiD™ Bead Concentration Chart (PN 4415131), supplied separately.

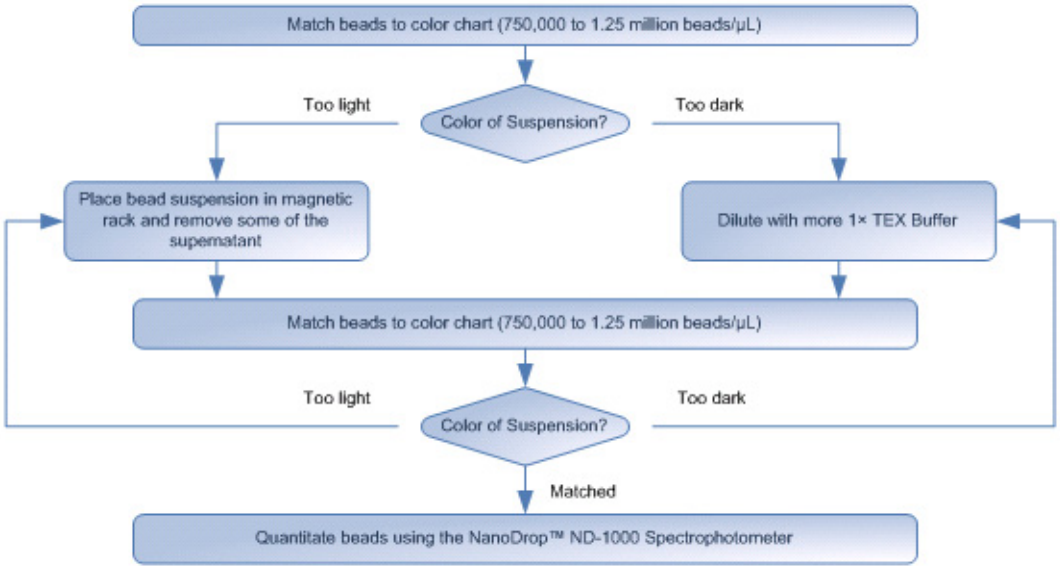


Figure 30 The SOLiD™ Bead Concentration Chart workflow.

6. When the bead concentration is within accurate range, quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer. Take 3 readings, then average them. Calculate the bead concentration using the appropriate standard curve (for more details, see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

7. Combine all diluted and undiluted beads.

STOPPING POINT. Store the beads at 4 °C in 1× TEX Buffer, or proceed to "Enrich for the templated beads" on page 48.

Enrich for the templated beads

Prepare the Denaturing Buffer solution

1. For each ePCR reaction, transfer 1.8 mL of Denaturing Buffer to a 15-mL conical tube.
2. Add 200 µL of Denaturant to the 1.8 mL of Denaturing Buffer, then cap the tube and vortex.

IMPORTANT! Prepare the prepared Denaturing Buffer solution fresh weekly.

Prepare 60% glycerol

1. With a 10-mL syringe, add 4 mL of nuclease-free water to a 15-mL conical tube.
2. With a 3-mL syringe, add 6 mL glycerol to the nuclease-free water by dispensing 3 mL glycerol twice with the syringe. Fill and dispense the glycerol slowly to ensure that the total volume of glycerol is dispensed.
3. Cap the tube, then vortex to mix the solution well.

IMPORTANT! Prepare the 60% glycerol fresh weekly.

Prepare the enrichment beads

1. Vortex the enrichment beads and immediately transfer 650 µL of the enrichment beads to a 1.5-mL LoBind tube.
2. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
3. Resuspend the enrichment beads in 900 µL of 1✕ Bind & Wash Buffer.
4. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
5. Repeat steps 3 and 4.
6. Resuspend the enrichment beads in 350 µL of 1✕ Bind & Wash Buffer.
7. Add 3.5 µL of 1 mM Enrichment Oligo to the tube of enrichment beads, then vortex and pulse-spin the tube.
8. Rotate the tube at room temperature for 30 minutes.
9. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove the supernatant.
10. Resuspend the enrichment beads in 900 µL of 1✕ TEX Buffer.

11. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.

12. Repeat steps 10 and 11.

13. Resuspend the enrichment beads in 150 µL of 1✕ Low Salt Binding Buffer.

STOPPING POINT. Store the prepared enrichment beads at 4 °C in 1✕ Low Salt Binding Buffer, or proceed to "Prepare the templated beads for enrichment". Prepared enrichment beads should be used within one week of preparation.

Prepare the templated beads for enrichment

1. Place the tube in the magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

2. Resuspend the templated beads in 300 µL of prepared Denaturing Buffer solution, then let the suspension stand for 1 minute.

3. Place the tube in the magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

4. Repeat steps 2 and 3.

5. Resuspend the templated beads in 300 µL of 1✕ TEX Buffer.

6. Place the tube in the magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

7. Repeat steps 5 and 6.

8. Resuspend the templated beads in 150 µL of 1✕ TEX Buffer, then transfer the templated bead suspension to a new 0.5-mL LoBind tube.

9. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119).

Enrich for the templated beads

1. Transfer the prepared enrichment beads to the tube of templated beads, then vortex and pulse-spin the bead mixture.
2. Sonicate the enrichment-templated bead mixture using the Covalent Declump 3 program on the Covaris S2 System (for program conditions, see "Covalent Declump 3" on page 119), then pulse-spin the beads.
3. Incubate the bead mixture at 61 °C for 15 minutes. During the incubation, vortex and pulse-spin the bead mixture every 5 minutes including at the end of the incubation.
4. Immediately cool the beads on ice for 2 minutes.
5. Add 600 µL of *freshly prepared* 60% glycerol to a new 1.5-mL LoBind tube.
6. Gently pipette the bead mixture up and down the beads to mix, then load the entire volume *carefully* on top of the 60% glycerol solution. Do *not* vortex the tube
7. Centrifuge the tubes for 3 minutes at 21,000 × g (minimum 14,000 × g).
8. Add 1 mL of 1X TEX Buffer to a new 2.0-mL LoBind tube.
9. Transfer the top layer of beads into the tube with 1X TEX Buffer. Aspirate as little glycerol as possible to collect all of the beads at the top layer without touching the un-templated beads at the bottom of the tube. When you dispense the top layer of beads into the 1X TEX Buffer, dispense the beads into the bottom of the tube. Aspirate a small amount of 1X TEX buffer to clean the pipette tip.
10. Top off the tube with additional 1X TEX Buffer to the 2.0-mL mark, then vortex.
11. Centrifuge the tube for 1 minute at 21,000 × g (minimum 14,000 × g).

Note: Verify the beads are pelleted in case excess glycerol carried over to the 1X TEX Buffer creates a matrix that impedes pelleting of beads.

Proceed according to the table below (Table 9)

Table 9 Steps for pelleted or unpelleted beads

If the beads are…	Then perform steps…
Pelleted	12 *and* 13
Not pelleted	14 *to* 16

12. Remove the supernatant. Add 400 µL of 1X TEX Buffer to the tube of beads and vortex.
13. Proceed to "Isolate the P2-enriched beads" on page 51.
14. Transfer half of the tube volume to a new 2.0-mL LoBind tube, then add an additional 500 µL of 1X TEX Buffer to each tube. Vortex each tube.

15. Centrifuge the tubes for 1 minute at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.

16. Add 200 µL of 1X TEX Buffer to each tube, resuspend the beads, then pool the beads into one tube.

Isolate the P2-enriched beads

1. Centrifuge the tube for 1 minute at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.

   IMPORTANT! *Never* magnet the P2-enriched beads before adding prepared Denaturing Buffer solution to the beads. If you do, the templated beads linked to the enrichment beads are lost when the supernatant is removed.

2. Resuspend the pellet with 400 µL of prepared Denaturing Buffer solution, then let stand for 1 minute.

3. Place the tube in a magnetic rack for at least 1 minute until the supernatant is pure white or clear, then remove and discard the supernatant.

4. Repeat steps 2 and 3 until the supernatant is clear (all white enrichment beads have been removed).

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

6. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear. Remove and discard the supernatant.

7. Repeat steps 5 and 6.

8. Resuspend the beads in 200 µL 1X TEX Buffer. Vortex, pulse-spin, then transfer the bead solution to a 1.5-mL LoBind tube.

9. Rinse the 2.0-mL tube with 200 µL 1X TEX Buffer and transfer the rinse to the 1.5-mL LoBind tube.

10. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119). Pulse-spin the beads.

11. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

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

13. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

14. If the supernatant appears cloudy due to residual enrichment beads, repeat steps 12 and 13 until the supernatant is clear.

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

STOPPING POINT. Store the prepared enrichment beads at 4 °C in 1X TEX Buffer, or proceed to "Modify the 3′ ends".

Modify the 3′ ends

Extend the 3′ ends with Terminal Transferase and Bead Linker

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 (for program conditions, see "Covalent Declump 3" on page 119). Pulse-spin the beads.
2. For each ePCR reaction, prepare 500 µL of 1X Terminal Transferase Reaction Buffer according to Table 10:

Table 10 Prepare 1× Terminal Transferase Reaction Buffer

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

Note: The 1X Terminal Transferase Reaction Buffer should be clear. If the solution becomes colored, discard then prepare fresh buffer using a new lot of material.

3. Add 1 µL of 50 mM Bead Linker to 49 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. After the supernatant clears, remove and discard the supernatant.
5. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer, then transfer the beads to a new 1.5-mL LoBind tube.
6. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
7. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer.
8. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
9. Resuspend the beads in 178 µL of 1X Terminal Transferase Reaction Buffer.
10. Add 20 µL of 1 mM Bead Linker solution.

11. Sonicate the beads using the Covalent Declump 3 program on the Covaris S2 System (for program conditions, see "Covalent Declump 3" on page 119). Pulse-spin the beads.

12. Add 2 µL of Terminal Transferase (20 U/µL) and vortex. Pulse-spin the beads.

13. Place the tube on a rotator and rotate for 2 hours at 37 °C.

14. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

15. Resuspend the beads in 400 µL of 1× TEX Buffer.

16. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

17. Resuspend the beads in 400 µL of 1× TEX Buffer.

STOPPING POINT. Store the templated beads at 4 °C in 1× TEX Buffer, or proceed to "Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer" or "Quantitate the beads using a hemocytometer" on page 106.

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

1. If necessary, generate a standard curve (see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

2. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin the beads.

3. Use the SOLiD™ Bead Concentration Chart (Applied Biosystems PN 4415131) to estimate the bead concentration of the beads (see Figure 31).

4. Adjust the volume of beads so that the color of the bead solution matches a color in the optimal range (750,000 beads/µL to 1.25 million beads/µL; see Figure 31 and Figure 32 on page 54).

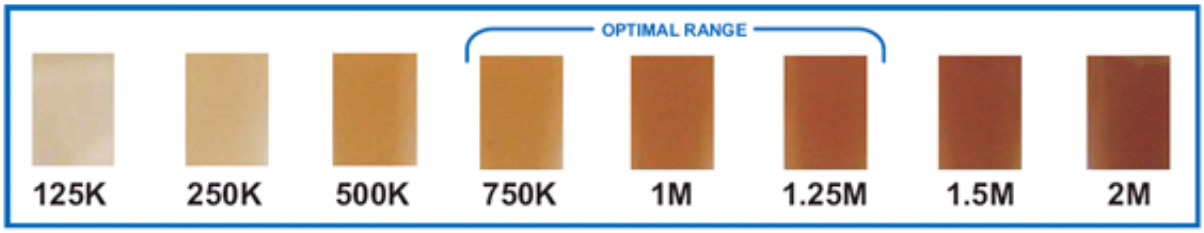


Figure 31 The SOLiD™ Bead Concentration Chart. For best results, use the SOLiD™ Bead Concentration Chart (PN 4415131), supplied separately.

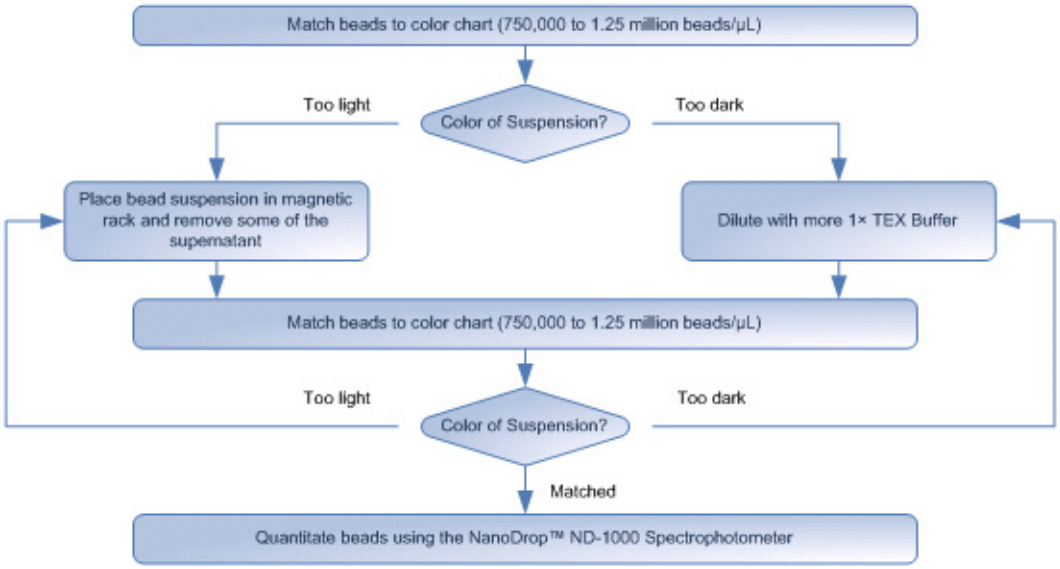


Figure 32 The SOLiD™ Bead Concentration Chart workflow.

5. When the bead concentration is within accurate range, quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer. Take 3 readings, then average them. Calculate the bead concentration using the appropriate standard curve (for more details, see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

STOPPING POINT. Store the templated beads at 4 °C in 1X TEX Buffer, or proceed to bead deposition and sequencing [refer to the *Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Guide* (PN 4442357)].

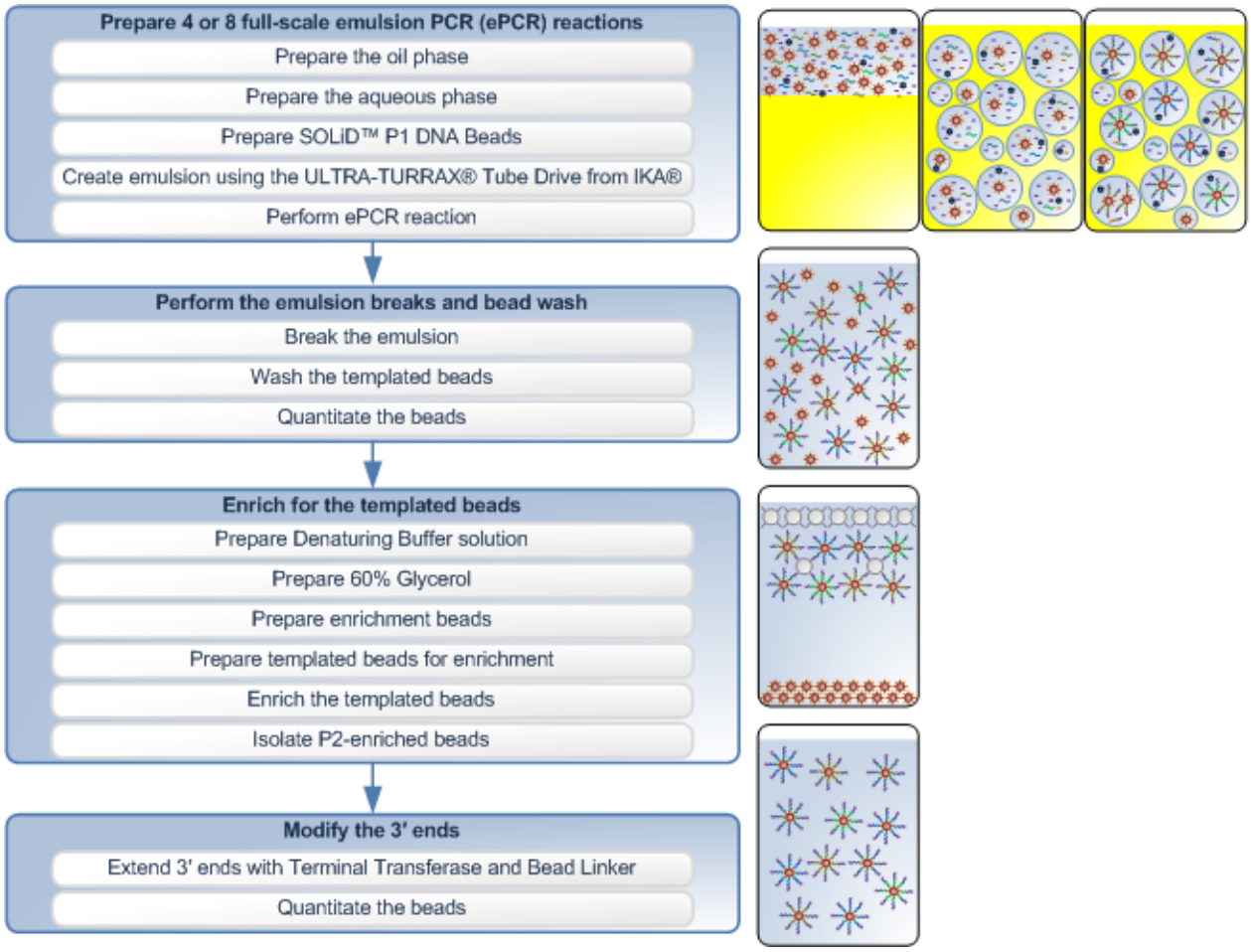
Section 2.3 Prepare templated beads (macro-scale)

Materials and equipment required

See Appendix A on page 77 for a list of equipment, kits, and consumables necessary for this procedure.

Workflow

See the descriptions of the steps below the workflow.



Prepare 4 or 8 full-scale emulsion PCR (ePCR) reactions

The oil phase and aqueous phase of the emulsion are prepared separately, then emulsified using the ULTRA-TURRAX™ Tube Drive from IKA®. Each emulsion is seeded with 1.6 billion SOLiD™ P1 DNA Beads, then transferred into a single, 96-well plate for cycling. Depending on the output required for your experiment, perform 4 or 8 ePCR reactions. Different library template lengths require different numbers of cycles for thermal cycling.

Perform the emulsion break and bead wash

The emulsion break uses 2-butanol to purify emulsified templated beads from the oil phase following amplification. The beads are washed to remove any residual 2-butanol, oil, and aqueous phase containing PCR reagents. There are two methods available to break the emulsion. In the *standard* method, a multi-channel pipettor is used to add and mix 2-butanol into the emulsion in each well of the 96-well plate. The pipettor is then used to transfer the solution into a 50-mL reservoir. In the *alternative* method, the

SOLiD™ Emulsion Collection Tray is placed over the 96-well plate, then the plate is centrifuged. Centrifuging the plate forces the emulsion from each well to a single reservoir. After centrifugation, 2-butanol is added to the reservoir. For both methods, the broken emulsion is transferred to a 50-mL tube for further processing.

Enrich for the templated beads

The templated bead enrichment procedure isolates beads with full-length extension products following ePCR. Beads with full-length extension products are isolated by oligo hybridization using the sequence of the P2 primer. Both monoclonal and polyclonal beads are enriched. The procedure is designed to enrich the templated beads derived from four or eight ePCR reactions containing 1.6 billion SOLiD™ P1 DNA Beads each (6.4 billion SOLiD P1 DNA Beads for four ePCR reactions or 12.8 billion SOLiD P1 DNA Beads for eight ePCR reactions).

Modify the 3′ ends

The P2-enriched beads are extended with a Bead Linker by Terminal Transferase.

Tips

General

- Syringes are required to accurately measure viscous reagents. Aspirate the volume very slowly from the reagent bottle to ensure that no air bubbles are trapped within the syringe. The best practice is to draw some reagent into the syringe, dispense the entire reagent back to the reagent bottle, then draw the correct volume of reagent. The volume should be measured to the point where the plunger contacts the side of the syringe.
- Perform all steps requiring 0.5-mL, 1.5-mL, and 2.0-mL tubes with Eppendorf LoBind tubes.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols. Applied Biosystems recommends the Eppendorf 5417R tabletop microcentrifuge.

SOLiD™ P1 DNA Beads

- Do not freeze SOLiD™ P1 DNA Beads or templated beads. Store the SOLiD™ P1 DNA Beads at 4 °C in 1✕ TEX Buffer.
- If beads remain in the original tube after transfer, you can use a small additional volume of the appropriate buffer to recover the remaining beads. Do not exceed a total volume of 1.3 mL for a 1.5-mL LoBind tube.

Covaris™ S2 System

- The procedures are optimized for the Covaris™ S2 System. The Covaris S2 System must be specially adapted to prepare beads for the Applied Biosystems SOLiD™ 3 Plus System. Do not use the Covaris S1 sonicator or an unadapted Covaris S2 System for bead preparation. For more information, contact an Applied Biosystems SOLiD™ System applications specialist.
- Ensure that the Covaris™ S2 System is degassed, that no bubbles are present in the system, and that the instrument and tube are properly aligned for appropriate sonication of beads.

- To ensure optimal sonication by the Covaris™ S2 System, use the appropriate adaptor with the Covaris S2 System. For sample volumes ≤ 200 µL, use a 0.5-mL LoBind tube and 0.65-mL tube adaptor. For sample volumes between 200 µL and 600 µL, use a 1.5-mL LoBind tube and 1.5-mL tube adaptor. For sample volumes between 600 µL and 1.2 mL, use a 2.0-mL LoBind tube and the same adaptor as used for the 1.5-mL tubes. Place the tube collar at the indicator line of the adaptor.

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 36 to prepare 4 full-scale emulsion PCR reactions. Four ePCR reactions provide an adequate bead yield for two quadrants of a 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" on page 44 to perform the emulsion break and bead wash procedure on each of the four emulsion PCR 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)

Prepare the Denaturing Buffer solution

1. For each enrichment (4 plates to be combined), transfer 5.4 mL of Denaturing Buffer to a 15-mL conical tube.
2. Add 600 µL of Denaturant to the 5.4 mL of Denaturing Buffer, then cap the tube and vortex.

IMPORTANT! Prepare the prepared Denaturing Buffer solution fresh weekly.

Prepare 60% glycerol

1. With a 10-mL syringe, add 6 mL of nuclease-free water to a 15-mL conical tube.
2. With a 3-mL syringe, add 9 mL of glycerol to the nuclease-free water by dispensing 3 mL of glycerol three times with the syringe. Fill and dispense the glycerol slowly to ensure that the total volume of glycerol is dispensed.
3. Cap the tube, then vortex to mix the solution well.

IMPORTANT! Prepare the 60% glycerol fresh weekly.

Prepare the enrichment beads

1. Vortex the enrichment beads, then immediately transfer 1250 µL of the enrichment beads to each of two 2.0-mL LoBind tubes.
2. Centrifuge the beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.

3. Resuspend the enrichment beads in 500 µL of 1X Bind & Wash Buffer per tube.
4. 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 1X Bind & Wash Buffer.
5. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
6. Resuspend the enrichment beads in 500 µL of 1X Bind & Wash Buffer.
7. Add 5 µL of 1 mM Enrichment Oligo, then vortex and pulse-spin the enrichment beads.
8. Rotate the tube at room temperature for 30 minutes.
9. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
10. Resuspend the enrichment beads in 1 mL of 1X TEX Buffer.
11. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
12. Repeat steps 10 and 11.
13. Resuspend the enrichment beads in 500 µL of 1X Low Salt Binding Buffer.

STOPPING POINT. Store the prepared enrichment beads at 4 °C in 1X Low Salt Binding Buffer, or proceed to "Prepare the templated beads for enrichment". Prepared enrichment beads should be used within one week of preparation.

Prepare the templated beads for enrichment

1. Place a 1.5-mL LoBind tube in a magnetic rack.
2. Transfer the suspension of templated beads from the first ePCR reaction to the tube in the magnetic rack.
3. Rinse the bottom of the first tube of templated beads with 100 µL of 1X TEX Buffer, then transfer the rinse to the tube in the magnetic rack.
4. Wait for at least 1 minute. After the solution clears, remove and discard the supernatant.
5. Transfer the suspension of templated beads from the next ePCR reaction to the tube in the magnetic rack.
6. Rinse that tube with 100 µL of 1X TEX Buffer, then transfer the rinse to the tube in the magnetic rack.
7. Wait for at least 1 minute. After the solution clears, remove and discard the supernatant.

8. Repeat steps 5 to 7 until all templated beads are in the LoBind tube in the magnetic rack.

9. Resuspend the templated beads in 450 µL of prepared Denaturing Buffer solution, then let the mixture stand for 1 minute.

10. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

11. Repeat steps 9 and 10.

12. Resuspend the beads in 750 µL of 1× TEX Buffer.

13. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

14. Repeat steps 12 and 13.

15. Resuspend the beads in 500 µL of 1× TEX Buffer.

16. Sonicate the beads with the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119).

Enrich the templated beads

1. Transfer all (500 µL) of the enrichment bead suspension to the 1.5-mL tube with the templated beads, vortex to mix, then pulse-spin the tube.
2. Sonicate the enrichment-templated bead mixture using the Covalent Declump 3 program on the Covaris S2 System (for program conditions, see "Covalent Declump 3" on page 119), then pulse-spin the beads.
3. Incubate the bead mixture at 61 °C for 15 minutes. During the incubation, vortex and pulse-spin the bead mixture every 5 minutes including at the end of the incubation.
4. Immediately cool the beads on ice for 2 minutes.
5. Add 7 mL of *freshly prepared* 60% glycerol to a new 50-mL conical polypropylene tube.
6. Use a 1-mL pipettor tip to pipette the bead mixture up and down to mix, then load the entire volume of bead mixture *carefully* on top of the 60% glycerol solution. Do *not* vortex the tube.
7. Centrifuge the tubes for 10 minutes at 2284 × g.
8. Add 10 mL of 1✕ TEX Buffer to a new 50-mL conical polypropylene tube.
9. Transfer the top layer of beads into the tube with 1✕ TEX Buffer. Aspirate as little glycerol as possible to collect all of the beads at the top layer without touching the un-templated beads at the bottom of the tube. When you dispense the top layer of beads into the 1✕ TEX Buffer, dispense the beads into the bottom of the tube. Aspirate a small amount of 1✕ TEX buffer to clean the pipette tip.
10. Top off the tube with additional 1✕ TEX Buffer to the 25-mL mark, then vortex the tube.
11. Centrifuge the tube for 10 minutes at 2284 × g.

Note: Verify that the beads are pelleted in case excess glycerol carried over to the 1✕ TEX Buffer creates a matrix that impedes pelleting of beads.

12. Proceed according to the Table 11:

Table 11 Steps for pelleted or unpelleted beads

If the beads are…	Then…
Pelleted	Remove and discard the supernatant, then proceed to "Isolate the P2-enriched beads" on page 63.
Not pelleted	Perform steps 13 to 15.

13. Carefully remove as much supernatant as possible without pipetting up the beads.

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

15. Repeat steps 11 to 12.

Isolate the P2-enriched beads

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

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

3. Place the tube in a magnetic rack for at least 1 minute until the supernatant is pure white or clear, then remove and discard the supernatant.

   IMPORTANT! *Never* magnet the P2-enriched beads before adding prepared Denaturing Buffer solution to the beads. If you do, the templated beads linked to the enrichment beads are lost when the supernatant is removed.

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

5. Repeat steps 3 and 4 until the supernatant is clear (all white enrichment beads have been removed).

6. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear. Remove and discard the supernatant.

7. Resuspend the beads in 1 mL of 1X TEX Buffer.

8. Repeat steps 6 and 7 *twice*.

9. Sonicate the enrichment-templated bead mixture using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin the beads.

10. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear. Remove and discard the supernatant.

11. Resuspend the beads in 1 mL of 1X TEX Buffer.

12. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear. Remove and discard the supernatant.

13. If the supernatant appears cloudy due to residual enrichment beads, repeat steps 11 and 12 until the supernatant is clear.

14. Resuspend the beads in 1 mL of 1✕ TEX Buffer.

STOPPING POINT. Store the prepared enrichment beads at 4 °C in 1✕ TEX Buffer, or proceed to "Modify the 3′ ends (4 ePCR reactions)" on page 65.

Modify the 3′ ends (4 ePCR reactions)

Extend the 3′ ends with Terminal Transferase and Bead Linker

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 (for program conditions, see "Covalent Declump 3" on page 119). Pulse-spin the beads.
2. Prepare the appropriate volume of 1× Terminal Transferase Reaction Buffer (1.5 mL per 4 ePCR reactions; see Table 12):

Table 12 Four ePCR reactions: prepare 1× Terminal Transferase Reaction Buffer

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

Note: The 1× Terminal Transferase Reaction Buffer should be clear. If the solution becomes colored, discard then prepare fresh buffer using a new lot of material.

3. Add 2 µL of 50 mM Bead Linker to 98 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. After the supernatant clears, remove and discard the supernatant.
5. Resuspend the beads in 300 µL of 1× 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. After the supernatant clears, remove and discard the supernatant.
7. Resuspend the beads in 300 µL of 1× Terminal Transferase Reaction Buffer.
8. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
9. Resuspend the beads in 712 µL of 1× Terminal Transferase Reaction Buffer.
10. Add 80 µL of 1 mM Bead Linker solution to the tube.
11. Sonicate the beads using the Covalent Declump 3 program on the Covaris S2 System (for program conditions, see "Covalent Declump 3" on page 119). Pulse-spin the beads.

12. Add 8.0 µL of Terminal Transferase (20 U/µL) to the tube, vortex, then pulse-spin the beads.

13. Seal the tube with Parafilm, then place the tube on a rotator and rotate for 2 hours at 37 °C.

14. Pulse-spin the tube.

15. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

16. Resuspend the beads in 400 µL of 1✕ TEX Buffer.

17. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

18. Resuspend the beads in 400 µL of 1✕ TEX Buffer.

STOPPING POINT. Store the templated beads at 4 °C in 1✕ TEX Buffer, or proceed to "Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer" or "Quantitate the beads using a hemocytometer" on page 106.

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

1. If necessary, generate a standard curve (see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

2. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin the beads.

3. Use the SOLiD™ Bead Concentration Chart (Applied Biosystems PN 4415131) to estimate the bead concentration of the beads (see Figure 33).

4. Adjust the volume of beads so that the color of the bead solution matches a color in the optimal range (750,000 beads/µL to 1.25 million beads/µL; see Figure 33 and Figure 34 on page 67).

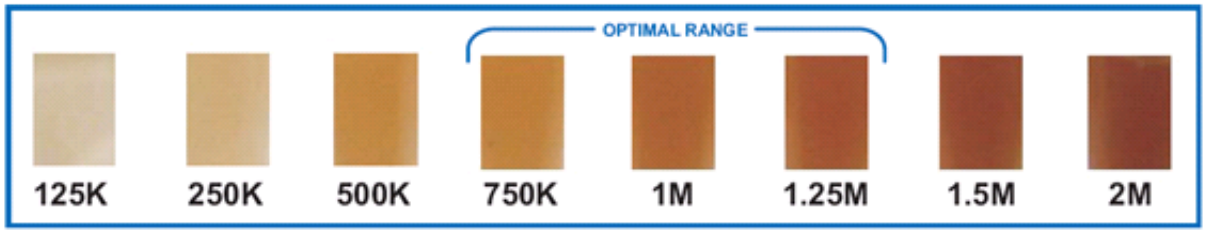


Figure 33 The SOLiD™ Bead Concentration Chart. For best results, use the SOLiD™ Bead Concentration Chart (PN 4415131), supplied separately.

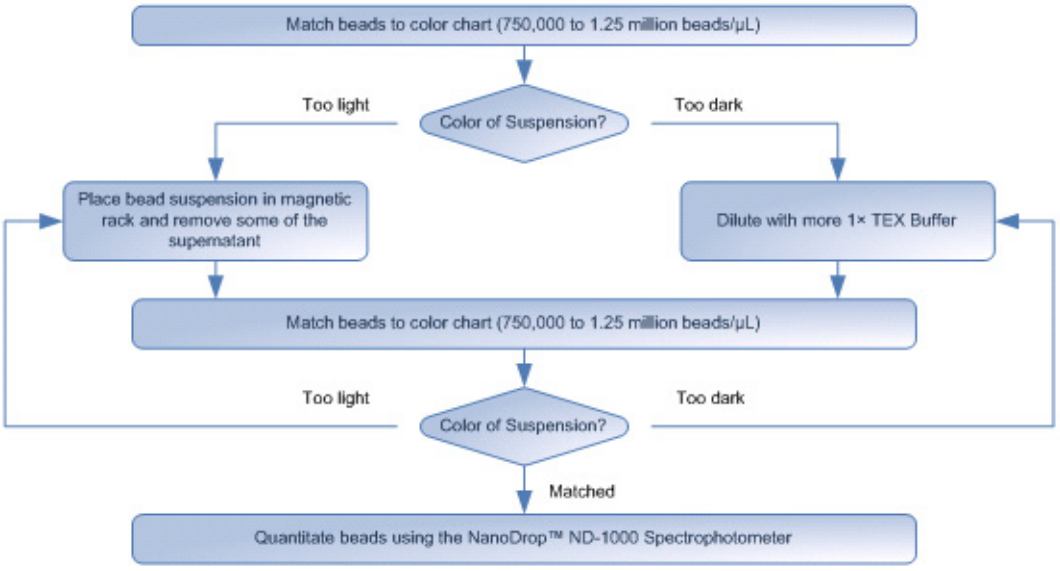


Figure 34 The SOLiD™ Bead Concentration Chart workflow

5. When the bead concentration is within accurate range, quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer. Take 3 readings, then average them. Calculate the bead concentration using the appropriate standard curve (for more details, see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

STOPPING POINT. Store the templated beads at 4 °C in 1X TEX Buffer, or proceed to bead deposition and sequencing [refer to the *Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Guide* (PN 4442357)].

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

Prepare the emulsion PCR (ePCR)

See "Prepare the full-scale emulsion PCR (ePCR) reaction" on page 36 to prepare 8 emulsion PCR reactions. Eight ePCR reactions 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" on page 44 to perform the emulsion break and bead wash procedure on each of the 8 emulsion PCR reactions. 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)

Prepare the Denaturing Buffer solution

1. For each enrichment (8 plates to be combined), transfer 5.4 mL of Denaturing Buffer to a 15-mL conical tube.
2. Add 600 µL of Denaturant to the 5.4 mL of Denaturing Buffer, then cap the tube and vortex.

IMPORTANT! Prepare the prepared Denaturing Buffer solution fresh weekly.

Prepare 60% glycerol

1. With a 10-mL syringe, add 6 mL of nuclease-free water to a 15-mL conical tube.
2. With a 3-mL syringe, add 9 mL of glycerol to the nuclease-free water by dispensing 3 mL of glycerol three times with the syringe. Fill and dispense the glycerol slowly to ensure that the total volume of glycerol is dispensed.
3. Cap the tube, then vortex to mix the solution well.

IMPORTANT! Prepare the 60% glycerol fresh weekly.

Prepare the enrichment beads

1. Vortex the enrichment beads, then immediately transfer 1250 µL of the enrichment beads to each of four 2.0-mL LoBind tubes.
2. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.

3. Resuspend the enrichment beads in 500 µL of 1✕ Bind & Wash Buffer per tube.
4. Combine the contents of two tubes into a single tube, resulting in two 2.0-mL tubes containing enrichment beads, each tube with 1 mL of 1✕ Bind & Wash Buffer.
5. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
6. Resuspend the enrichment beads in 500 µL of 1✕ Bind & Wash Buffer per tube.
7. Add 5 µL of 1 mM Enrichment Oligo per tube, then vortex and pulse-spin the enrichment beads.
8. Rotate the tubes at room temperature for 30 minutes.
9. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
10. Resuspend the enrichment beads in 1 mL of 1✕ TEX Buffer per tube.
11. Centrifuge the enrichment beads for 2 minutes at 21,000 × g (minimum 14,000 × g), then remove and discard the supernatant.
12. Repeat steps 10 and 11.
13. Resuspend the enrichment beads in 500 µL of 1✕ Low Salt Binding Buffer per tube. Combine the enrichment beads from both tubes into one 2.0-mL LoBind tube. The final volume is 1 mL.

STOPPING POINT. Store the prepared enrichment beads at 4 °C in 1✕ Low Salt Binding Buffer, or proceed to "Prepare the templated beads for enrichment" on page 60. Prepared enrichment beads should be used within one week of preparation.

Prepare the templated beads for enrichment

1. Place a 1.5-mL LoBind tube in a magnetic rack.
2. Transfer the suspension of templated beads from the first ePCR reaction to the tube in the magnetic rack.
3. 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.
4. Wait for at least 1 minute. After the solution clears, remove and discard the supernatant.
5. Transfer the suspension of templated beads from the next ePCR reaction to the tube in the magnetic rack.
6. Rinse that tube with 100 µL of 1✕ TEX Buffer, then transfer the rinse to the tube in the magnetic rack.
7. Wait for at least 1 minute. After the solution clears, remove and discard the supernatant.
8. Repeat steps 5 to 7 until all templated beads from four tubes are in the LoBind tube in the magnetic rack.
9. Repeat steps 1 to 8 for the remaining four tubes of templated beads.
10. Resuspend the templated beads in each tube with 450 µL of prepared Denaturing Buffer solution, then let stand for 1 minute.
11. Place the tubes in a magnetic rack for at least 1 minute. After the solution clears, remove then discard the supernatant.
12. Repeat steps 10 and 11.
13. Resuspend the beads in each tube with 750 µL of 1✕ TEX Buffer.
14. Place the tubes in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.
15. Repeat steps 13 and 14.
16. Resuspend the beads in each tube with 500 µL of 1✕ TEX Buffer.
17. Sonicate the beads with the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119).

Enrich the templated beads

1. Transfer 500 µL of enrichment bead suspension to each 1.5-mL tube of templated beads, then vortex the tubes.
2. Sonicate the enrichment-templated bead mixture using the Covalent Declump 3 program on the Covaris S2 System (for program conditions, see "Covalent Declump 3" on page 119), then pulse-spin the beads.
3. Incubate the bead mixture at 61 °C for 15 minutes, then vortex and pulse-spin the bead mixture every 5 minutes including at the end of the incubation.
4. Immediately cool the beads on ice for 2 minutes.
5. Add 7 mL of *freshly prepared* 60% glycerol to a new 50-mL conical polypropylene tube.
6. Use a 1-mL pipettor tip to pipette the bead mixture up and down to mix, then load the entire volume of bead mixture *carefully* on top of the 60% glycerol solution. Do *not* vortex the tube. Transfer the bead mixture from both tubes.
7. Centrifuge the tube for 10 minutes at 2284 × g.
8. Add 10 mL of 1X TEX Buffer to a new 50-mL conical polypropylene tube.
9. Transfer the top layer of beads into the tube with 1X TEX Buffer. Aspirate as little glycerol as possible to collect all of the beads at the top layer without touching the un-templated beads at the bottom of the tube. When you dispense the top layer of beads into the 1X TEX Buffer, dispense the beads into the bottom of the tube. Aspirate a small amount of 1X TEX buffer to clean the pipette tip.
10. Top off the tube with additional 1X TEX Buffer to the 25-mL mark, then vortex the tube.
11. Centrifuge the tube for 10 minutes at 2284 × g.

Note: Verify that the beads are pelleted in case excess glycerol carried over to the 1X TEX Buffer creates a matrix that impedes pelleting of beads.

12. Proceed according to the Table 13:

Table 13 Steps for pelleted or unpelleted beads

If the beads are…	Then…
Pelleted	Remove and discard the supernatant, then proceed to "Isolate the P2-enriched beads" on page 72.
Not pelleted	Perform steps 13 to 15.

13. Carefully remove as much supernatant as possible without pipetting up the beads.

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

15. Repeat steps 11 and 12.

Isolate the P2-enriched beads

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

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

3. Place the tube in a magnetic rack for at least 1 minute until the supernatant is pure white or clear, then remove and discard the supernatant.

   IMPORTANT! *Never* magnet the P2-enriched beads before adding prepared Denaturing Buffer solution to the beads. If you do, the templated beads linked to the enrichment beads are lost when the supernatant is removed.

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

5. Repeat steps 3 and 4 until the supernatant is clear (all white enrichment beads have been removed).

6. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear. Remove and discard the supernatant.

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

8. Repeat steps 6 and 7 *twice*.

9. Sonicate the enrichment-templated bead mixture using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin the beads.

10. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear. Remove and discard the supernatant.

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

12. Place the tube in a magnetic rack for at least 1 minute until the supernatant is clear. Remove and discard the supernatant.

13. If the supernatant appears cloudy due to residual enrichment beads, repeat steps 11 and 12 until the supernatant is clear.

14. Resuspend the beads in 1 mL of 1X TEX Buffer.

STOPPING POINT. Store the prepared enrichment beads at 4 °C in 1X TEX Buffer, or proceed to "Modify the 3′ ends (8 ePCR reactions)".

Modify the 3′ ends (8 ePCR reactions)

Extend the 3′ ends with Terminal Transferase and Bead Linker

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 (for program conditions, see "Covalent Declump 3" on page 119). Pulse-spin the beads.

2. Prepare the appropriate volume of 1X Terminal Transferase Reaction Buffer (2.4 mL per 8 ePCR reactions; see Table 14).

Table 14 Eight ePCR reactions: prepare 1× Terminal Transferase Reaction Buffer

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

Note: The 1X Terminal Transferase Reaction Buffer should be clear. If the solution becomes colored, discard then prepare fresh buffer using a new lot of material.

3. Add 4 µL of 50 mM Bead Linker to 196 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. After the supernatant clears, remove and discard the supernatant.

5. Resuspend the beads in 300 µL of 1X 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. After the supernatant clears, remove and discard the supernatant.

7. Resuspend the beads in 300 µL of 1X Terminal Transferase Reaction Buffer.

8. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.

9. Resuspend the beads in 1424 µL of 1X Terminal Transferase Reaction Buffer.

10. Add 160 µL of 1 mM Bead Linker solution to the tube.
11. Transfer 792 µL of bead solution to a new 2.0-mL LoBind tube.
12. Sonicate the beads using the Covalent Declump 3 program on the Covaris S2 System (for program conditions, see "Covalent Declump 3" on page 119). Pulse-spin the beads.
13. Add 8 µL of Terminal Transferase (20 U/µL) to each tube, vortex, then pulse-spin the beads.
14. Seal the tubes with Parafilm, then place the tubes on a rotator and rotate for 2 hours at 37 °C.
15. Pulse-spin the tubes, then pool the beads in one LoBind tube.
16. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
17. Resuspend the beads in 400 µL of 1✕ TEX Buffer.
18. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
19. Resuspend the beads in 400 µL of 1✕ TEX Buffer.

STOPPING POINT. Store the templated beads at 4 °C in 1✕ TEX Buffer, or proceed to "Quantitate the beads with the SOLiD™ Bead Concentration Chart and the NanoDrop® ND-1000 Spectrophotometer" or "Quantitate the beads using a hemocytometer" on page 106.

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

1. If necessary, generate a standard curve (see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).
2. Sonicate the beads using the Covalent Declump 1 program on the Covaris S2 System (for program conditions, see "Covalent Declump 1" on page 119), then pulse-spin the beads.
3. Use the SOLiD™ Bead Concentration Chart (Applied Biosystems PN 4415131) to estimate the bead concentration of the beads (see Figure 35 on page 75).
4. Adjust the volume of beads so that the color of the bead solution matches a color in the optimal range (750,000 beads/µL to 1.25 million beads/µL; see Figure 35 on page 75 and Figure 36 on page 75).

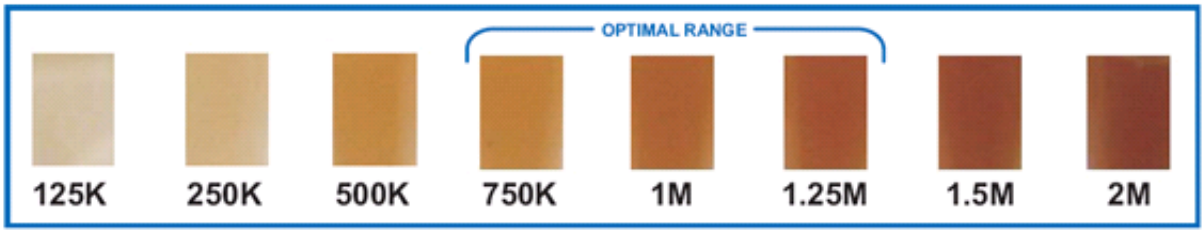


Figure 35 The SOLiD™ Bead Concentration Chart. For best results, use the SOLiD™ Bead Concentration Chart (PN 4415131), supplied separately.

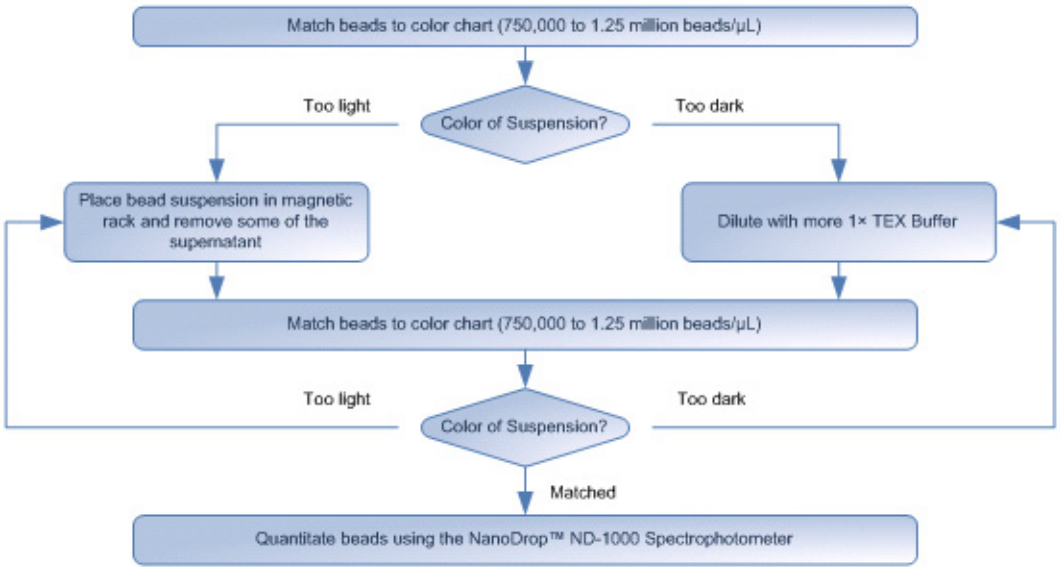


Figure 36 The SOLiD™ Bead Concentration Chart workflow.

5. When the bead concentration is within accurate range, quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer. Take 3 readings, then average them. Calculate the bead concentration using the appropriate standard curve (for more details, see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

STOPPING POINT. Store the templated beads at 4 °C in 1X TEX Buffer, or proceed to bead deposition and sequencing [refer to the *Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Guide* (PN 4442357)].

A Required Materials

This appendix covers:

Prepare templated beads (mini-scale)

Table 15 Required Applied Biosystems reagent kits

Item (part number)‡	Components	Kit component(s) used In…
SOLiD™ ePCR Kit V2, 20 Mini-Reactions (4407756)	Magnesium Chloride Emulsion Oil Emulsion Stabilizer 1 Emulsion Stabilizer 2 Bead Block Solution 10× PCR Buffer dNTP Mix AmpliTaq Gold DNA Polymerase, UP ePCR primer 1 ePCR primer 2 SOLiD™ P1 DNA Beads	Emulsion PCR
SOLiD™ Buffer Kit, 20 Mini-Reactions (4407759)	1× Bead Wash Buffer 2-Butanol§	Emulsion break and bead wash
	1× Bind & Wash Buffer 1× Low Salt Binding Buffer	Enrichment
	1× Low TE Buffer	Emulsion PCR, 3′-end modification
	1× TEX Buffer	Emulsion PCR, emulsion break and bead wash, enrichment, 3′- end modification
SOLiD™ Bead Enrichment Kit, 20 Mini-Reactions (4407757)	Glycerol Denaturing Buffer Denaturant Enrichment Oligo Enrichment Beads	Enrichment
SOLiD™ Bead Deposition Kit, 20 Mini-Reactions (4407758)	10× Terminal Transferase Buffer 10× Cobalt Chloride Terminal Transferase Bead Linker	3′-end modification
	Overlay Buffer Deposition Buffer	Bead deposition

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.
§ The tube is labeled as “butanol” in the kit.

Table 16 Required equipment

Item‡	Source
ULTRA-TURRAX® Tube Drive from IKA®§ (115 V for U.S. customers) (230 V for international customers) The system includes: SOLiD™ ePCR Tubes and Caps, 10-pack	Applied Biosystems 4400335 (115 V and 230 V)
96-well GeneAmp® PCR System 9700 (thermal cycler)	• Applied Biosystems N8050200 (Base) • Applied Biosystems 4314443 (Block)‡
Covaris™ S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris™ S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, see "Covaris™ S2 System Materials Summary," *SOLiD™ 3 Plus System Site Preparation Guide*.	• Applied Biosystems 4387833 (110 V) • Applied Biosystems 4392718 (220 V) or Covaris
6-Tube Magnetic Stand	Applied Biosystems AM 10055
Microcentrifuge 5417R, refrigerated, without rotor	• Eppendorf# 022621807 (120 V/60 Hz) • Eppendorf‡ 022621840 (230 V/50 Hz)
FA-45-24-11, fixed-angle rotor, 24 × 1.5/2 mL, including aluminum lid, aerosol-tight	Eppendorf# 022636006
Repeater® Xstream	Eppendorf 022460811

Table 16 Required equipment

Item‡	Source
Repeater® Plus Pipette	Eppendorf 022260201
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Labquake Rotisserie Rotator, Barnstead/Thermolyne	VWR 56264-312
Fume hood	Major Laboratory Supplier (MLS)
Tabletop Centrifuge	MLS
Microscope	MLS
Vortexer	MLS
Picofuge	MLS
Incubator (37 °C)	MLS
Incubator (61 °C)	MLS
12-channel multi-channel pipettor	MLS
Pipettors, 2 µL	MLS
Pipettors, 20 µL	MLS
Pipettors, 200 µL	MLS
Pipettors, 1000 µL	MLS

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.
§ Applied Biosystems ships one ULTRA-TURRAX® Tube Drive from IKA® per instrument.
# Or equivalent but validation of the equipment for library preparation is required.

Table 17 Required consumables

Item‡	Source
SOLiD™ ePCR Tubes and Caps, 10 pack (15-mL tubes)	Applied Biosystems 4400401
MicroAmp® Optical 96-Well Reaction Plates	Applied Biosystems N8010560
Clear Adhesive Film: MicroAmp® Optical Adhesive Film, *or* Clear Seal Diamond Heat Sealing Film	Applied Biosystems 4360954 Thermo Scientific AB-0812
Nuclease-free Water (1 L)	Applied Biosystems AM9932
50-mL high-clarity polypropylene conical centrifuge tube, 9400 RCF rating, sterile	Becton-Dickinson 352070
1-mL BD™ slip-tip disposable tuberculin syringe	Becton-Dickinson 309602

Table 17 Required consumables

Item‡	Source
5-mL Combitips Plus	Eppendorf 022496107
10-mL Combitips Plus	Eppendorf 022496123
0.5-mL LoBind Tubes	Eppendorf 022431005
1.5-mL LoBind Tubes	Eppendorf 022431021
2.0-mL LoBind Tubes	Eppendorf 022431048
Polypropylene wide-mouth jars (0.5 oz., 15 mL, 38-mm cap)	Nalgene 2118-9050
Ethylene glycol	American Bioanalytical AB00455-01000
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit§	Thermo Scientific PR-1
10-mL serological pipettes	Major Laboratory Supplier (MLS)
15-mL conical polypropylene tubes	MLS
3-mL syringes	MLS
10-mL syringes	MLS
50-mL reservoirs	MLS
Razor blades	MLS
Filtered pipettor tips	MLS
Ice	MLS

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.

§ The NanoDrop® Conditioning Kit is useful for “reconditioning” the sample measurement pedestals to a hydrophobic state if they become “unconditioned” (see NanoDrop user’s manual for more information). The PR-1 kit consists of a container of specially formulated polishing compound and a supply of convenient applicators.

Prepare templated beads (full-scale and macro-scale)

Table 18 Required Applied Biosystems reagent kits

Item (part number)‡	Components	Kit component(s) used in…
SOLiD™ ePCR Kit V2 (4400834)	Magnesium Chloride Emulsion Oil Emulsion Stabilizer 1 Emulsion Stabilizer 2 Bead Block Solution 10× PCR Buffer dNTP Mix AmpliTaq Gold DNA Polymerase, UP ePCR primer 1 ePCR primer 2 SOLiD™ P1 DNA Beads	Emulsion PCR
SOLiD™ Buffer Kit (4387918)	1X Bead Wash Buffer 2-Butanol§	Emulsion break and bead wash
	1X Bind & Wash Buffer 1X Low Salt Binding Buffer	Enrichment
	1X Low TE Buffer	Emulsion PCR, 3′-end modification
	1X TEX Buffer	Emulsion PCR, emulsion break and bead wash, enrichment, 3′- end modification
SOLiD™ Bead Enrichment Kit (4387894)	Glycerol Denaturing Buffer Denaturant Enrichment Oligo Enrichment Beads	Enrichment
SOLiD™ Bead Deposition Kit (4387895)	10X Terminal Transferase Buffer 10X Cobalt Chloride Terminal Transferase Bead Linker	3′-end modification
	Overlay Buffer Deposition Buffer	Bead deposition

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.
§ The tube is labeled as “butanol” in the kit.

Table 19 Required equipment

Item‡	Source
ULTRA-TURRAX® Tube Drive from IKA®§ (115 V for U.S. customers) (230 V for international customers) The system includes: SOLiD™ ePCR Tubes and Caps, 10-pack	Applied Biosystems 4400335 (115 V and 230 V)
96-well GeneAmp® PCR System 9700 (thermal cycler)	• Applied Biosystems N8050200 (Base) • Applied Biosystems 4314443 (Block)‡
Covaris S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, see "Covaris™ S2 System Materials Summary," *SOLiD™ 3 Plus System Site Preparation Guide*.	• Applied Biosystems 4387833 (110 V) • Applied Biosystems 4392718 (220 V) or Covaris
6-Tube Magnetic Stand	Applied Biosystems AM 10055
Microcentrifuge 5417R, refrigerated, without rotor	• Eppendorf# 022621807 (120 V/60 Hz) • Eppendorf‡ 022621840 (230 V/50 Hz)
FA-45-24-11, fixed-angle rotor, 24 × 1.5/2 mL, including aluminum lid, aerosol-tight	Eppendorf# 022636006
Repeater® Xstream	Eppendorf 022460811

Table 19 Required equipment

Item‡	Source
Repeater® Plus Pipette	Eppendorf 022260201
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Labquake Rotisserie Rotator, Barnstead/Thermolyne	VWR 56264-312
Fume hood	Major Laboratory Supplier (MLS)
Tabletop Centrifuge	MLS
Microscope	MLS
Vortexer	MLS
Picofuge	MLS
Incubator (37 °C)	MLS
Incubator (61 °C)	MLS
12-channel multi-channel pipettor	MLS
Pipettors, 2 µL	MLS
Pipettors, 20 µL	MLS
Pipettors, 200 µL	MLS
Pipettors, 1000 µL	MLS

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.
§ Applied Biosystems ships one ULTRA-TURRAX® Tube Drive from IKA® per instrument.
# Or equivalent but validation of the equipment for library preparation is required.

Table 20 Required consumables

Item‡	Source
SOLiD™ ePCR Tubes and Caps, 10 pack (15-mL tubes)	Applied Biosystems 4400401
MicroAmp® Optical 96-Well Reaction Plates	Applied Biosystems N8010560
Clear Adhesive Film: MicroAmp® Optical Adhesive Film, *or* Clear Seal Diamond Heat Sealing Film	Applied Biosystems 4360954 Thermo Scientific AB-0812
Nuclease-free Water (1 L)	Applied Biosystems AM9932
50-mL high-clarity polypropylene conical centrifuge tube, 9400 RCF rating, sterile	Becton-Dickinson 352070

Table 20 Required consumables

Item‡	Source
1-mL BD™ slip-tip disposable tuberculin syringe	Becton-Dickinson 309602
5-mL Combitips Plus	Eppendorf 022496107
10-mL Combitips Plus	Eppendorf 022496123
0.5-mL LoBind Tubes	Eppendorf 022431005
1.5-mL LoBind Tubes	Eppendorf 022431021
2.0-mL LoBind Tubes	Eppendorf 022431048
Polypropylene wide-mouth jars (0.5 oz., 15 mL, 38-mm cap)	Nalgene 2118-9050
Ethylene glycol	American Bioanalytical AB00455-01000
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit§	Thermo Scientific PR-1
10-mL serological pipettes	Major Laboratory Supplier (MLS)
15-mL conical polypropylene tubes	MLS
3-mL syringes	MLS
10-mL syringes	MLS
50-mL reservoirs	MLS
Razor blades	MLS
Filtered pipettor tips	MLS
Parafilm	MLS
Ice	MLS

‡ Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.

§ The NanoDrop® Conditioning Kit is useful for “reconditioning” the sample measurement pedestals to a hydrophobic state if they become “unconditioned” (see NanoDrop user’s manual for more information). The PR-1 kit consists of a container of specially formulated polishing compound and a supply of convenient applicators.

B Calculation of the Emulsion PCR Library Concentration

This appendix covers:

Objective

This appendix provides a mathematical formulation to determine optimal library concentration for ePCR targeting a given clonality metric, *P2%*, without workflow analysis. All library concentrations were measured by TaqMan qPCR assay using the 7500 Real-Time PCR System (standard or fast modules). Emulsion PCR reactions are set up as described in the standard protocol with different amounts of input library templates. Pre-enriched P2% values were obtained and plotted against the corresponding input DNA concentrations to examine their relationship. Data were sorted by two ePCR cycling conditions: 40 cycles and 60 cycles. (General guidelines are 40 cycles for fragment library or 2 × 25 bp mate-paired library, 60 cycles for 2 × 50 bp mate-paired library.) Empirical equations were generated by fitting these data into polynomial curves in which library concentration for ePCR is determined by the desired P2% and the size of the library.

Titration of the diluted library samples by ePCR

The diluted library samples used for ePCR titrations were taken from the same sample tubes used for TaqMan® qPCR assay quantitation.

1. Five SOLiD™ System libraries were chosen for this study with different sizes (150 bp to 317 bp) and from different library constructions (fragment and mate-paired). All libraries were diluted to about 50 pg/µL and quantitated by TaqMan qPCR assay using the 7500 Standard or Fast Real-Time PCR System (see Table 21).

Table 21 SOLiD™ System libraries used to calculate the optimal library concentration for ePCR

Library	Size (bp)	Library Type	Organism
1	156	Fragment	*E. coli*
2	154	Mate-paired	*E. coli*
3	250	Mate-paired	Human
4	250	Mate-paired	Human
5	317	Mate-paired	*E. coli*

2. After obtaining the stock concentrations by qPCR, all libraries were subjected to ePCR titration assays. In order to compare between the libraries, all concentrations were calculated in molar (pM) instead of mass (pg/µL). Two ePCR cycling conditions were employed: 40 cycles and 60 cycles. Amplified beads were recovered by standard ePCR process and pre-enriched P2% (coated beads/total beads) was measured by P2-Cy3 hybridization (see Table 22 on page 89, Table 23 on page 89, and Figure 37 on page 90).

Table 22 Pre-enriched *P2%* against input DNA concentrations from quantitation by 7500 *Fast* Real-Time PCR System.

Library concentration (pM)	Pre-enriched P2%				
	Library 1	Library 2	Library 2	Library 3	Library 4
0.25	5.3	4.7	7.0	—	6.1
0.31	—	—	—	8.1	—
0.5	9.1	9.0	10.7	—	11.2
0.62	—	—	—	13.0	—
1	16.1	16.7	17.8	—	19.1
1.23	—	—	—	22.3	—
1.5	20.0	21.2	23.1	—	24.3
1.85	—	—	—	27.0	—
ePCR cycling	40 cycles	40 cycles	60 cycles	60 cycles	60 cycles

Table 23 Pre-enriched *P2%* against input DNA concentrations from quantitation by 7500 *Standard* Real-Time PCR System.

Library concentration (pM)	Pre-enriched P2%			
	Library 1	Library 3	Library 4	Library 5
0.25	—	—	—	5.6
0.5	10.8	10.2	9.6	10.8
1.0	18.8	17.0	18.4	18.0
1.5	—	—	—	26.2
ePCR cycling	40 cycles	60 cycles	60 cycles	60 cycles

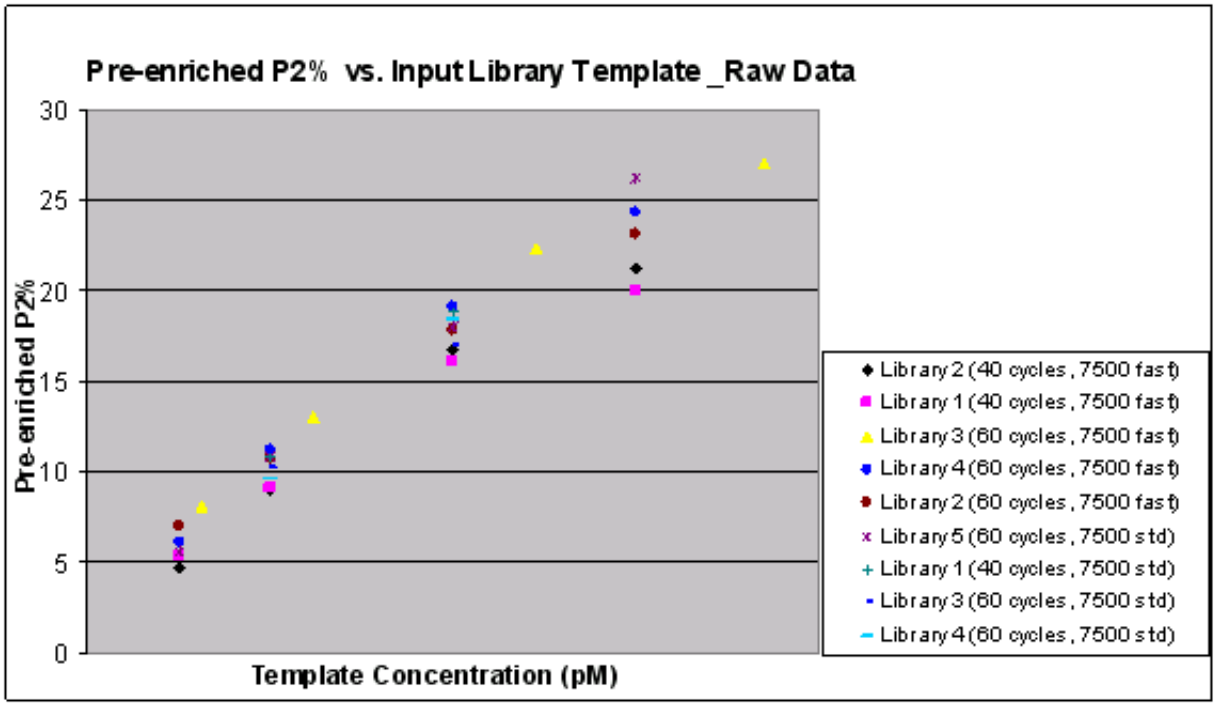


Figure 37 Plot of pre-enriched P2% against corresponding input DNA concentration.

3. Two equations (I and II below) were generated by fitting the curves in Figure 37 to describe the relationship between pre-enriched *P2%* and the amount of input template after 40 cycles or 60 cycles of ePCR reactions:

Equation I

$$P2\% = -3.9946 \times [DNA]_{(pM)}^2 + 21.414 \times [DNA]_{(pM)} + 1.2648 \qquad 60 \text{ cycles}$$

Equation II

$$P2\% = -7.242 \times [DNA]_{(pM)}^2 + 25.281 \times [DNA]_{(pM)} - 0.9831 \qquad 40 \text{ cycles}$$

Therefore, to target a specific *P2%* (15-20% is generally recommended), the optimal input DNA concentration (in pM) can be calculated from Equation I or II depending on ePCR cycling numbers. This molar concentration can then be converted into pg/µL by using Equation A:

Equation A

$$X \text{ pg/µL DNA} = [\text{DNA}]_{(\text{pM})} \frac{660 \text{ pg}}{1 \text{ pmol}} \times \frac{1 \text{ L}}{10^6 \text{ µL}} \times \text{Size}$$

Equations I and II can be applied to library concentrations measured by the SYBR® Green Assay.

C

Supplemental Procedures

This appendix covers:

Program the Eppendorf Repeater® Xstream Pipettor

The Eppendorf Repeater® Xstream pipettor has been preset to use with IKA®-based emulsions and the 10-mL Combitip Plus. Follow the procedure below only if you need to reprogram the pipettor.

Materials and equipment required

Table 24 Required equipment

Item	Source
Repeater® Xstream	Eppendorf 022460811

Table 25 Required consumables

Item	Source
10-mL Combitips Plus	Eppendorf 022496123

Procedure

1. Attach a 10-mL Combitip Plus on the Eppendorf Repeater Xstream pipettor.
2. Set the top dial to pipette mode: **Pip**.
3. Push the left blue **select** button. The screen displays “Set volume.”
4. Toggle the right blue **+/-** button to set the pipettor fill volume to **5.6 mL** (or other appropriate volume as specified in the procedure).
5. Push the left blue **select** button. The screen displays “up (▲) speed.”
6. Toggle the right blue **+/-** button to set histogram to **scale 5** (five colored bars: mid-range).
7. Push the left blue **select** button. The screen displays “down (▼) speed.”
8. Toggle the right blue **+/-** button to set histogram to **scale 1** (one colored bar: slowest).
9. Push the left blue **select** button to finish programming.
10. Push the *round lower center* blue button to save/store program.
11. Use the programmed Eppendorf Repeater Xstream pipettor with IKA®-based emulsions

Break the emulsion with the SOLiD™ Emulsion Collection Tray

The SOLiD™ Emulsion Collection Tray and tabletop centrifuge are used instead of a multi-channel pipettor to transfer the beads from a 96-well plate to the tray. 2-butanol is added to the tray and mixed into the emulsion.

Materials and equipment required

Table 26 Required equipment

Item	Source
Tabletop centrifuge	MLS (major laboratory supplier)‡
Fume hood	Major Laboratory Supplier (MLS)
Vortexer	MLS

‡ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Table 27 Required consumables

Item	Source
SOLiD™ Emulsion Collection Tray Kit	Applied Biosystems PN 4415129
SOLiD™ Buffer Kit – Butanol	Applied Biosystems PN 4389770‡
50-mL high-clarity polypropylene conical centrifuge tube, 9400 RCF rating, sterile	Becton-Dickinson PN 352070
10-mL serological pipettes	Major Laboratory Supplier (MLS)
Tape	MLS

‡ The part number for the complete SOLiD™ Buffer Kit is 4387918.

Procedure

1. Place the SOLiD™ Emulsion Collection Tray on top of the ePCR 96-well plate (see Figure 38).

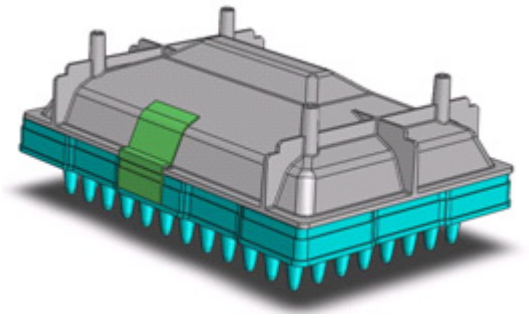


Figure 38 The SOLiD™ Emulsion Collection Tray taped to a 96-well reaction plate.

2. Seal the pieces together with tape on all four sides and flip the entire apparatus so that the 96-well plate is upside-down over the collection tray immediately prior to centrifugation (see Figure 39).

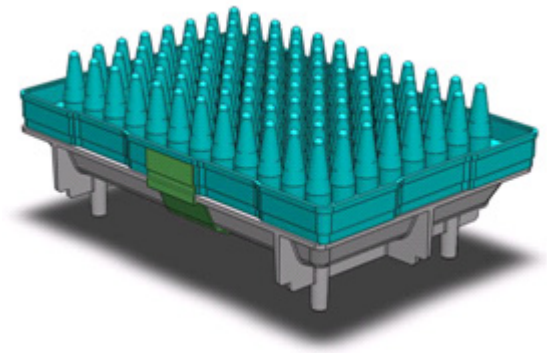


Figure 39 The inverted plate assembly.

Centrifuge the inverted plate and reservoir for 2 minutes at 550 × g according to recommended centrifuge settings (see Table 28).

Table 28 Recommended centrifuge settings

Adjustable Parameter	Recommend Setting
Acceleration (independent)	High
Deceleration (independent)	Low
Acceleration/Deceleration (single setting)	Mid

3. After centrifugation is complete, remove the plate assembly from the centrifuge and place on the lab bench. Hold the assembly steady, then gently remove the tape and 96-well plate from the collection tray.

Note: Ensure that the centrifuge is working properly and maintained regularly. Use anti-slip pads in the centrifuge carriers whenever possible.

4. In a fume hood, add 10 mL of 2-butanol to the collection tray using a serological pipette.
5. Pipette the emulsion up and down until the solution is homogeneous.
6. Transfer all the emulsion and 2-butanol to a 50-mL conical tube.
7. Rinse the reservoir with an additional 6 mL of 2-butanol to ensure that all residual beads are recovered.
8. Cap the tube, then vortex to mix the solution.
9. Centrifuge the tube at 2000 × g for 5 minutes.
10. Gently decant the 2-butanol-oil phase into a waste bottle. With the tube inverted, place the tube onto paper towels to drain residual 2-butanol-oil.
11. Wait 5 minutes to ensure that all the oil is removed.
12. Return to "Wash the templated beads" in the appropriate section for preparing templated beads.

Quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer

Materials and equipment required

Table 29 Required equipment

Item	Source
Covaris S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, see "Covaris™ S2 System Materials Summary," *SOLiD™ 3 Plus System Site Preparation Guide*.	Applied Biosystems PN 4387833 (110 V) PN 4392718 (220 V) or Covaris Inc.
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Pipettors	Major Laboratory Supplier (MLS)

Table 30 Required consumables

Item	Source
SOLiD™ Buffer Kit – 1X TEX Buffer	Applied Biosystems PN 4389776‡
Nuclease-free water (1 L)	Applied Biosystems PN AM9932
CF-1 Calibration Fluid Kit§	Thermo Scientific CF-1
PR-1 Conditioning Kit	Thermo Scientific PR-1

Table 30 Required consumables

Item	Source
0.5-mL LoBind tubes	Eppendorf 022431005
Filtered pipettor tips	Major Laboratory Supplier (MLS)

‡ The part number for the complete SOLiD™ Buffer Kit is 4387918.
§ The NanoDrop® Conditioning Kit is useful for "reconditioning" the sample measurement pedestals to a hydrophobic state if they become "unconditioned." (See the NanoDrop user's manual for more information.) The PR-1 kit consists of a container of specially formulated polishing compound and a supply of convenient applicators.

Procedure

1. Ensure that the NanoDrop ND-1000 Spectrophotometer is properly calibrated. Use the CF-1 Calibration Fluid Kit if necessary.

2. Open the NanoDrop ND-1000 Spectrophotometer software a dialog box displays (see Figure 40).

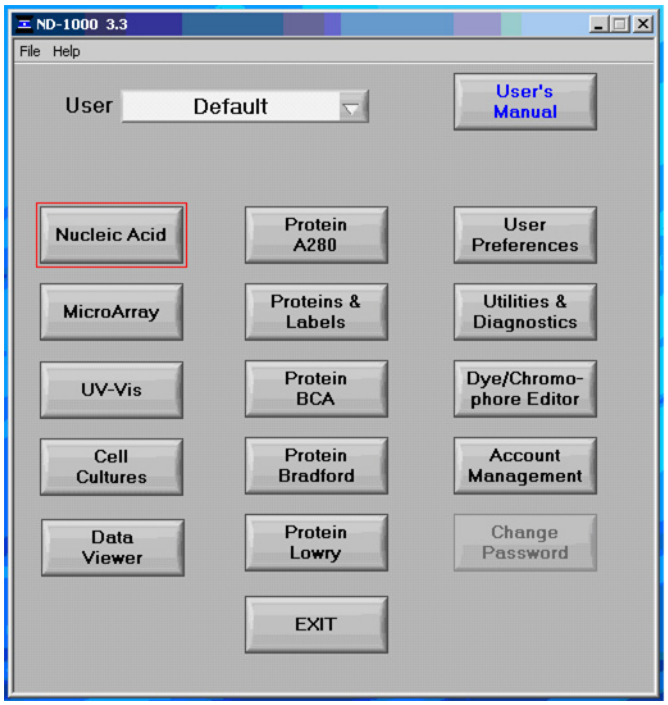


Figure 40 NanoDrop® ND-1000 Spectrophotometer software dialog box (from: http://nanodrop.com/nd-1000-software.html)

3. Select the **Cell Cultures** button.

4. Lift the sampling arm and load 2 µL of nuclease-free water onto the lower measurement pedestal and lower the sampling arm (see Figure 41 on page 100).

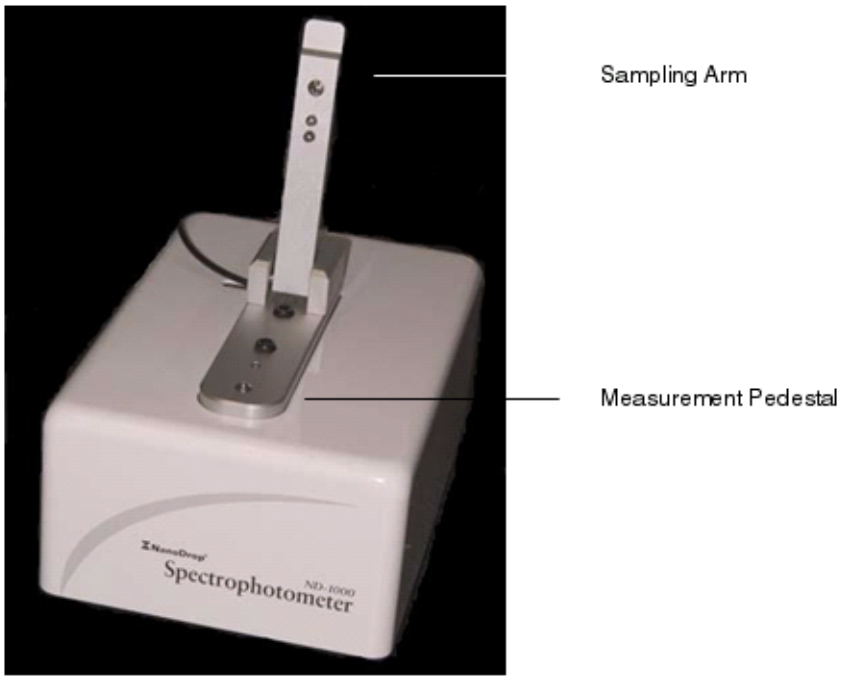


Figure 41 Components of the NanoDrop® ND-1000 Spectrophotometer.

5. In the dialog box, click **OK**, then allow the instrument to initialize.
6. Lift the sampling arm and use a Kimwipe® to remove water from the measurement pedestal and the sampling arm.
7. Load 2 µL of the same buffer that was used to resuspend the beads onto the sampling pedestal, then lower the sampling arm.
8. Click **Blank**, then allow the instrument to take a measurement (see Figure 42 on page 101).

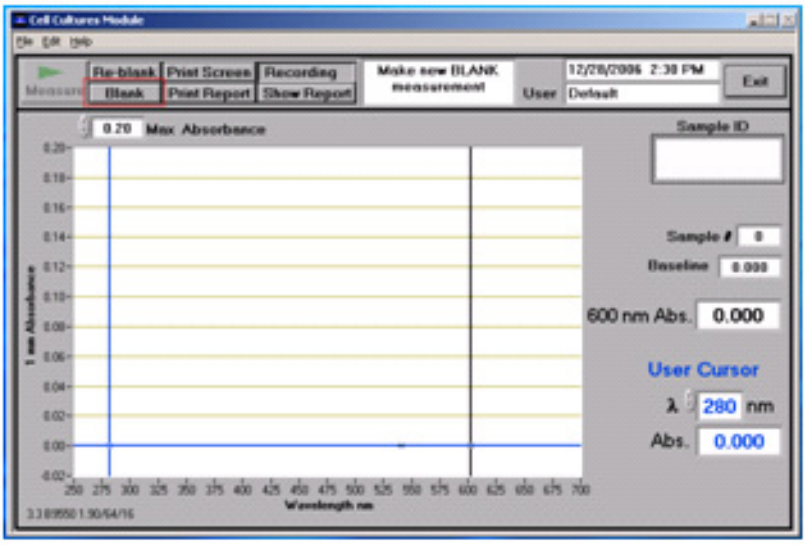


Figure 42 NanoDrop® ND-1000 Spectrophotometer software measurement dialog box.

9. Lift the sampling arm and wipe away the buffer from the sampling arm and measurement pedestal with a Kimwipe. The instrument is now ready to take readings.

10. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System (for program conditions, see "Covalent Declump 3" on page 119), then pulse-spin the beads. Proceed immediately to the next step.

11. If necessary, make a dilution of beads in 1✕ TEX Buffer.

12. Lift the sampling arm and load 2 µL of beads onto the lower measurement pedestal and lower the sampling arm.

13. Enter the sample name in the Sample ID field and click **Measure**. The A600 readings should be between 0.2 and 1 absorbance unit. Depending on the absorbance, perform one of these steps:
    - If the absorbance reading is >1 abs, dilute beads until the absorbance reading is within the correct range.
    - If the absorbance reading is <0.2 abs, place the tube of beads in the magnetic rack and resuspend them in half the volume of buffer. Be sure to sonicate the beads again according to step 10.

14. Record the absorbance for each sample.

15. Use a Kimwipe® to clean the sample from the sampling arm and the measurement pedestal.

16. Repeat steps 12 to 15 two more times for a total of three readings.

17. Repeat steps 9 to 16 for any remaining samples.

18. (Optional) Save the data as a text document:
    a. Click **Show Report** to open the Data Viewer.
    b. Select **Reports** ▸ **Save Report As**.
    c. Click the **Export Report Table Only** button to save the file in the desired location (see Figure 43).

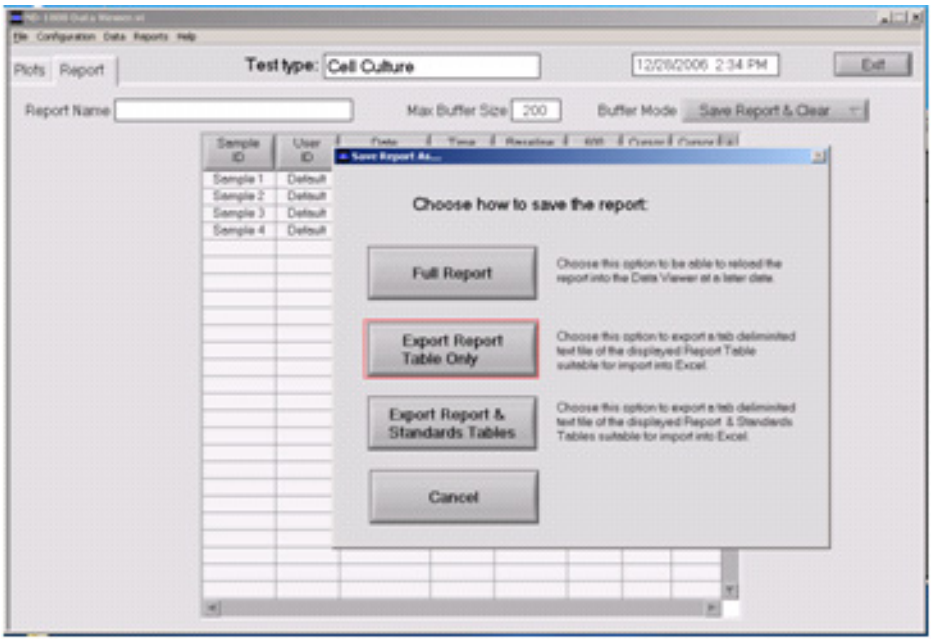


Figure 43 Save Report Software Dialog Box on the NanoDrop® ND-1000 Spectrophotometer.

19. Average the three A600 readings for each sample and calculate the bead concentrations using the appropriate standard curve (see "Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer" on page 103).

Generate a standard curve for calculating bead concentration using the NanoDrop® ND-1000 Spectrophotometer

Materials and equipment required

Table 31 Required equipment

Item	Source
Covaris S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, see "Covaris™ S2 System Materials Summary," *SOLiD™ 3 Plus System Site Preparation Guide*.	Applied Biosystems PN 4387833 (110 V) PN 4392718 (220 V) or Covaris
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Hemocytometer	Major Laboratory Supplier (MLS)
Clicker counter	MLS
Pipettors	MLS

Table 32 Required consumables

Item	Source
SOLiD™ Buffer Kit – 1X TEX Buffer	Applied Biosystems PN 4389776‡
SOLiD™ ePCR Kit – P1 DNA Beads	Applied Biosystems PN 4392175§
0.5-mL LoBind tubes	Eppendorf 022431005
Filtered pipettor tips	MLS

‡ The part number for the complete SOLiD™ Buffer Kit is 4387918.

§ The part number for the complete SOLiD™ ePCR Kit V2 is 4400834.

Procedure

1. Sonicate either P1 DNA Beads or surplus templated beads using the Covalent Declump 3 program on the Covaris™ S2 System (for program conditions, "Covalent Declump 3" on page 119), then pulse-spin the beads.
2. Dilute the beads to a concentration of between 10,000 and 100,000 beads/µL.
3. Place the glass coverslip on the hemocytometer.
4. Pipette 10 µL of diluted beads into the groove of the hemocytometer. Allow the beads to settle for 5 minutes.
5. Count an average of 4 squares of the 25 squares that form the larger center square. Use a clicker counter and count the beads only within the triple lines of the square.
6. Calculate the concentration of beads using the following formula:

   Bead concentration = (average beads in square) × 250 × (dilution factor)

   Example

   Bead concentration = (240 beads) × 250 × 100 = 6.0 × 10^6 beads/µL
7. Rinse, then dry the hemocytometer.
8. According to the hemocytometer counts, dilute ePCR beads in 1✕ TEX to make 10 µL of the following concentrations: 200 K, 400 K, 600 K, 800 K, 1 M, and 1.2 M beads/µL, where K = 10^3 and M = 10^6.
9. Take readings on the NanoDrop® ND-1000 Spectrophotometer for each bead concentration (see "Quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer" on page 98). The lowest absorbance reading should be < 0.2 and the largest absorbance readings should be > 1. If the above dilution series does not meet these criteria, create additional dilutions).
10. Using analysis software such as Microsoft® Office Excel®, average the NanoDrop readings for each concentration and graphically plot absorbance versus bead concentration. A linear trend line gives the equation of the standard curve, $y = mx + b$ (see Figure 4), where:

    y: Absorbance at 600 nm

    m: Slope of the line

    x: Bead concentration (beads/µL)

    b: y-intercept (determined by extrapolating standard curve) (see Figure 44 on page 105)

    Examples

    SOLiD™ P1 DNA Beads: (A600) = (8×10^{-7}) × (Concentration) + 0.08

    Enrichment Beads: (A600) = (4×10^{-6}) × (Concentration) + 0.04

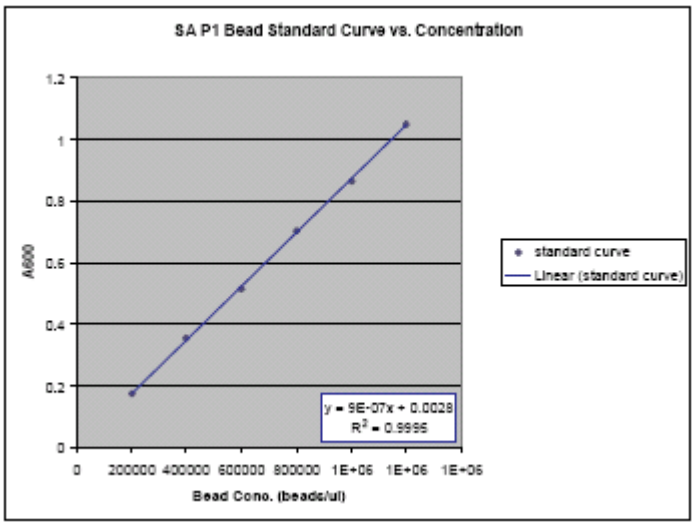


Figure 44 Standard curve generated from NanoDrop® readings of a titration of beads.

11. For added accuracy, repeat steps 1 to 9 with new dilutions and average the resulting curves.

12. Create an Excel analysis worksheet to convert a NanoDrop reading to concentration. The formula is:

$$\text{Concentration} = \frac{(\text{A600 Measurement}) - (y\text{-intercept})}{\text{slope}}$$

Quantitate the beads using a hemocytometer

Materials and equipment required

Table 33 Required equipment

Item	Source
Covaris S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris S2 sonicator • Latitude™ laptop from Dell® Inc. • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTUBEs (1 pack of 25) For system materials summary, see "Covaris™ S2 System Materials Summary," *SOLiD™ 3 Plus System Site Preparation Guide*.	Applied Biosystems PN 4387833 (110 V) PN 4392718 (220 V) or Covaris
Hemocytometer	Major Laboratory Supplier (MLS)
Clicker counter	MLS
Pipettors	MLS‡

‡ For the MSDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Table 34 Required consumables

Item	Source
SOLiD™ Buffer Kit – 1X TEX Buffer	Applied Biosystems PN 4389776‡
0.5-mL LoBind tubes	Eppendorf 022431005
Filtered pipettor tips	Major Laboratory Supplier (MLS)

‡ The part number for the complete SOLiD™ Buffer Kit is 4387918.

Procedure

1. Sonicate the beads using the Covalent Declump 3 program on the Covaris™ S2 System (for program conditions, see "Covalent Declump 3" on page 119), then pulse-spin the beads.
2. Make a dilution of beads in 1✕ TEX Buffer (1:100 dilution recommended for post-emulsion break quantitation and 1:10 dilution recommended for post-3′ end-modification quantitation).
3. Place the glass coverslip on the hemocytometer.
4. Pipette 10 µL of diluted beads into the groove of the hemocytometer. Allow the beads to settle for 5 minutes.
5. Count an average of 4 squares of the 25 squares that form the larger center square. Use a clicker counter and count the beads only within the triple lines of the square.
6. Calculate the concentration of beads using the following formula:

   Bead concentration = (average beads in square) × 250 × (dilution factor)

   Example

   Bead concentration = (240 beads) × 250 × 100 = 6.0×10^6 beads/µL
7. Rinse, then dry the hemocytometer.

D Library Concentration Conversion

Fragment Library:

Assuming average length of 175 bp

$$X \text{ pg/µL DNA} = \frac{500 \text{ pmol}}{1 \text{ L}} \times \frac{660 \text{ pg}}{1 \text{ pmol}} \times \frac{1 \text{ L}}{10^6 \text{ µL}} \times 175 = 60 \text{ pg/µL DNA}$$

Mate-Paired Library (2 × 25 bp):

Assuming average length of 150 bp

$$X \text{ pg/µL DNA} = \frac{500 \text{ pmol}}{1 \text{ L}} \times \frac{660 \text{ pg}}{1 \text{ pmol}} \times \frac{1 \text{ L}}{10^6 \text{ µL}} \times 150 = 50 \text{ pg/µL DNA}$$

Mate-Paired Library (2 × 50 bp):

Assuming average length of 290 bp

$$X \text{ pg/µL DNA} = \frac{500 \text{ pmol}}{1 \text{ L}} \times \frac{660 \text{ pg}}{1 \text{ pmol}} \times \frac{1 \text{ L}}{10^6 \text{ µL}} \times 290 = 96 \text{ pg/µL DNA}$$

E Checklists and workflow tracking forms

This appendix covers:

Workflow checklists: prepare templated beads

	Equipment	Reagents	Preparation steps
Emulsion PCR (ePCR)	☐ Covaris™ S2 System ☐ ULTRA-TURRAX® Tube Drive from IKA® ☐ Thermal cycler ☐ Xstream Pipettor ☐ Repeater Plus Pipette ☐ Magnetic rack ☐ Vortexer ☐ Picofuge ☐ 3-mL syringe ☐ 1-mL syringe ☐ 10-mL syringe ☐ 5-mL Combitip Plus ☐ 10-mL Combitip Plus ☐ 1.5-mL LoBind tubes ☐ 50-mL conical polypropylene tubes ☐ Pipettors ☐ Filtered pipettor tips ☐ 96-well PCR plates ☐ Wide-mouthed jars ☐ Clear adhesive film ☐ Razor blades ☐ Ice	☐ Library template ☐ SOLiD™ ePCR Tube ☐ SOLiD™ P1 DNA Beads ☐ Emulsion Stabilizer 1 ☐ Emulsion Stabilizer 2 ☐ Emulsion Oil ☐ ePCR Primer 1 ☐ ePCR Primer 2 ☐ 10× PCR Buffer ☐ dNTP Mix ☐ Magnesium chloride ☐ 1× Low TE Buffer ☐ 1× TEX Buffer ☐ Bead Block Solution ☐ Nuclease-free water	☐ Turn on Covaris™ S2 System (including chiller and degasser) ☐ Thaw library template, ePCR Primer 1, ePCR Primer 2, dNTP Mix, 10× PCR Buffer
Emulsion break and bead wash	☐ Covaris™ S2 System ☐ NanoDrop™ ND-1000 ☐ Hemocytometer ☐ Microscope ☐ Fume hood ☐ Tabletop centrifuge ☐ Microcentrifuge ☐ Magnetic rack ☐ Vortexer ☐ Picofuge ☐ Multi-channel pipettor ☐ 50-mL reservoirs ☐ 50-mL conical polypropylene tubes ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips ☐ Paper towels	☐ 2-butanol ☐ 1× Bead Wash Buffer ☐ 1× TEX Buffer	☐ Turn on Covaris™ S2 System (including chiller and degasser)

Template bead preparation checklist, continued

	Equipment	Reagents	Preparation steps
Templated bead enrichment	☐ Covaris™ S2 System ☐ Incubator (61 °C) ☐ Microcentrifuge ☐ Rotator ☐ Magnetic rack ☐ Vortexer ☐ Picofuge ☐ 0.5-mL syringe ☐ 1.5-mL syringe ☐ 2.0-mL syringe ☐ 3-mL syringe ☐ 10-mL syringe ☐ 15-mL conical polypropylene tubes ☐ Pipettors ☐ Filtered pipettor tips ☐ Ice	☐ Enrichment Beads ☐ Enrichment Oligo ☐ Denaturing Buffer ☐ Denaturant ☐ Glycerol ☐ 1× Bind & Wash Buffer ☐ 1× TEX Buffer ☐ 1× Low Salt Binding Buffer ☐ Nuclease-free water	☐ Turn on Covaris™ S2 System (including chiller and degasser) ☐ Turn on 61 °C incubator ☐ Thaw Enrichment Oligo
3'-End modification	☐ Covaris™ S2 System ☐ NanoDrop™ ND-1000 ☐ Hemocytometer ☐ Microscope ☐ Incubator (37 °C) ☐ Rotator ☐ Magnetic rack ☐ Vortexer ☐ Picofuge ☐ 1.5-mL LoBind tubes ☐ Pipettors ☐ Filtered pipettor tips	☐ 10× Terminal Transferase Buffer ☐ 10× Cobalt chloride ☐ Terminal Transferase ☐ Bead Linker ☐ 1× Low TE Buffer ☐ 1× TEX Buffer ☐ Nuclease-free water	☐ Turn on Covaris™ S2 System (including chiller and degasser) ☐ Turn on 37 °C incubator ☐ Thaw 10× Terminal Transferase Buffer and Bead Linker

Workflow tracking: prepare templated beads (mini-scale or full-scale)

Sample:			
Quantitation			
After emulsion break & bead wash		After 3'-end modification	
Lot numbers			
Emulsion PCR (ePCR)		**Templated Bead Enrichment**	
SOLiD™ ePCR Kit Box 1 of 3		SOLiD™ Bead Enrichment Kit Box 1 of 3	
SOLiD™ ePCR Kit Box 2 of 3		SOLiD™ Bead Enrichment Kit Box 2 of 3	
SOLiD™ ePCR Kit Box 3 of 3		SOLiD™ Bead Enrichment Kit Box 3 of 3	
Emulsion Stabilizer 1		Denaturing Buffer	
Emulsion Stabilizer 2		Denaturant	
Emulsion Oil		Glycerol	
SOLiD™ ePCR Tube		Enrichment Beads	
ePCR Primer 1		Enrichment Oligo	
ePCR Primer 2		1× Bind & Wash Buffer	
1× Low TE Buffer		1× TEX Buffer	
10× PCR Buffer		1× Low Salt Binding Buffer	
dNTP Mix		**3'-End modification**	
Magnesium chloride		SOLiD™ Bead Deposition Kit 1 of 3	
AmpliTaq Gold DNA Polymerase, UP		10× Terminal Transferase Buffer	
SOLiD™ P1 DNA Beads		10× Cobalt chloride	
Bead Block Solution		Bead Linker	
1× TEX Buffer		Terminal Transferase	
Emulsion break and bead wash		1× TEX Buffer	
2-butanol			
SOLiD™ Emulsion Collection Tray			
1× Bead Wash Buffer			
1× TEX Buffer			

Sample:			
Quantitation			
After emulsion break & bead wash		After 3'-end modification	
Lot numbers			
Emulsion PCR (ePCR)		**Templated bead enrichment**	
SOLiD™ ePCR Kit Box 1 of 3		SOLiD™ Bead Enrichment Kit Box 1 of 3	
SOLiD™ ePCR Kit Box 2 of 3		SOLiD™ Bead Enrichment Kit Box 2 of 3	
SOLiD™ ePCR Kit Box 3 of 3		SOLiD™ Bead Enrichment Kit Box 3 of 3	
Emulsion Stabilizer 1		Denaturing Buffer	
Emulsion Stabilizer 2		Denaturant	
Emulsion Oil		Glycerol	
SOLiD™ ePCR Tube		Enrichment Beads	
ePCR Primer 1		Enrichment Oligo	
ePCR Primer 2		1× Bind & Wash Buffer	
1× Low TE Buffer		1× TEX Buffer	
10× PCR Buffer		1× Low Salt Binding Buffer	
dNTP Mix		**3'-End modification**	
Magnesium chloride		SOLiD™ Bead Deposition Kit 1 of 3	
AmpliTaq Gold DNA Polymerase, UP		10× Terminal Transferase Buffer	
SOLiD™ P1 DNA Beads		10× Cobalt chloride	
Bead Block Solution		Bead Linker	
1× TEX Buffer		Terminal Transferase	
Emulsion break and bead wash		1× TEX Buffer	
2-butanol			
SOLiD™ Emulsion Collection Tray			
1× Bead Wash Buffer			
1× TEX Buffer			

Workflow tracking: prepare templated beads (macro-scale)

Sample:			
Quantitation			
After emulsion break & bead wash (Plate 1)		After 3'-end modification	
After emulsion break & bead wash (Plate 2)			
After emulsion break & bead wash (Plate 3)			
After emulsion break & bead wash (Plate 4)			
After emulsion break & bead wash (Plate 5)			
After emulsion break & bead wash (Plate 6)			
After emulsion break & bead wash (Plate 7)			
After emulsion break & bead wash (Plate 8)			
Lot numbers			
Emulsion PCR (ePCR)		**Templated bead enrichment**	
SOLiD™ ePCR Kit Box 1 of 3		SOLiD™ Bead Enrichment Kit Box 1 of 3	
SOLiD™ ePCR Kit Box 2 of 3		SOLiD™ Bead Enrichment Kit Box 2 of 3	
SOLiD™ ePCR Kit Box 3 of 3		SOLiD™ Bead Enrichment Kit Box 3 of 3	
Emulsion Stabilizer 1		Denaturing Buffer	
Emulsion Stabilizer 2		Denaturant	
Emulsion Oil		Glycerol	
SOLiD™ ePCR Tube		Enrichment Beads	
ePCR Primer 1		Enrichment Oligo	
ePCR Primer 2		1× Bind & Wash Buffer	
1× Low TE Buffer		1× TEX Buffer	
10× PCR Buffer		1× Low Salt Binding Buffer	
dNTP Mix		**3'-End modification**	
Magnesium chloride		SOLiD™ Bead Deposition Kit 1 of 3	
AmpliTaq Gold DNA Polymerase, UP		10× Terminal Transferase Buffer	
SOLiD™ P1 DNA Beads		10× Cobalt chloride	
Bead Block Solution		Bead Linker	
1× TEX Buffer		Terminal Transferase	
Emulsion break and bead wash		1× TEX Buffer	
2-butanol			
SOLiD™ Emulsion Collection Tray			
1× Bead Wash Buffer			
1× TEX Buffer			

F The Covaris™ S2 System

This appendix covers:

Operation notes

Note: For important instrument safety information, refer to the Covaris™ S2 System manual.

Fill the tank

Fill the tank with fresh deionized water to the proper fill line (fill-line level “15” is recommended). The water should cover the visible part of the tube.

Degas the water

Degas the water for 30 minutes. To maintain degassed water, keep the pump continuously on during operation and sample processing.

Set the chiller

Set the chiller temperature to between 2 to 5 °C to ensure that the temperature reading in the water bath displays 5 °C. The circulated water chiller should be supplemented with 20% ethylene glycol.

Perform required maintenance of the Covaris™ S2 System

The Covaris S2 System requires regular maintenance to work properly. Perform the tasks in the table below (see Table 35):

Table 35 Required maintenance of the Covaris™ S2 System

Required maintenance task	Frequency to perform task
Degas water for 30 minutes prior to use	Before every use
Change water	Daily
Clean with bleach	Every two weeks

Covaris™ S2 programs

Bead Block Declump

Table 36 Bead Block Declump: 1 cycle Treatment 1 followed by 1 cycle Treatment 2

	Treatment 1	Treatment 2
Duty Cycle	1%	5%
Intensity	5	5
Cycles/Burst	50	100
Time	5 sec	60 sec
Target wattage power performance estimate (W)‡	2	10

‡ Not programmed

Covalent Declump 1

Table 37 Covalent Declump 1: 1 cycle Treatment 1 followed by 1 cycle Treatment 2

	Treatment 1	Treatment 2
Duty Cycle	2%	5%
Intensity	6	9
Cycles/Burst	100	100
Time	5 sec	30 sec
Target wattage power performance estimate (W)‡	4	15

‡ Not programmed

Covalent Declump 3

Table 38 Covalent Declump 3: 3 cycles Treatment 1 followed by 1 cycle Treatment 2

	Treatment 1	Treatment 2
Duty Cycle	2%	5%
Intensity	6	9
Cycles/Burst	100	100
Time	5 sec	30 sec
Target wattage power performance estimate (W)‡	4	15

‡ Not programmed

G Instrument Warranty Information

This appendix covers:

Computer configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited product warranty

Applied Biosystems warrants that all standard components of the SOLiD™ 3 Plus Analyzer, IKA® ULTRA-TURRAX® Tube Drive, the Covaris™ S2 System, APC UPS, and the recirculating chiller will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. Applied Biosystems warrants the Genomic Solutions HydroShear® DNA Shearing Device will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will replace a defective HydroShear DNA Shearing Device during the warranty period. The following parts of the HydroShear DNA Shearing Device are use- replaceable and not covered by the warranty on the HydroShearDNA Shearing Device: shearing assembly, syringes, syringe adapters, syringe shields, and output tubing. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its SOLiD 3 Plus Analyzer, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days from the date the warranty begins. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on or in the instrument are themselves warranted to be free of defects in materials and workmanship for a period of ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the operating software of the product, if any, will be free of defects in materials and workmanship under normal use. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media.

Unless indicated herein, Applied Biosystems makes no warranty whatsoever in regard to products or parts furnished by third parties, including but not limited to the non-APC- branded UPS or APC UPS, Covaris S2, Genomic Solutions HydroShear DNA Shearing Device, Recirculating Chiller, and IKA ULTRA-TURRAX purchased or obtained from a third party. Such products or parts will be subject to the warranties, if any, of their respective manufacturers to the extent they are 'transferable or otherwise available to Applied Biosystems' buyer.

Applied Biosystems at its sole discretion may refuse to provide buyer with support or service for buyer's use of Covaris S2 in a method not described in a SOLiD System protocol.

Applied Biosystems does not warrant that the operation of the instrument or its operating software will be uninterrupted or be error-free.

Warranty period effective date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for hardware and software installed by Applied Biosystems personnel. For all hardware and software installed by the buyer or anyone other than Applied Biosystems, and for all other products, the applicable warranty period begins the date the product is delivered to the buyer.

Warranty claims

Warranty claims must be made within the applicable warranty period, or, for chemicals or other consumable products, within thirty (30) days after receipt by the buyer unless otherwise specified in the documentation accompanying the product.

Warranty exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specifications or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; modification or repair of the product not authorized by Applied

Biosystems; relocation or movement of the instrument by buyer or by any third party not acting on behalf of Applied Biosystems; or intrusive activity, including without limitation, computer viruses, hackers or other unauthorized interactions with instrument or software that detrimentally affects normal operations.

Parts in contact with any liquid are considered wetted and may be deemed user-replaceable and not be covered by the above warranties, including, but not limited to, seals, filters, gaskets, shearing assemblies, valves, syringes, syringe adapters, syringe shields, and output tubing.

Warranty limitations

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THIS WARRANTY IS LIMITED TO THE BUYER OF THE PRODUCT FROM APPLIED BIOSYSTEMS AND IS NOT TRANSFERABLE.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or willful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Damages, claims, and returns

Damages If shipping damage to the product is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims After a damage inspection report is received by Applied Biosystems, Applied Biosystems will process the claim unless other instructions are provided.

Returns Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the address designated by the Applied Biosystems representative.

H Safety

This appendix covers:

Instrumentation safety

General instrument safety

Note: For important instrument safety information, refer to the *Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Guide* (PN 4442357) and the Covaris™ S2 System manual. For general safety information, see the "Preface" on page vii.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs). See "About MSDSs" on page 130.

Cleaning or decontaminating the instrument

CAUTION! Using a cleaning or decontamination method other than that specified by the manufacturer may result in damage to the instrument.

Physical hazard safety

Solvents and pressurized fluids

WARNING! PHYSICAL INJURY HAZARD. Always wear eye protection when working with solvents or any pressurized fluids.

- Be aware that PEEK™ tubing is a polymeric material. Use caution when working with any polymer tubing that is under pressure.

  Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames if you use flammable solvents.
- Do not use PEEK tubing that has been severely stressed or kinked.
- Do not use PEEK tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause PEEK tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40 mL/min) may cause a static charge to build up on the surface of the tubing. Electrical sparks may result.

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 130.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.

- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

  IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Glossary

3′-end modification	Process by which dUTP is added to the 3′ end of the P2 Adaptor on the templated beads using a terminal transferase reaction
aqueous phase	Emulsion component comprised of SOLiD™ P1 DNA Beads, library template, primers, DNA polymerase, dNTPs, and water
broken emulsion	An emulsion in which the aqueous phase appears at the bottom of a PCR plate well prior to emulsion break
Denaturing Buffer solution (or prepared Denaturing Buffer solution)	Solution made up of Denaturing Buffer and Denaturant used to make the template on templated beads single-stranded and to dissociate templated beads from enrichment beads during the enrichment step
emulsion break	Process by which the micro-reactors in an emulsion are broken using 2-butanol to allow processing of templated beads
emulsion PCR (ePCR)	Process by which DNA fragments are clonally amplified onto beads in individual droplets in an emulsion
enrichment	Process by which templated beads are isolated from non-amplifying beads using enrichment beads
enrichment beads	Polystyrene beads with a single-stranded P2 Adaptor attached to capture templated beads
full-scale templated bead preparation	Templated bead preparation process that yields 150 to 300 million templated beads
library	Set of DNA tags prepared from the same biological sample to be sequenced on the SOLiD™ System
macro-scale templated bead preparation	Templated bead preparation process that yields 600 million to 2.4 billion templated beads
micro-reactor	Droplet of aqueous phase in the emulsion in which amplification takes place
mini-scale templated bead preparation	Templated bead preparation process that yields 75 to 150 million templated beads
monoclonal bead	Templated bead with a single template

monoclonal micro-reactor	Micro-reactor containing a single template
monoclonal bead	Templated bead with a single template
multi-bead micro-reactor	Micro-reactor containing multiple beads
non-amplifying bead	SOLiD P1 DNA bead with no template
non-clonal micro-reactor	Micro-reactor containing no template
oil phase	Emulsion component of oil and emulsifiers
optimal library concentration	Library template concentration that gives the best sequencing results
P2-enriched beads	Enriched, templated beads
polyclonal bead	Templated bead with a multiple templates
pulse-spin	Place the tube in a picofuge and spin for a few seconds to bring down any beads or liquid stuck on the walls of the tube.
remove the supernatant	Use a pipette to carefully remove the liquid from the tube without disturbing any beads.
resuspend the beads	The beads can be resuspended in one of two ways: • Gently pipette the solution up and down until the beads are suspended. Using a slower speed to aspirate and expel the solution minimizes the amount of beads that stick to the inside of the pipette tip. • Vortex the solution until all of the beads are suspended. Place the beads in a picofuge, then pulse-spin the beads for a few seconds to bring down any beads stuck on the walls of the tube. Do not over-spin the beads, or the beads pellet.
SOLiD™ P1 DNA Beads	Bead with P1 Adaptor attached
sonicate the beads	Place the tube containing the beads in the appropriate tube holder, then place the holder in the Covaris™ S2 System. Next, run the appropriate Covaris S2 program.
templated bead preparation	Process of adding library template to beads by emulsion PCR, enriching the beads to remove beads without template, and modifying the 3′ end of the template on the beads to prepare for bead deposition and sequencing
templated beads	SOLiD P1 DNA Beads with amplified library template attached

titration	Library template concentration used to prepare an emulsion
workflow analysis (WFA) run	Type of run on the SOLiD™ System in which a small portion of templated beads are deposited and analyzed to test for templated bead quality

Related documentation

Document	Part number	Description
Applied Biosystems SOLiD™ 3 Plus System Library Preparation Guide	4442697	Describes how to prepare fragment and mate-paired libraries for templated bead preparation and sequencing on the SOLiD™ 3 Plus System.
Applied Biosystems SOLiD™ 3 Plus System Library Preparation Quick Reference Card	4442698	Provides brief, step-by-step procedures for preparing libraries.
Applied Biosystems SOLiD™ 3 Plus System Templated Bead Preparation Quick Reference Card	4442696	Provides brief, step-by-step procedures for preparing templated beads by emulsion PCR (ePCR), required before sequencing on the SOLiD™ 3 Plus System.
Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Guide	4442357	Describes how to load and run the SOLiD™ 3 Plus System for sequencing.
Applied Biosystems SOLiD™ 3 Plus System Instrument Operation Quick Reference Card	4442358	Provides brief, step-by-step procedures for loading and running the SOLiD™ 3 Plus System.
Applied Biosystems SOLiD™ 3 Plus System Site Preparation Guide	4444009	Provides all the information that you need to set up the SOLiD™ 3 Plus System.
Applied Biosystems SOLiD™ SETS Software v3.5 Getting Started Guide	4444007	Provides an alternate platform to monitor runs, modify settings and reanalyze previous runs that are performed on the SOLiD System.
Applied Biosystems SOLiD™ ICS Software v3.5 Help	—	Describes the software and provides procedures for common tasks (see the Instrument Control Software).
Applied Biosystems SOLiD™ Analysis Tools (SAT) v3.5 Reference Guide	4443929	Provides advanced technical information on how to modify pipelines for in-depth sequencing analysis using the SOLiD System.
Applied Biosystems SOLiD™ BioScope Software v1.0 Getting Started Guide	4442694	Provides a bioinformatics analysis framework for flexible application analysis (data-generated mapping, SNPs, count reads) from sequencing runs.

Note: For additional documentation, see "How to obtain support" on page viii.

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating *only* to documentation. To order documents, download PDF files, or for help with a technical question, see "How to obtain support" on page viii.

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Part Number 4442695 Rev. A 10/2009

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