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Agencourt CleanSEQ Dye-Terminator Removal Protocol

Agencourt CleanSEQ is a SPRI (Solid Phase Reversible Immobilization) magnetic bead-based sequencing purification system with a simple three-step protocol. The Agencourt CleanSEQ method can be performed directly in the thermal cycling plate and requires no centrifugation or filtration. The system efficiently purifies sequencing products to deliver superior quality sequencing data.

NOTE For CleanSEQ Application Notes, search the following documents online:

- *CleanSEQ for use with the ABI PRISM 3100*
- *The MD Anderson SPRI Experience: Using Agencourt CleanSEQ and Agencourt AMPure in HLA Sequencing-Based Typing*

Beckman Coulter has developed optimized CleanSEQ protocols for common sequencing dye sets, including Life Technologies and Beckman Coulter. Instructions for these sequencing chemistries are located in the following sections:

- **Life Technologies:** *CleanSEQ for Life Technologies BigDye Terminator Protocol*
- **Beckman Coulter:** *CleanSEQ Protocol for Use with Beckman Coulter DTCS Quick Start Chemistry*

NOTE For ET Terminator users, a CleanSEQ for MegaBACE protocol is available at:
https://www.beckmancoulter.com/wsrportal/bibliography?docname=Protocol+000411v001.pdf

In addition to the CleanSEQ protocols, this document contains a list of *CleanSEQ FAQs*, *Abbreviations*, and a *Glossary*.

Usage

NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC APPLICATIONS.

The Agencourt CleanSEQ system is a rapid, high performance dye-terminator removal process based on Solid Phase Reversible Immobilization (SPRI) technology. The paramagnetic bead format requires no centrifugation or filtration and is easily performed manually or fully automated for high throughput dye-terminator removal.

Reagent Contents

- Agencourt CleanSEQ Reagent

Sequencing Reaction Yields

Table 1 provides the number of reactions that each CleanSEQ kit yields, depending on plate format and quantity of reagent.

Table 1 CleanSEQ Quantity/Yield Per Plate Format

Plate Format	Approximate Number of Sequencing Reactions for 8 mL, PN A29151	Approximate Number of Sequencing Reactions for 50 mL, PN A29154
96-Well Format	800	5,000
384-Well Format	1,600	10,000

Storage Conditions

- Store at 4°C upon arrival, for up to eighteen months (see the bottle label for expiration date).
- Mix CleanSEQ well before using.

Statement of Warnings

The following is a list of potential hazards to consider when using CleanSEQ.

IMPORTANT For complete product safety information, refer to the CleanSEQ safety data sheet (PN **A29161**), located on www.beckmancoulter.com.

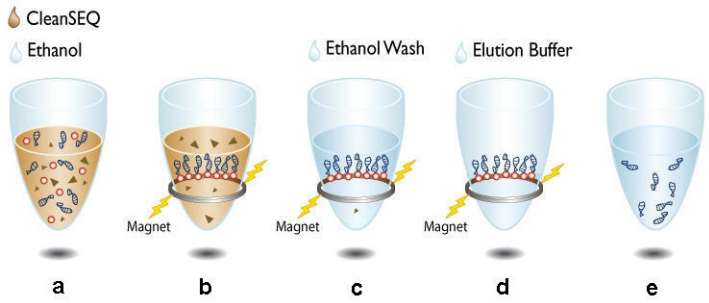
- Sodium azide forms explosive compounds with heavy metals. This product contains concentrations of azide <0.1% (w/w) which with repeated contact with lead and copper commonly found in plumbing drains may result in the build up of shock sensitive compounds.
- If product is inhaled, move exposed individual to fresh air. If individual is not breathing, begin artificial respiration immediately and obtain medical attention.
- If product enters eyes, wash eyes gently under running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occur, obtain medical attention.
- In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If pain or irritation occur, obtain medical attention.
- If ingested, wash mouth out with water. If irritation or discomfort occurs, seek medical attention.
- Dispose of waste product, unused product and contaminated packaging in compliance with federal, state and local regulations. If unsure of the applicable requirements, contact the authorities for information. Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).

Process Overview

The CleanSEQ dye-terminator removal protocol involves the following three main steps (Figure 1):

1. Bind sequencing extension products to magnetic beads **(a)**; then separate on a magnet plate **(b)**.
2. Wash beads **(c)** to remove unincorporated dyes, nucleotides, salts, and other contaminants.
3. Elute the DNA using an aqueous buffer **(d)** **(e)**.

Figure 1 Process Overview



CleanSEQ for Life Technologies BigDye Terminator Protocol

NOTE CleanSEQ can be used with all BigDye Terminator versions (1.1 and 3.1).

The CleanSEQ for Life Technologies BigDye Terminator Protocol contains two different protocols, one for each of the available plate options. Follow the instructions in the applicable section:

- *Protocol for 96-Well Plate Format*
- *Protocol for 384-Well Plate Format*

NOTE To determine which procedure is appropriate for achieving the desired sequencing reaction yield, refer to Table 1.

Protocol for 96-Well Plate Format

Materials Required

This section provides a complete listing of the materials required for completing the CleanSEQ for Life Technologies BigDye Terminator protocol for the 96-well plate format:

- For **Consumables and Accessories**, refer to Table 2.
- For **Reagents**, refer to Table 3.

Table 2 Consumables and Accessories — 96-Well Format

Item	Manufacturer	Part Number/Description
SPRIPlate Magnetic Plate	Beckman Coulter	• **A29164**, Agencourt SPRIPlate 96R – Ring Magnet Plate OR • **A32782**, Agencourt SPRIPlate 96R – Ring Super Magnet Plate
Reaction Plate	ThermoFisher Scientific, Inc.	• **AB-0800**, 96-Well Cycling Plate OR • **AB-1400**, 96-Well Cycling Plate **NOTE** For product information, see www.thermofisher.com or www.fishersci.com.
Direct Inject Magnet (*Optional*)	Beckman Coulter	• **A29173**, Agencourt Direct Inject Plate (96-Well Plate) **NOTE** Use of the Direct Inject Magnet is optional, and using it eliminates the need to perform step 12 of the procedure below.
Multichannel Pipettes	N/A	For 10 µL and 100 µL (minimum) or 20 µL and 200 µL volumes

Table 3 Reagents — 96-Well Format

Item	Manufacturer/Part Number	Description	Notes
CleanSEQ	Beckman Coulter, • **A29151** (8 mL) or • **A29154** (50 mL)	Agencourt CleanSEQ Kit - Dye Terminator Removal	Shake vigorously before use to ensure the magnetic beads are fully resuspended.
Ethanol	• American Bioanalytical, **AB-00138** or • equivalent[a]	85% Ethanol, Made From Non-Denatured Ethanol	• Make 25 mL of 85% ethanol per 96-well plate. • Ethanol is used for binding in the CleanSEQ protocol, so it is critical that the 85% ethanol be made fresh. • To avoid loss of product, make only enough to be used in 1-3 days and store in a tightly capped container. • *For product information, see www.americanbio.com.*
Elution Buffer	• Ambion, **AM9937** or • American Bioanalytical, **AB00502** *(diluted with Ambion,* **AM9937**) or • equivalent[a]	Reagent grade water or 0.1mM EDTA (pH 8.0)	• The optimal elution buffer varies, depending on reaction conditions.

a. An equivalent is a product with the same designation or purity specifications.

Procedure for the 96-Well Format

NOTE For troubleshooting the Life Technologies BigDye Terminator protocol, refer to *Troubleshooting Life Technologies BigDye Terminator Sequencing Reactions*.

1 Shake the CleanSEQ reagent to fully resuspend the magnetic beads before using. The reagent should appear homogenous and consistent in color. Ensure that no visible bead pellet remains in the bottle.

2 Add 10 µL of CleanSEQ reagent to each sample; use 10 µL of CleanSEQ regardless of the sequencing reaction volume.

3 Add 85% ethanol to each sample according to Table 4. Pipette mix seven times, or until the solution is homogenous throughout each well (ethanol floats to the top of the sample, while the CleanSEQ sinks to the bottom).

IMPORTANT Mix the layers well to completely bind the sequencing products to the magnetic beads.

Table 4 Calculating the Volume of Ethanol for a 96-Well Plate Format

Sequencing Reaction Volume (µL)	Volume of 85% Ethanol (µL)
5	31
10	42
15	52
20	62
25	73

To calculate other sample volumes not listed in Table 4, use the formula below:

- Volume of 85% Ethanol = 2.077 × (10 µL + Sample Volume)

4 Place the sample plate onto a Beckman Coulter Agencourt SPRIPlate 96R for 3-5 minutes, or until the solution is clear. The magnetic beads form a ring or a crescent on the side of the well.

IMPORTANT Perform this step while the plate is seated on the magnet.

5 Aspirate the cleared solution (supernatant) from the plate and discard; ensure that all liquid is removed from every well. To avoid disturbing the beads, place the pipette tip at the bottom of the well when aspirating. Remove as much supernatant as possible since it contains excess fluorescent dye and contaminants.

IMPORTANT Perform this step while the plate is seated on the magnet. It is not necessary to mix or resuspend the beads during this step.

6 Dispense 100 µL of 85% ethanol into each well. Wait at least 30 seconds to allow the beads to resettle before continuing to the next step.

IMPORTANT Perform this step while the plate is seated on the magnet.

7 Completely remove the ethanol and discard. Ensure that all liquid is removed from every well. To avoid disturbing the beads, place the pipette tip at the bottom of the well when aspirating. Remove as much ethanol as possible, since it contains excess fluorescent dye and contaminants.

8 Repeat steps 6 and 7 for a total of two 85% ethanol washes.

9 Let the samples air-dry for 10 minutes at room temperature. The sample plate can be seated on or off the magnet while drying.

OR

Continue to the next step, as the optimal time should be determined experimentally, based on your specific conditions.

NOTE Excessive drying can lead to degradation of the fluorescent dye.

10 Add 40 µL of elution buffer into each well. See Table 5 and incubate the plate for 2–5 minutes at room temperature to elute. If using the Beckman Coulter 96 Direct Inject Magnet Plate* (eliminates final plate transfer), elute with 70–80 µL elution buffer.

NOTE Consider the following while eluting samples:

- Elution of the sequencing products is rapid. It is not necessary for the beads to go back into solution for complete recovery.
- If the elution volume is doubled when eluting in EDTA, then the concentration must be reduced by half so that the number of EDTA molecules is not increased.
- Do not denature samples prior to loading on capillary sequencers.

The suggested elution buffers are:

- 0.1mM EDTA (pH 8.0)†
- reagent grade water

Water is used to give maximum signal, while EDTA is used to lower the signal in cases where the signal is too strong. The appropriate elution buffer varies depending on:

- the sensitivity of the sequencing detector
- the amount of BigDye used per sequencing reaction type
- the type of template

Use Table 5 as a general guideline for choosing an elution buffer.

Table 5 Guideline for Choosing an Elution Buffer

Sequencing Reaction Type	Life Technologies 3100[a]/3130/3500/3730	Life Technologies 3700
>2 µL BigDye with PCR Products	0.1mM EDTA	0.1mM EDTA
<2 µL BigDye with PCR Products	0.1mM EDTA	DiH_2O
>2 µL BigDye with Plasmids	0.1mM EDTA	DiH_2O
<2 µL BigDye with Plasmids	DiH_2O	DiH_2O

a.For Life Technologies 3100 users or other users currently eluting in formamide, see the application note *CleanSEQ for use with the ABI PRISIM 3100* at https://www.beckmancoulter.com/wsrportal/bibliography?docname=Agencourt_CleanSEQ_3100_AppNote.pdf

* Refer to Direct Inject Protocol at www.beckmancoulter.com; PN A29173.

† Use 0.05mM EDTA in place of 0.1mM EDTA when using Direct Inject Magnet.

11 Allow the sample plate to separate on the magnet for 3–5 minutes or until the solution is clear.

IMPORTANT Complete this step only if **NOT** using the Beckman Coulter Agencourt Direct Inject Plate (96-Well Plate, PN **A29173**). This step is not necessary when loading on Life Technologies 3700.

12 **OPTIONAL**: Transfer 35 µL of the clear sample into a new plate for loading on the detector.

Leave 5–10 µL of liquid behind to prevent transfer of beads into the final plate. Residual beads can interfere with injection, causing late starts or failed injections. If this occurs, simply re-transfer the samples away from the beads and re-inject.

13 Seal the samples and store at 4°C, for up to 24 hours, prior to loading. If the samples will not be loaded within 24 hours, store them at –20°C. Samples can be kept at –20°C for approximately one month.

Protocol for 384-Well Plate Format

Materials Required

This section provides a complete listing of the materials required for completing the CleanSEQ for Life Technologies BigDye Terminator protocol for the 384-well plate format:

- For **Consumables and Accessories**, refer to Table 6.
- For **Reagents**, refer to Table 7.

Table 6 Consumables and Accessories — 384-Well Format

Item	Manufacturer	Part Number/Description
SPRIPlate Magnetic Plate	Beckman Coulter	• **A29165**, Agencourt SPRIPlate 384 Magnet Plate
Reaction Plate	ThermoFisher Scientific, Inc.[a]	• **AB-1111**, 384-Well Hardshell Cycling Plate
Direct Inject Magnet[b] (*Optional*)	Beckman Coulter	• **A29166**, Agencourt Direct Inject Plate (384-Well Plate)
Multichannel Pipettes	N/A	For 10 µL and 100 µL (minimum) or 20 µL and 200 µL volumes.

a. For information, see www.thermofisher.com or www.fishersci.com.

b. Use of the Direct Inject Magnet is optional. Use of the Direct Inject Magnet eliminates the need to perform step 12 in the procedure below.

Table 7 Reagents — 384-Well Format

Item	Manufacturer/Part Number	Description	Notes
CleanSEQ	Beckman Coulter, • **A29151** (8 mL) or • **A29154** (50 mL)	Agencourt CleanSEQ Kit - Dye Terminator Removal	Shake vigorously before use to ensure the magnetic beads are fully resuspended.
Ethanol	• American Bioanalytical, **AB-00138** or • equivalent[a]	85% ethanol made from non-denatured ethanol	• Make 25 mL of 85% ethanol per 96-well plate. • Ethanol is used for binding in the CleanSEQ protocol, so it is critical that the 85% ethanol be made fresh. • To avoid loss of product, make only enough to be used in 1-3 days and store in a tightly capped container. • *For product information, see www.americanbio.com.*
Elution Buffer	• Life Technologies, **AM9937** (Ambion) or • American Bioanalytical, **AB00502** (*diluted with Ambion, AM9937*) or • equivalent[a]	Reagent grade water or 0.1mM EDTA (pH 8.0)	• The optimal elution buffer varies depending on reaction conditions. • *For product information, see www.lifetechnologies.com and www.americanbio.com.*

a. An equivalent is a product with the same designation or purity specifications.

Procedure for the 384-Well Format

NOTE For troubleshooting the Life Technologies BigDye Terminator protocol, refer to *Troubleshooting Life Technologies BigDye Terminator Sequencing Reactions*.

1 Shake the CleanSEQ Reagent to fully resuspend the magnetic beads before using. The reagent should appear homogenous and consistent in color. Ensure that no visible bead pellet remains in the bottle.

2 Add 5 µL of CleanSEQ Reagent to each sample; use 5 µL of CleanSEQ regardless of the sequencing reaction volume.

3 Add 85% ethanol to each sample according to Table 8. Pipette mix seven times, or until the solution is homogenous throughout each well (ethanol floats to the top of the sample, while the CleanSEQ sinks to the bottom).

IMPORTANT Mix the layers well to completely bind the sequencing products to the magnetic beads.

Table 8 Calculating the Volume of Ethanol for a 384-Well Plate Format

Sequencing Reaction Volume (µL)	Volume of 85% Ethanol (µL)
5	14.3
10	21.4

To calculate other sample volumes not listed in Table 8, use the formula below:

- Volume of 85% ethanol = 1.428 × (5 µL + Sample Volume)

4 Place the sample plate onto a Beckman Coulter Agencourt SPRIPlate 384 Magnet Plate (PN **A29165**) for 2–3 minutes or until the solution is clear. The magnetic beads accumulate to the side of the well.

IMPORTANT Perform this step while the plate is seated on the magnet.

5 Aspirate the cleared solution (supernatant) from the plate and discard; ensure that all liquid is removed from every well. To avoid disturbing the beads, place the pipette tip at the bottom of the well when aspirating. Remove as much supernatant as possible since it contains excess fluorescent dye and contaminants.

IMPORTANT Perform this step while the plate is seated on the magnet.

6 Dispense 30 µL of 85% ethanol into each well.

- **OPTIONAL**: Pipette mix seven times to rinse the beads. Wait at least 30 seconds to allow the beads to resettle before continuing to the next step.

IMPORTANT Perform this step while the plate is seated on the magnet.

7 Completely remove the ethanol and discard. Ensure that all liquid is removed from every well. To avoid disturbing the beads, place the pipette tip at the bottom of the well when aspirating. Remove as much ethanol as possible, since it contains excess fluorescent dye and contaminants.

8 Repeat steps 6 and 7 for a total of two 85% ethanol washes.

9 Let the samples air-dry for 10 minutes at room temperature, or continue to the elution step. Determine the optimal time experimentally, based on your specific conditions.

The sample plate can be situated on or off the magnet while drying.

NOTE Excessive drying can lead to degradation of the fluorescent dye.

10 Add 15–30 µL of elution buffer into each well. See Table 9 and incubate the plate for 2–5 minutes at room temperature to elute.

IMPORTANT Consider the following while eluting samples:

- Elution of the sequencing products is rapid. It is not necessary for the beads to go back into solution for complete recovery.
- Do not denature samples prior to loading on capillary sequencers.

The suggested elution buffers are:

- 0.1mM EDTA (pH 8.0)
- reagent grade water

Water is used to give maximum signal, while EDTA is used to lower the signal in cases where the signal is too strong. The appropriate elution buffer varies depending on:

- the sensitivity of the sequencing detector
- the amount of BigDye used per sequencing reaction type
- the type of template

Use Table 9 as a general guideline for choosing an elution buffer.

Table 9 Guideline for Choosing an Elution Buffer

Sequencing Reaction Type	Life Technologies 3100/3130/3500/3730	Life Technologies 3700
>2 µL BigDye with PCR Products	0.1mM EDTA	0.1mM EDTA
<2 µL BigDye with PCR Products	0.1mM EDTA	DiH_2O
>2 µL BigDye with Plasmids	0.1mM EDTA	DiH_2O
<2 µL BigDye with Plasmids	DiH_2O	DiH_2O

11 Allow the sample plate to separate on the magnet for 3–5 minutes, or until the solution is clear.

IMPORTANT Complete this step only if **NOT** using the Beckman Coulter (**PN A29166**) Agencourt Direct Inject Plate (384-Well Plate). This step is not necessary when loading on Life Technologies 3700.

12 **OPTIONAL:** Transfer clear sample into a new plate for loading on the detector. Leave 2–5 µL of liquid behind to prevent transfer of beads into the final plate. Residual beads can interfere with injection, causing late starts or failed injections. If this occurs, re-transfer the samples away from the beads and re-inject.

Troubleshooting Life Technologies BigDye Terminator Sequencing Reactions

Table 10 Troubleshooting Guide for Life Technologies BigDye Terminator Only

Symptom	Reason	Solution
Dye Blobs (*dye peaks usually at 70 and 100 bases*)	Insufficient Supernatant Removal	• Check the plate visually, and make sure that the supernatant and ethanol washes are removed completely. • Aspirate a second time, if necessary.
	Too Much BigDye	• Use less BigDye per sequencing reaction. • Contact reagentsupport@beckman.com, if needed.
	Formamide Elution	• Elute in water or EDTA. • See Protocol and Application Note: *CleanSEQ on ABI 3100* (www.beckmancoulter.com).
	Ethanol Concentration Too High	• Add the correct amount of ethanol for the sequencing reaction volume, and make sure that ethanol is correctly mixed (see Table 4 and Table 8).
Low Signal (*signal intensity is similar to intensity of background noise*)	Insufficient Mix	• Ensure that the appropriate number of mixes are performed. • Visually inspect the wells to ensure reactions look homogeneous.
	Bead Loss	• Ensure that no beads are aspirated during supernatant removal, but if this happens, dispense back the supernatant. Attempt again with a smaller volume after beads have re-settled.
	Low Ethanol Concentration	• Ensure ethanol and stock bottle is made fresh at least weekly. • Ensure that the correct volume is added for sequencing reaction volume. • Measure ethanol and water in separate containers before combining.
Overload (*signal intensity is extremely high, may appear as flat peaks in electropherogram)*	Too Much BigDye	• Use less BigDye per sequencing reaction; contact reagentsupport@beckman.com, if needed. • Transfer only part of the eluant for loading. • If the signal is only high in the beginning of the electropherogram and then rapidly decreases, elute in EDTA. **NOTE** If the signal is still too high after eluting in 0.1 mM EDTA, a higher concentration of EDTA can be used. The ideal concentration may need to be determined experimentally.

Table 10 Troubleshooting Guide for Life Technologies BigDye Terminator Only *(Continued)*

Symptom	Reason	Solution
C and/ or G Shoulders (*bump on the right side of C or G bases; might actually look like an underlying base to the base on the right*)	Degradation	• Increase the elution buffer to 70-80 µL (only applies to the 96-well format). • If increasing the elution buffer does not help, or if using a 384-well format, elute in Melatonin or TCEP instead: **IMPORTANT** If you normally elute in water, then make up the Melatonin or TCEP as indicated below. If you normally elute in EDTA (due to high signal), then instead of using water in the recipes below, use 0.1 mM (or the concentration you normally use) of EDTA. **To elute in Melatonin:** **1.** Dissolve 26.135 mg melatonin (Sigma, PN **M5250**) in 1.25 mL isopropanol (for example, American Bioanalytical, PN AB00866). **2.** Fill to 50 mL with water. **3.** Store melatonin solution at 2–8°C. OR **To elute in TCEP:** **1.** Add 100 µL Thermo Fisher PI-77720 to 1250 mL nuclease-free water (Ambion, PN **AM9932**). **2.** Invert 10 times. • If elution in TCEP or melatonin does not help, the degradation might happen during the purification. Add 200 µL of TCEP to each liter of 85% ethanol used for binding and washing. • Minimize any time the samples are dry without any liquid in the well.
Top Heavy Reads (*signal starts out very strong, but quickly diminishes and reads are short*)	Sequencing Reaction Produces a Lot of Short Sequencing Fragments	• Spike EDTA into the reaction and reload. EDTA competes for injection with primarily short sequencing fragments. The concentration of EDTA to add depends on the severity of the overload. In a 96-well format, spike in 20–40 µL of 0.1–1 mM EDTA; in a 384-well format, use less, but use a higher concentration. • If this happens frequently, consider cutting the amount of primer added to the sequencing reaction at least by half.

CleanSEQ Protocol for Use with Beckman Coulter DTCS Quick Start Chemistry

Materials Required

This section provides a complete listing of the materials required for completing the CleanSEQ for use with Beckman Coulter DTCS Quick Start Chemistry protocol:

- For **Consumables and Accessories**, refer to Table 11.
- For **Reagents**, refer to Table 12.

Table 11 Consumables and Accessories

Item	Manufacturer	Part Number/Description
Magnetic Plate	Beckman Coulter	• **A32782**, SPRIPlate 96R-Ring Super Magnet Plate
Sample Plate	Beckman Coulter	• **609801**, Plate, Microtiter, 96-well, V-Bottom, Polypropylene, 200 µL
Multichannel Pipettes	N/A	For 10 µL and 100 µL (minimum) or 20 µL and 200 µL volumes.

Table 12 Reagents

Item	Manufacturer/Part Number	Description	Notes
CleanSEQ	Beckman Coulter, • **A29151** (8 mL) or • **A29154** (50 mL)	Agencourt CleanSEQ Kit - Dye Terminator Removal	• For product information, see www.beckmancoulter.com.
Mineral Oil	Sigma, **M5904**	BioReagent, for molecular biology, light oil	• *For product information, see www.sigmaaldrich.com.*
Ethanol	• American Bioanalytical, **AB-00138** or • equivalent[a]	85% Ethanol (made from non-denatured ethanol)	• Make 25 mL of 85% ethanol per 96-well plate. • Ethanol is used for binding in the CleanSEQ protocol, so it is critical that the 85% ethanol be made fresh. • To avoid loss of product, make only enough to be used in 1–3 days and store in a tightly capped container. • *For product information, see www.americanbio.com.*
Sample Loading Solution (SLS)	Beckman Coulter, **608120**	GenomeLab DTCS Quick Start Kit	Supplied with the Beckman Coulter Dye Terminator Cycle Sequencing Quick Start Kit.

a. An equivalent is a product with the same designation or purity specifications.

Procedure

NOTE For troubleshooting the CleanSEQ for Use with Beckman Coulter DTCS Quick Start Chemistry protocol, refer to *Troubleshooting the CleanSEQ Protocol for use with Beckman Coulter DTCS Quick Start Chemistry*.

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1 Beckman Coulter recommends the use of Beckman Coulter CEQ sample plates (PN **609801**) for sequencing reactions. If non-Beckman Coulter sample plates were used, transfer each entire sequencing reaction mixture into a well of the Beckman Coulter sample plate.

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2 Gently shake the CleanSEQ bottle (PN **A29151** and PN **A29154)** to re-suspend the magnetic beads.

NOTE There should be no residue at the bottom of the bottle. The reagent should appear homogenous and consistent in color. Ensure that no visible bead pellet remains in the bottle.

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3 Add 10 µL of CleanSEQ to every sample, regardless of the sequencing reaction volume.

4 Add an amount of fresh 85% ethanol to the sample according to Table 13.

NOTE Make 25mL of 85% ethanol per 96-well plate.

- Ethanol is used for binding in the CleanSEQ protocol, so it is critical that the 85% ethanol be made fresh.
- To avoid loss of product, make only enough to be used in 1–3 days and store in a tightly capped container.

Table 13 Proportional Amount of Ethanol to Add for a Given Sequencing Reaction Volume

Sequencing Reaction Volume (µL)	Volume of 85% Ethanol (µL)
10	42
20	62

To calculate other sample volumes not listed in Table 13, use the formula below:

- Volume of 85% Ethanol = 2.077 × (10 µL + Sample Volume)

5 Adjust the pipette to the appropriate volume, pipette up and down seven times or vortex at medium speed to suspend the beads and the reaction mix.

6 Place the sample plate onto a Beckman Coulter SPRIPlate 96R-Ring Super Magnet Plate (PN **A32782**) for 3-5 minutes, or until the solution is clear. The magnetic beads form a ring or a crescent on the side of the well.

7 Adjusting the pipette to the appropriate volume, carefully and completely aspirate the cleared supernatant from the sample plate and discard the supernatant.

8 Add 100 µL of 85% ethanol and incubate for 30 seconds.

9 Completely remove the supernatant and discard as described in step 7.

10 Remove the sample plate from the magnet and immediately and add 40 µL of Sample Loading Solution (SLS) (PN **608082**) to each sample well.

IMPORTANT Do not dry the beads! Drying of the beads leads to loss of sample and lower signal intensities.

11 Adjusting the pipette to the appropriate volume, pipette up and down seven times or vortex at medium speed until the beads are in suspension.

IMPORTANT This step is critical to prevent the negatively charged beads from being injected into the capillary and causing current crashes.

12 Pellet the magnetic beads to the bottom of each well:

a. Cover the SPRI-magnetic plate completely with a piece of paper as a barrier between the wells and magnetic rings.

b. Place the sample plate on top of the magnetic plate covered with paper. All beads in the solution will be attracted to the bottom within two minutes.

13 Overlay the samples with a drop of mineral oil (Sigma, PN **M5904**).

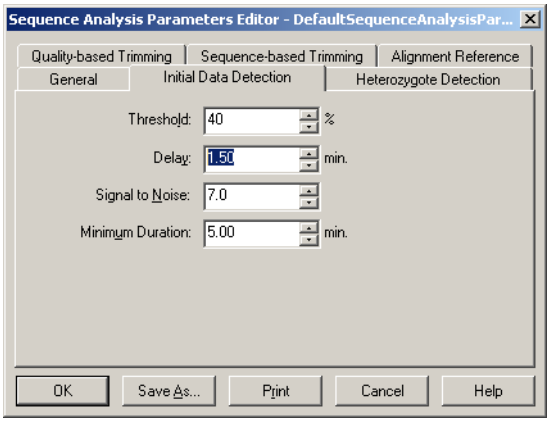
14 Load the plate onto the CEQ and run with standard methods.

15 Edit the Sequence Analysis Parameters to increase the delay migration time from 0.75 to 1.5 minutes; see instructions in the applicable the user guide:

- *CEQ 8000 User's Guide* (PN A16637), rev AA, p. 151
- *CEQ 8800 User's Guide* (PN A16638), rev AA, p. 161
- *GenomeLab GeXP User's Guide* (PN A29142) rev AB, p. 114

16 Click **OK** to analyze the results with these new parameters. See Figure 2.

Figure 2 Sequence Analysis Parameters Editor



This step helps to exclude the noise peaks generated from the leftover free nucleotides and dye in the sequencing reactions.

Troubleshooting the CleanSEQ Protocol *for use with Beckman Coulter DTCS Quick Start Chemistry*

Table 14 Troubleshooting Guide for the CleanSEQ Protocol (for use with Beckman Coulter DTCS Quick Start Chemistry)

Symptom	Reason	Solution
No Signals, But Dye Present	Wrong DNA Templates	• Ensure that the correct DNA templates are used.
	Wrong Primers	• Ensure that the correct primers are used.
	Poor Primer Design	• Ensure that the primers do not form the secondary structures. • Alternatively, use the Beckman Coulter Method Development Kit (PN 60800).
	Reagent Problem	• Ensure that the reagents are added properly.
	Degradation of Dye, or Instrument Problem	• Contact dnasupport@absciex.com for further help specific to DTCS chemistry or GeXP hardware or software.
Low Signals	Primer to Template Ratio Problem	• Recommend primer concentration in a standard sequencing reaction is at 30:1 to 40:1 (Primers: templates).
	Beads are Over Dried	• Ensure not to over dry the beads before adding the SLS buffer. If perform 96 samples, process 24 samples per batch, aspirate the ethanol completely and add the SLS buffer immediately.
	Low/Wrong Ethanol Concentration	• Ensure to use freshly made 85% good quality ethanol. • Ensure that correct 85% ethanol volume is added for the sequencing reaction volume.
	Bead Loss	• Ensure that no beads are aspirated during supernatant removal, but if this happens, re-suspend the supernatant and aspirate the supernatant with a smaller volumes after beads have resettled.
Overload Signals (*dye signal is saturated*)	Too Much Template	• Reduce sample injection. • Reduce cycle number. • Reduce template amount.
	Too Much Primer	• Reduce sample injection. • Reduce cycle number. • Reduce primer amount.

CleanSEQ FAQs

Q — Can I elute BigDye sequencing reactions in Formamide?

A — Formamide reduces the signal of BigDye sequencing reactions and when it gets in contact with beads it causes dye blobs. Beckman Coulter therefore recommends elution in water or EDTA. See the application note on using a mineral overlay if evaporation is a concern. See https://www.beckmancoulter.com/wsrportal/bibliography?docname=Agencourt_CleanSEQ_3100_AppNote.pdf.
The mineral oil overlay can also be used on Life Technologies 3730.

If formamide is the only option, elute the sample in water, transfer it away from the beads, dry it down and resuspend it in formamide.

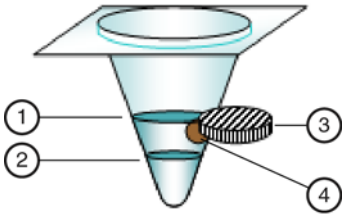
Q — Can beads damage my capillaries?

A — No. A large amount of beads can interfere with injection, but they do not cause permanent damage. The beads are too small to clog the capillaries. Beckman Coulter recommends a transfer away from the beads or the use of a direct inject magnet to prevent failed injections. A small amount of bead carryover into the plate loaded on the detector is not a concern.

Q — How is the direct inject magnet used?

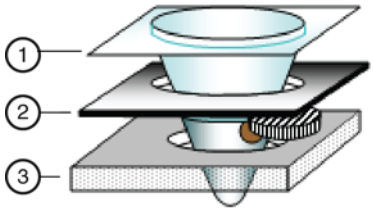
A — The direct inject magnet is placed between the loading plate and the loading cassette, where it holds the beads to the side. Use one direct inject magnet for each plate you plan to load into the stacker at the same time. See Figure 1.3, Figure 1.5, Figure 1.4, and Figure 1.6.

Figure 1.3 Well Containing Elution Buffer at 40 µL or 80 µL



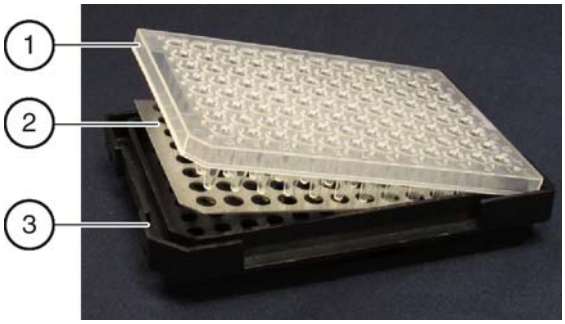
1. 80 µL Elution
2. 40 µL Elution
3. Magnet
4. Bead Pellet

Figure 1.4 Well Inserted Through the Plate Base and Direct Inject Magnet



1. Sample Plate
2. 96 Direct Inject Magnet
3. Life Technologies Plate Base

Figure 1.5 96-Well Direct Inject Magnet



1. PCR Plate
2. 96-Well Direct Inject Plate
3. Base

Also contains (not shown)

- Retainer
- Seal

Figure 1.6 384-Well Direct Inject

1. Retainer
2. PCR Plate
3. 384-Well Direct Inject Magnet
4. Base

Q — Which Life Technologies plate bases can be used with the direct inject magnet?

A — The 96-well direct inject magnet fits into Life Technologies 4334873 and 4334875. It does NOT fit with 4367473 and 4367469 for FAST PCR plates. The 384-well direct inject magnet fits 4334877 (heat seal), but does not fit 4334874 (septa seal).

Q — Why does the protocol recommend 5 µL of CleanSEQ per well in 384-well format and 10 µL in 96-well format?

A — The volumes were determined by experiment. If less reagent is used, the signal becomes inconsistent, and samples with inherently lower signal have a higher chance of failing.

Q — Why do I not need to resuspend the beads during the ethanol washes?

A — The amount of beads in the reagent is low enough that a simple rinse will be effective in most cases.

Q — How does a change in elution volume affect the data?

A — For the 3100, 3130, 3730, it does not have a significant impact. The injection is electrokinetic, and no volume is transferred; consequently, the detector has access to all the sequencing products in a well. If EDTA is used for elution, the total number of EDTA molecules will determine the signal. So, if a certain EDTA concentration is used, and increasing the volume is considered, the EDTA concentration would be reduced by half if the volume is doubled. For the 3700, the elution volume will impact signal and should be taken into consideration.

Q — The kit was left at room temperature for an extended amount of time. Can I still use it?

A — The reagent is quite stable, but can only be guaranteed if it is stored at the recommended temperature.

Q — My kit has expired. Can I still use it?

A — Beckman Coulter only guarantees the performance of the product until the expiration date. The reagent is quite stable. If used past the expiration date, the data quality should be monitored closely.

Q — I accidentally froze the kit. Is it okay to use?

A — From numerous customer reports and Beckman Coulter's own experience, freezing CleanSEQ does not impact performance.

Q — How long can sequencing products be stored after cleanup and before detection?

A — At +4°C, a day or two; at –20°C, weeks to months. Samples that are not eluted are more stable at –20°C than samples that are eluted. Be sure that the plate is sealed well.

Q — How long do you recommend to dry the samples before elution?

A — It depends on the environment of the lab. Most labs can skip elution entirely because trace ethanol does not have a detrimental effect on sequencing data, but zero minutes up to 20 minutes works best.

Q — How long should samples elute before being transferred away from the beads?

A — Most of the sequencing products elute off rapidly if they are BigDye products; longer than two minutes should not give additional signal.

Q — Does it help if I resuspend the beads for elution?

A — In most cases a passive elution without mixing is perfectly fine; however, some labs have found an increase in signal when mixing the beads during elution. This is most likely due to the elution buffer causing the DNA to adhere to the beads more strongly than normal.

Q — Can mineral oil overlay be used in 384-well format to avoid formamide elution?

A — Yes. Elute in 12µL and add 5-8µL mineral oil. Do not increase the total volume over 20 µL if septa seal is used in a 384-well format.

Abbreviations

µL — microliter

CEQ — CEQ 8000 Series Genetic Analysis System

DiH_2O — deionized water

DNA — deoxyribonucleic acid

DTCS — Dye Terminator Cycle Sequencing

EDTA — ethylenediaminetetraacetate

ET — Dye Terminator chemistry

mM — milli molar

PCR — Polymerase Chain Reaction

PN — part number

SPRI — Solid Phase Reversible Immobilization

TCEP — Tris (2-carboxyethyl) phosphine

Glossary

bind — To combine with, form a chemical bond with, or be taken up by, as an enzyme with its substrate.

buffer — A substance that minimizes change in the acidity of a solution when an acid or base is added to the solution.

capillary sequencer — DNA sequencer using capillary electrophoresis for separation.

contaminants — A substance in a particular specimen that may skew diagnostic or research results.

direct inject magnet — Beckman Coulter Magnet Plates allowing injection of the sample from the cleanup plate still containing magnetic beads.

dye blob — Unincorporated dyes obscuring part of the sequence in the electropherogram.

dye terminator — A nucleotide linked to a fluorescent dye that is missing the 3' OH group and thus ending synthesis of the DNA strand.

dye-terminator removal — Purification step eliminating unincorporated dyes.

electropherogram — Graphical depiction of data obtained by electrophoresis in automated sequencing.

elute — To extract (one material) from another, usually by means of a solvent.

ethanol — Also called ethyl alcohol, grain alcohol, ethanol. a colorless, volatile, flammable liquid, C_2H_5OH.

fluorescent dye — chemical compound re-emitting light after light excitation.

isopropanol — Common name for a chemical compound with the molecular formula C_3H_8O or C_3H_7OH. It is a colorless, flammable chemical compound with a strong odor.

magnetic beads — Beads with iron content that are attracted by a magnetic field.

magnet plate — Plate containing magnets that are used to attract the magnetic beads in a solution.

nucleotide — Any of various compounds consisting of a nucleoside combined with a phosphate group and forming the basic constituent of DNA and RNA.

plasmid — A circular, double-stranded unit of DNA that replicates within a cell independently of the chromosomal DNA and is most often found in bacteria; it is used in recombinant DNA research to transfer genes between cells.

precipitation — The process of formation of a solid previously held in solution or suspension in a liquid.

protocol — An explicit, detailed plan of an experiment, procedure, or test.

sequencing extension products — Strands of DNA produced by cycle sequencing reactions.

sequencing reaction — Enzymatic reaction that creates sequencing products.

supernatant — The liquid lying above a layer of precipitated insoluble material.

unincorporated dye — Dye terminator molecules not used by the sequencing reaction.

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