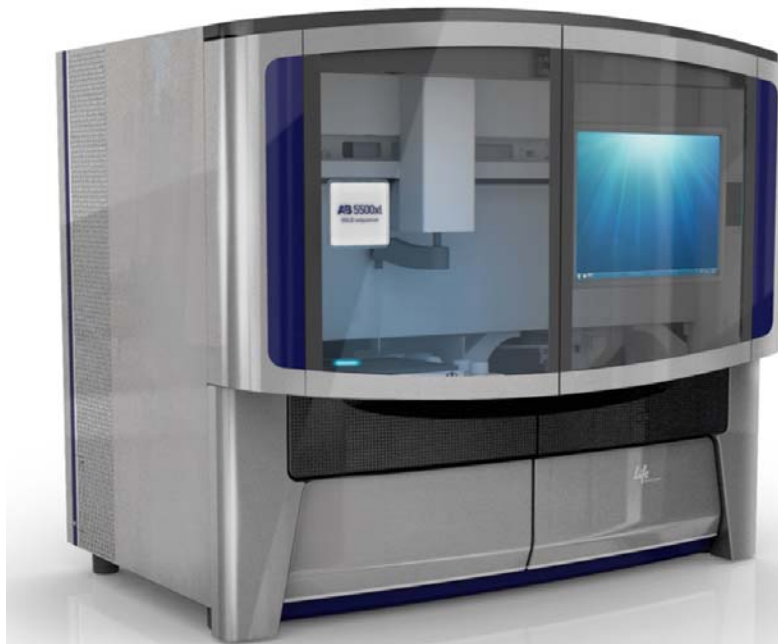


5500 Series Genetic Analysis Systems

SOLiD® FlowChip Preparation for Higher Deposition Densities
AB Demonstrated Protocol



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Preface

Applied Biosystems (AB) protocols for the 5500 Series Genetic Analysis Systems can be described by the following categories in the table below. This is an AB Demonstrated protocol.

Category	Description
AB Supported (S)	Testing and validation have been performed by Applied Biosystems for this protocol on this instrument system. The technical support and field application specialists have been trained to support this protocol.
AB Demonstrated (A)	Applied Biosystems has tested this protocol but no validation was performed for this instrument system. Certain components of the protocol workflow such as reagent kits and other protocols for preparation of reagents may not be available through Applied Biosystems. Supporting documentation such as application notes may be available from Applied Biosystems and/or third parties. Limited support is available from Applied Biosystems.
Customer Demonstrated (C)	The performance of this protocol has not been evaluated on this instrument by Applied Biosystems. However, at least one customer or third party has reported successfully performing this protocol on this instrument. Applied Biosystems cannot guarantee instrument and reagent performance specifications with the use of customer demonstrated protocols. However supporting documentation from Applied Biosystems and/or third parties may be available and Applied Biosystems may provide basic guidelines in connection with this protocol.
Experimental/Not Supported (N)	This protocol has not been evaluated and/or tested on this instrument by either Applied Biosystems or its customers. Applied Biosystems cannot warranty that attempting to utilize this protocol will not adversely affect the functionality of the instrument and other Applied Biosystems products. Data generated by this protocol on this instrument may not be fully representative of typical results. Applied Biosystems does not provide any support for this protocol.

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About this protocol

This protocol provides a method to deposit beads at higher deposition densities and enable greater throughput per sequencing run. In addition to requirements in the deposition procedure in the *5500 Series Genetic Analyzers User Guide* (PN 4456991), this procedure requires more templated beads for deposition, as well as centrifuging the SOLiD® FlowChip after filling the lanes with beads.

Prepare for bead deposition

Your beads should be prepared using the SOLiD® EZ Bead® System and 3'-modified before using them for deposition. For more information, refer to the *SOLiD® EZ Bead® Enricher Getting Started Guide* (PN 4443496).

Required materials

- P100 calibrated pipette and tips
- P200 calibrated pipette and tips
- P1000 calibrated pipette and tips
- Magnetic rack
- Covaris® S220 or Covaris® S2 Sonicator with adapter for Eppendorf 1.5-mL DNA LoBind® Tubes
- Vortexer
- Incubator
- PicoFuge® Microcentrifuge or other mini - centrifuge
- SOLiD® FlowChip Deposition Kit (contains SOLiD® FlowChip Deposition Buffer 1 and six tubes of SOLiD® Sequencing Control Beads, one for each lane)
- SOLiD® FlowChip, 6-Lanes package (contains the FlowChip in a deposition cassette, and two adhesive strips for sealing the holes in the FlowChip)
- Lint-free wipes
- Eppendorf 1.5-mL DNA LoBind® Tubes
- Tabletop Centrifuge



NOTE Use Eppendorf LoBind® Tubes to perform all steps requiring 1.5-mL tubes. LoBind Tubes from other vendors may have a chemical coating that can have adverse effects on bead deposition.

Set up the equipment

1. Ensure that the Covaris® sonicator water bath is cooled to 5 °C and degassed. For details about the Covaris® System and the protocols used for bead preparation, see Appendix B, “Covaris® S2 and S220 Systems” on page 89 of the *5500 Series Genetic Analyzers User Guide* (PN 4456991).
2. Set the incubator temperature to 37 °C.

Thaw the FlowChip

Remove the sealed FlowChip from the freezer and allow it to thaw to room temperature before opening the package. This takes three to five minutes.

The FlowChip is sealed inside a plastic bead deposition cassette. Do *not* remove the FlowChip from the deposition cassette before depositing beads.



CAUTION! The FlowChip is made of glass and is fragile. To avoid cutting yourself or breaking the FlowChip, when handling the FlowChip use caution.

Troubleshooting: Putting the FlowChip back in the cassette

If you have removed the FlowChip from the deposition cassette before you have filled the lanes, put it back in the cassette using the following steps:

1. Handle the FlowChip by the edges to avoid smearing or damaging the surface.
2. Ensure that the O-rings are centered precisely above the holes in the FlowChip.
3. Place the FlowChip in the cassette with the corners lined up so that the FlowChip is level and seated completely in the cassette.
4. Gently snap the cover of the cassette down over the FlowChip.

Important tips on FlowChip storage

- Store unused FlowChips at -15°C to -25°C to ensure optimal bead deposition and to minimize loss of P2-enriched beads.
- After thawing, do not leave the FlowChip at room temperature for more than 24 hours.
- After depositing the beads and incubating the FlowChip, either store the FlowChip at 4°C for up to two weeks, or place it immediately on the sequencer. Do not store FlowChips with deposited beads in the freezer.

Calculate the volume of beads

1. To quantitate the beads, use one of the following: (a) the NanoDrop® ND-1000 Spectrophotometer, see “NanoDrop® ND-1000 Spectrophotometer protocols” on page 99 of the *5500 Series Genetic Analyzers User Guide* (PN 4456991); (b) the Work Flow Analysis run; or (c) the Applied Biosystems SOLiD® Bead Concentration Chart (PN 4415131).
2. Record the lane volume from the FlowChip box. This will be used as the final resuspension volume.
3. Calculate and record the volume of stock beads (your sample beads) that are needed to deposit in each lane using the following formula:

$$\text{Bead Volume} = (400,000,000 \text{ beads}) / \text{sample bead concentration}$$

Example:

$$(400,000,000 \text{ beads}) / (1,000,000 \text{ beads}/\mu\text{L}) = 400 \mu\text{L of sample beads}$$

Prepare the beads

Prepare the beads and add them to the SOLiD® Sequencing Control beads



NOTE

SOLiD® Sequencing Control beads must be present in each deposited lane. To ensure even distribution of SOLiD® Sequencing Control beads, always prepare one tube of sample beads per lane using one tube of SOLiD® Sequencing Control beads. Do not prepare samples for different lanes in the same tube, even if the same sample will be used.

1. Using your stock (sample) beads stored at 4 °C, place the sample tube in the 1.5-mL tube adapter and sonicate the stock tube using Covaris® sonicator module Covalent Declump 1.
2. To lock the tube in the adapter:
 - a. Place the top of the tube against the spring-loaded plunger as shown below (no beads are shown in this sample tube).



- b. Push the plunger up and slide the tube in, allowing the spring action to lock the tube in place.
3. Remove the tube from the sonicator adapter and pulse-spin 2 to 3 seconds in a microfuge to bring down any liquid on the sides of the tube. Do not pellet the beads.
 4. Aspirate the volume of beads that you calculated using the Bead Formula from step 3 in the previous section.
 5. If you are depositing beads for a sequencing run, transfer the beads to one SOLiD® Sequencing Control beads tube (in the SOLiD® FlowChip Deposition Kit).

Wash the beads three times

First wash

1. Place the tube in a magnetic rack and wait one minute for the solution to become clear.



NOTE

Magnetic bead capture may take longer than a minute. If beads do not pellet well on the magnet, then slowly pipette some of the supernatant up and down in the bottom of the tube to help the beads pellet against the magnet.



2. Carefully remove the supernatant and resuspend the beads in 400 μ L of SOLiD® FlowChip Deposition Buffer 1.
3. Remove the tube from the rack and vortex the tube thoroughly.
4. Transfer the volume to a new 1.5-mL DNA LoBind® Tube and pulse-spin the tube briefly (2 to 3 seconds). Do not pellet the beads.

Second wash

1. Place tube in a magnetic rack and wait one minute for the solution to become clear.



NOTE

If you place the LoBind® Tube in the magnetic rack with the hinge of its cap facing the rack (as shown above), it will be easier to open the tube when you pipette.



NOTE

Magnetic bead capture may take longer than a minute. If beads do not pellet well on the magnet, then slowly pipette some of the supernatant up and down in the bottom of the tube to help the beads pellet against the magnet.

2. Remove the supernatant and resuspend the beads in 400 μ L of SOLiD® FlowChip Deposition Buffer 1.
3. Vortex the tube thoroughly, then pulse-spin the tube briefly (2 to 3 seconds). Do not pellet the beads.

Final wash

1. Place the tube onto the magnetic rack and wait at least one minute for the solution to become clear before removing supernatant.



NOTE Magnetic bead capture may take longer than a minute. If the beads do not pellet well on the magnet, slowly pipette some of the supernatant up and down in the tube to help the beads pellet against the magnet.

2. Resuspend the beads in 400 µL of SOLiD® FlowChip Deposition Buffer 1.
3. Vortex the tube thoroughly, then pulse-spin the tube briefly (2 to 3 seconds). Do not pellet the beads.

Final buffer removal and bead resuspension

1. Place the tube onto the magnetic rack and wait at least one minute for the solution to become clear before removing supernatant. The solution may not seem to clear as quickly as the first two washes. This is to be expected.

2. Use a P200 pipette set to 200 µL with regular P200 tips to remove all of the supernatant.



NOTE Having a volume greater than suggested will result in a lower bead density than was targeted.

3. Capture the beads and resuspend in a volume of SOLiD® FlowChip Deposition Buffer 1. Resuspend beads into the appropriate volume, as listed on the FlowChip package label.
4. Vortex the tube thoroughly, then pulse-spin the tube briefly (2 to 3 seconds). Do not pellet the beads.
5. Place the tube in the Covaris® sonicator and select the Deposition_Declump protocol.
6. Take the tube out of the sonicator and pulse-spin the tube again briefly (2 to 3 seconds). Do not pellet the beads.



NOTE Deposit the beads immediately after sonication. Do not let the beads sit in the tube after you have sonicated them. We suggest you use pipette tips that fit snugly in the fill holes in the deposition cassette.

Deposit the beads on the FlowChip

Remove adhesive strips from the deposition cassette

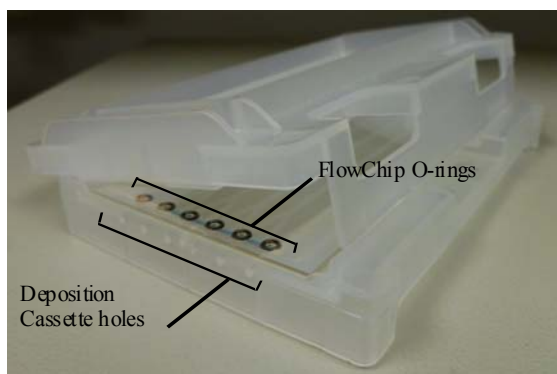
To allow accurate filling of the lanes, the FlowChip is contained in a deposition cassette. This cassette has six holes at each end, covered with an adhesive strip on the left and right outside edges. The FlowChip also has six holes at each end, and these holes are sealed with small black O-rings.

1. Carefully peel back the adhesive strips covering the six holes at each end of the deposition cassette.
2. Discard both adhesive strips.

The figure on the next page shows the holes in the cassette, and the FlowChip inside with O-rings.



NOTE The purpose of this figure is only to show you the cassette with the FlowChip and O-rings inside. Do not open the cassette until you have filled the FlowChip.



Important tips on handling the FlowChip



CAUTION! The FlowChip is made of glass and is fragile. To avoid cutting yourself or breaking the FlowChip when handling the FlowChip, use caution.

- To ensure that beads adhere to the correct surface of the Flowchip, always hold the FlowChip level and do not flip the FlowChip over (holes up) during any portion of the protocol.
- To avoid contamination or fingerprints, always wear gloves and hold the FlowChip by the edges only.
- The FlowChip is fragile. Always handle it gently to avoid breaking it.
- Do not rub or wipe the FlowChip with any material to remove condensation. Wiping might scratch the FlowChip surface and interfere with imaging.

Confirm that the pipette tips fit the deposition cassette

It is important to use pipette tips that fit snugly into the holes in the deposition cassette without “bottoming out” or being loose. If the tip of the pipette is loose in the hole, the beads can leak out around it.

Check the fit of your pipette tip by inserting one of them in a hole in the cassette. If the pipette tip does not fit snugly, use a different tip.

Aspirate the beads

1. Make sure the FlowChip has reached room temperature.
2. Set the P100/P200 pipette to the bead resuspend volume + 3 μ L. Setting the pipette to a slightly larger volume than the resuspend volume helps ensure that all the beads are aspirated from the tube, and deposited into the lane.
3. Aspirate the beads. If a small bubble forms in the end of the pipette tip after pipetting all of the bead solution, this will not interfere with bead deposition.

Troubleshooting your deposition volume

- If the volume in the tube is larger than what is suggested: To improve bead concentration, re-capture the beads, resuspend in a volume 1 or 2 μL smaller than suggested on the package, re-sonicate the beads, and then move forward with the deposition.
- If the volume in the tube is smaller than what was suggested: Add 1 to 2 μL of deposition buffer, re-sonicate the beads, and then move forward with deposition.

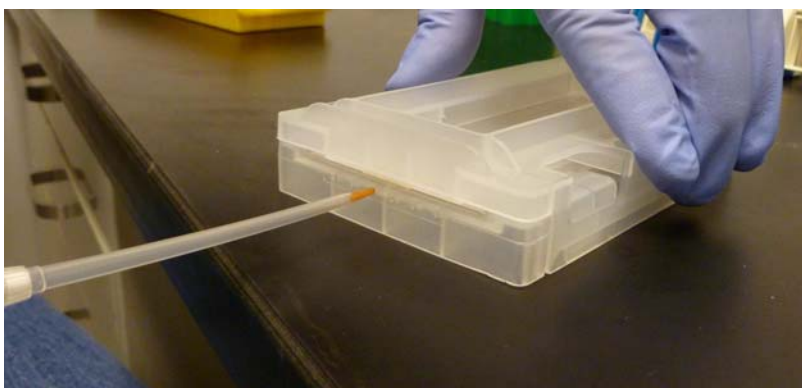
Fill the lanes in the FlowChip with beads

1. Hold the FlowChip in the deposition cassette flat on the bench.



IMPORTANT!

To ensure that beads adhere to the correct surface of the FlowChip, keep the FlowChip level and do not turn it upside down (holes up) during any portion of the protocol.



2. Firmly insert the tip of the pipettor into the hole located on the side of the FlowChip deposition cassette that corresponds to the lane you wish to fill. Avoid flexing the tip of the pipette. If the tip seems loose, put the beads back in the tube and change to a tip with a snug fit.
3. Dispense the beads into the lane in one gentle, smooth motion. The beads flow by capillary action to fill the lane.

Tips on filling the lane

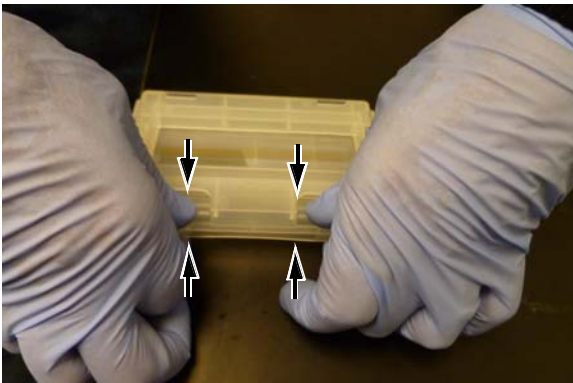
- A bubble at the beginning of the lane will not affect imaging.
- To avoid introducing bubbles in the middle of the lane, do not pause or pull back on the pipette.
- If a bubble is introduced into the lane, the beads may not adhere to the FlowChip and subsequent reagent and buffer washes can cause the bubble to dislodge beads.
- If the beads do not completely fill the lane, but are evenly distributed and fill about 90% of the lane, the sequencer will still be able to image the panels you have filled. However, imaging may take extra time because the sequencer will check the empty areas of the lane looking for missing beads.

Remove the FlowChip from the deposition cassette



CAUTION! The FlowChip is made of glass and is fragile. To avoid cutting yourself or breaking the FlowChip, handle the FlowChip with caution.

1. After you have deposited beads in the lanes, open the plastic FlowChip deposition cassette by placing a thumb on each indented clamp, reaching under the edge of the deposition cassette, and snapping it open.



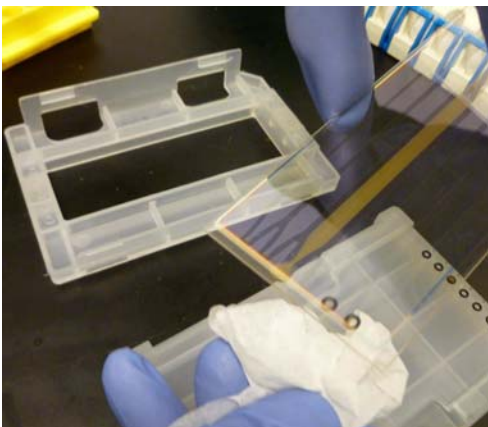
2. Holding the FlowChip by its edges, carefully lift it out of the deposition cassette.



NOTE As stated earlier, hold the FlowChip horizontally, by its edges only, and do not flip it over (holes up).

The O-rings from the deposition cassette may cling to the holes on the underside of the FlowChip.

3. If any O-rings have clung to the FlowChip, use a lint-free wipe to gently wipe the holes on the FlowChip, removing the O-rings and any excess fluid.



IMPORTANT!

Do not discard the O-rings or the deposition cassette as they will be used later in the protocol. Proceed immediately to centrifuging the FlowChip.

Centrifuge the FlowChip

1. Holding the FlowChip horizontal and by its edges, place the FlowChip in a tabletop centrifuge on a lint-free wipe with the ports facing down (the same configuration as it was in the cassette). Do not apply an adhesive strip. Be sure the FlowChip “notch” is away from the center of the rotor. Centrifuge at 805 x g for 5 minutes. Consult the manual specific to your centrifuge or rotor to convert g-forces to rpm, if needed.



IMPORTANT!

Spin the FlowChip so the ports are facing down. Spinning the FlowChip with the ports facing up will result in catastrophic bead loss.



IMPORTANT!

To ensure that beads do not dry out, proceed through the rest of the protocol immediately after the centrifugation is complete.

Return the FlowChip to the deposition cassette

1. While the FlowChip is in the centrifuge, place all the O-rings into the FlowChip deposition cassette in preparation for returning the FlowChip into the deposition cassette.
2. Remove the FlowChip from the centrifuge while holding the FlowChip horizontal and by its edges. Handle the FlowChip by the edges to avoid smearing or damaging the surface.
3. Ensure that the O-rings are centered precisely above the holes in the FlowChip.
4. Place the FlowChip in the cassette with the corners lined up so that the FlowChip is level and seated completely in the cassette.
5. Gently snap the cover of the cassette down over the FlowChip.

Fill the lanes in the FlowChip with Deposition Buffer

1. Set the P100/P200 pipette to the bead resuspend volume + 3 µL.
2. Aspirate the SOLiD® FlowChip Deposition Buffer 1. Try to fill the pipette tip completely, avoiding any bubble forming in the end of the pipette tip. A bubble may interfere with the lane being filled completely.
3. Hold the FlowChip in the deposition cassette flat on the bench:



IMPORTANT!

To ensure that beads adhere to the correct surface of the FlowChip, keep the FlowChip level and do not turn it upside down (holes up) during any portion of the protocol.

4. Firmly insert the tip of the pipettor into the hole located on the side of the FlowChip deposition cassette that corresponds to the lane you wish to fill. Avoid flexing the tip of the pipette. If the tip seems loose, change to a tip with a snug fit.
5. Dispense the SOLiD® FlowChip Deposition Buffer 1 into the lane in one gentle, smooth motion. The buffer flows by capillary action to fill the lane.
6. Apply an adhesive strip (found in the FlowChip box) over the six holes on both ends of the deposition cassette. Do not turn it upside down. This prevents the beads from drying out.

Incubate the FlowChip

1. Place the FlowChip in the incubator for 60 minutes at 37 °C.



IMPORTANT!

Make sure that you incubate the FlowChip in the deposition cassette with both ends of the deposition cassette sealed with adhesive strips and with the FlowChip ports facing down.



IMPORTANT!

Do not incubate the FlowChip for longer than 1 hour. Incubation for more than one hour will result in the beads drying out, affecting sequencing quality.

Remove the FlowChip from the deposition cassette



CAUTION!

The FlowChip is made of glass and is fragile. To avoid cutting yourself or breaking the FlowChip, handle the FlowChip with caution.

2. After you have incubated the FlowChip in the deposition cassette, open the plastic FlowChip deposition cassette by placing a thumb on each indented clamp, reaching under the edge of the deposition cassette, and snapping it open.
3. Holding the FlowChip by its edges, carefully lift it out of the deposition cassette.



NOTE

Hold the FlowChip horizontally, by its edges only, and do not flip it over (holes up).

The O-rings from the deposition cassette may cling to the holes on the underside of the FlowChip.

4. If any O-rings have clung to the FlowChip, use a lint-free wipe to gently wipe the holes on the FlowChip, removing the O-rings and any excess fluid.
5. After incubation, the FlowChip can be immediately placed on the sequencer following the instructions starting on page 29 of the *5500 Series Genetic Analyzers User Guide* (PN 4456991), or stored for up to two weeks at 4 °C.
Before placing the FlowChip in storage, “sandwich” it between two lint-free cloths and wrap it in aluminum foil to ensure that it’s not exposed to light.



NOTE

Do not store a FlowChip with deposited beads in the freezer.

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