Preparing Extension Products for Electrophoresis

Overview

Preparation of extension products for electrophoresis will vary depending on the cycle sequencing chemistry used.

Dye Terminator Chemistries

Unincorporated dye terminators must be removed before the samples can be analyzed by electrophoresis. Excess dye terminators in sequencing reactions obscure data in the early part of the sequence and can interfere with basecalling.

Several protocols for each sequencing chemistry are presented to offer a choice of reagents and process. We recommend performing controlled reactions with each method to determine the one that works best for you.

- Precipitation methods are cheaper and faster, but if performed poorly can leave unincorporated dye-labeled terminators that can obscure data at the beginning of the sequence.

  Refer to the *Precipitation Methods to Remove Residual Dye Terminators from Sequencing Reactions User Bulletin* (P/N 4304655). This document can be obtained from the Applied Biosystems WWW site (www.appliedbiosystems.com/techsupport).

- The spin column and 96-well plate procedures remove all excess terminators if performed correctly, but are more costly than precipitation methods.

Dye Primer Chemistries

The standard procedure is ethanol precipitation, which concentrates the sample. An Express Load option is also available for BigDye primers (see page 3-49).

Table 3-3 Recommended Methods for Preparing Extension Products for Electrophoresis

Chemistry	Recommended Methods	See Page
Rhodamine Dye Terminator and dRhodamine Terminator	Spin Column Purification	3-34
	96-Well Plate Purification Protocol	3-35
	Ethanol/Sodium Acetate Precipitation	3-41
	Ethanol/$MgCl_2$ Precipitation	3-43
	Shrimp Alkaline Phosphatase Digestion	3-45
BigDye Terminator	Spin Column Purification	3-34
	96-Well Plate Purification Protocol	3-35
	Isopropanol Precipitation	3-36
	Ethanol Precipitation for BigDye Terminators	3-38
	Ethanol/Sodium Acetate Precipitation	3-41
Fluorescein/ Rhodamine Dye Primer	Ethanol Precipitation for Fluorescein/Rhodamine Dye Primers	3-46
BigDye Primer	Ethanol Precipitation for BigDye Primers	3-47
	Express Load Option for BigDye Primers Run on 36-Lane Gels	3-49

To precipitate extension products in MicroAmp Trays: *(continued)*

Step	Action
9	Remove the tray and discard the paper towel. **Note** Pellets may or may not be visible. Vacuum drying of the samples is not necessary.

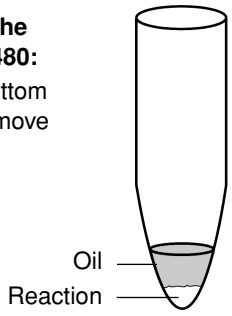
Precipitation in Microcentrifuge Tubes

Reagents and equipment required for this method:

- 1.5-mL microcentrifuge tubes
- Benchtop microcentrifuge, capable of reaching at least 14000 × *g*
- Vacuum centrifuge
- 75% Isopropanol (2-propanol) or 100% isopropanol (anhydrous) at room temperature

Note This procedure does not use salt.

To precipitate extension products in microcentrifuge tubes:

Step	Action
1	Pipet the entire contents of each extension reaction into a 1.5-mL microcentrifuge tube. **To remove reactions run on the TC1 or DNA Thermal Cycler 480:** Place the pipette tip into the bottom of the reaction and carefully remove the reaction from the oil.  **IMPORTANT** Transfer as little oil as possible.
2	Add one of the following: ♦ 80 µL of 75% isopropanol or ♦ 20 µL of deionized water and 60 µL of 100% isopropanol The final isopropanol concentration should be 60 ± 5%.
3	Close the tubes and vortex briefly.
4	Leave the tubes at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
5	Place the tubes in a microcentrifuge and mark their orientations. Spin the tubes for 20 minutes at maximum speed. **IMPORTANT** Proceed to the next step immediately. If not possible, then spin the tubes for 2 minutes more immediately before performing the next step.

To precipitate extension products in microcentrifuge tubes: *(continued)*

Step	Action
6	Carefully aspirate the supernatants with a separate pipette tip for each sample and discard. Pellets may or may not be visible. **IMPORTANT** The supernatants must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the tubes, the more unincorporated dye terminators will remain in the samples.
7	Add 250 µL of 75% isopropanol to the tubes and vortex them briefly.
8	Place the tubes in the microcentrifuge in the same orientation as in step 5 and spin for 5 minutes at maximum speed.
9	Aspirate the supernatants carefully as in step 6.
10	Dry the samples in a vacuum centrifuge for 10–15 minutes or to dryness. (Alternatively, place the tubes with the lids open in a heat block or thermal cycler at 90 °C for 1 minute.)

Ethanol Precipitation for BigDye Terminators

Note These procedures are for use with BigDye terminators only.

With ethanol precipitation, traces of unincorporated terminators may be seen at the beginning of the sequence data (up to base 40), but this is usually minimal. Some loss in the recovery of the smallest fragments may also be observed.

IMPORTANT Where 95% ethanol is recommended in precipitation protocols, purchase non-denatured ethanol at this concentration rather than absolute (100%) ethanol. Absolute ethanol absorbs water from the atmosphere, gradually decreasing its concentration. This can lead to inaccurate final concentrations of ethanol, which can affect some protocols.

Precipitation in 96-Well MicroAmp Trays

Reagents and equipment required:

- Variable speed table-top centrifuge with microtiter plate tray, capable of reaching at least 1400 × *g*
- Strip caps or adhesive-backed aluminum foil tape (3M Scotch Tape 425-3)[1]
- 95% Ethanol (ACS reagent grade), non-denatured

Note This procedure does not use salt.

To precipitate extension products in MicroAmp Trays:

Step	Action
1	Remove the MicroAmp Tray from the thermal cycler. Remove the caps from each tube.
2	Add the following: ♦ 16 µL of deionized water ♦ 64 µL of non-denatured 95% ethanol The final ethanol concentration should be 60 ± 3%.

1. Contact 3M in the USA at (800) 364-3577 for your local 3M representative. Use of other tapes may result in leakage or contamination of the sample.

To precipitate extension products in MicroAmp Trays: *(continued)*

Step	Action
3	Seal the tubes with strip caps or by applying a piece of 3M Scotch Tape 425-3 adhesive-backed aluminum foil tape. Press the foil onto the tubes to prevent any leakage.
4	Invert the tray a few times to mix.
5	Leave the tray at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
6	Place the tray in a table-top centrifuge with tube-tray adaptor and spin it at the maximum speed, which must be ≥1400 × *g* but <3000 × *g*: ♦ 1400–2000 × *g*: 45 minutes ♦ 2000–3000 × *g*: 30 minutes **Note** A MicroAmp tube in a MicroAmp Tray can withstand 3000 × *g* for 30 minutes. **IMPORTANT** Proceed to the next step immediately. If not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
7	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the tray onto a paper towel folded to the size of the tray.
8	Place the inverted tray with the towel into the table-top centrifuge and spin at 700 × *g* for 1 minute.
9	Remove the tray and discard the paper towel. **Note** Pellets may or may not be visible. Vacuum drying of the samples is not necessary.

Precipitation in Microcentrifuge Tubes

Reagents and equipment required for this method:

- 1.5-mL microcentrifuge tubes
- Benchtop microcentrifuge, capable of reaching at least 14000 × *g*
- Vacuum centrifuge
- 95% Ethanol (ACS reagent grade), non-denatured

Note This procedure does not use salt.

To precipitate extension products in microcentrifuge tubes:

Step	Action
1	Pipet the entire contents of each extension reaction into a 1.5-mL microcentrifuge tube. **Note** If the TC1 or DNA Thermal Cycler 480 was used for thermal cycling, remove the reactions from the tubes as shown in step 1 on page 3-37.
2	Add the following: ♦ 16 µL of deionized water ♦ 64 µL of non-denatured 95% ethanol The final ethanol concentration should be 60 ± 3%.

To precipitate extension products in microcentrifuge tubes: *(continued)*

Step	Action
3	Close the tubes and vortex briefly.
4	Leave the tubes at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
5	Place the tubes in a microcentrifuge and mark their orientations. Spin the tubes for 20 minutes at maximum speed. **IMPORTANT** Proceed to the next step immediately. If not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
6	Carefully aspirate the supernatants with a separate pipette tip for each sample and discard. Pellets may or may not be visible. **IMPORTANT** The supernatants must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the tubes, the more unincorporated dye terminators will remain in the samples.
7	Add 250 µL of 70% ethanol to the tubes and vortex them briefly.
8	Place the tubes in the microcentrifuge in the same orientation as in step 5 and spin for 10 minutes at maximum speed.
9	Aspirate the supernatants carefully as in step 6.
10	Dry the samples in a vacuum centrifuge for 10–15 minutes or to dryness. (Alternatively, place the tubes with the lids open in a heat block or thermal cycler at 90 °C for 1 minute.)

Ethanol/Sodium Acetate Precipitation

IMPORTANT Use non-denatured 95% ethanol rather than absolute (100%) ethanol. Absolute ethanol absorbs water from the atmosphere, gradually decreasing its concentration. This can lead to inaccurate final concentrations of ethanol, which can affect some protocols.

Precipitation in 96-Well MicroAmp Trays

Reagents and equipment required:

- Variable speed table-top centrifuge with microtiter plate tray, capable of reaching at least 1400 × *g*
- Strip caps or adhesive-backed aluminum foil tape (3M Scotch Tape 425-3)[1]
- Sodium acetate (NaOAc), 3 M, pH 4.6 (P/N 400320)
- 95% Ethanol (ACS reagent grade), non-denatured

To precipitate extension products in MicroAmp Trays:

Step	Action
1	Remove the MicroAmp Tray from the thermal cycler. Remove the caps from each tube.
2	Add the following: ♦ 2.0 µL of 3 M sodium acetate (NaOAc), pH 4.6 ♦ 50 µL of 95% ethanol (EtOH) The final ethanol concentration should be 65%.
3	Seal the tubes with strip caps or by applying a piece of 3M Scotch Tape 425-3 adhesive-backed aluminum foil tape. Press the foil onto the tubes to prevent any leakage.
4	Invert the tray a few times to mix.
5	Leave the tray at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
6	Place the tray in a table-top centrifuge with tube-tray adaptor and spin it at the maximum speed, which must be ≥1400 × *g* but <3000 × *g*: ♦ 1400–2000 × *g*: 45 minutes ♦ 2000–3000 × *g*: 30 minutes **Note** A MicroAmp tube in a MicroAmp Tray can withstand 3000 × *g* for 30 minutes. **IMPORTANT** Proceed to the next step immediately. If not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
7	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the tray onto a paper towel folded to the size of the tray.
8	Place the inverted tray with the towel into the table-top centrifuge and spin at 700 × *g* for 1 minute.
9	Add 150 µL of 70% ethanol to each pellet.

1. Contact 3M in the USA at (800) 364-3577 for your local 3M representative. Use of other tapes may result in leakage or contamination of the sample.

To precipitate extension products in MicroAmp Trays: *(continued)*

Step	Action
10	Cap or seal the tubes, then invert the tray a few times to mix.
11	Spin the tray for 10 minutes at maximum speed.
12	Repeat steps 7 and 8.
13	Remove the tray and discard the paper towel. **Note** Pellets may or may not be visible. Vacuum drying of the samples is not necessary.

Precipitation in Microcentrifuge Tubes

Reagents and equipment required:

- 1.5-mL microcentrifuge tubes
- Benchtop microcentrifuge, capable of reaching at least 14000 × *g*
- Vacuum centrifuge
- Sodium acetate (NaOAc), 3 M, pH 4.6 (P/N 400320)
- 95% Ethanol (ACS reagent grade), non-denatured

Step	Action
1	For each sequencing reaction, prepare a 1.5-mL microcentrifuge tube containing the following: ♦ 2.0 µL of 3 M sodium acetate (NaOAc), pH 4.6 ♦ 50 µL of 95% ethanol (EtOH) **Note** If the TC1 or DNA Thermal Cycler 480 was used for thermal cycling, remove the reactions from the tubes as shown in step 1 on page 3-37.
2	Pipet the entire contents of each extension reaction into a tube of sodium acetate/ethanol mixture. Mix thoroughly.
3	Vortex the tubes and leave at room temperature for 15 minutes to precipitate the extension products. Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
4	Spin the tubes in a microcentrifuge for 20 minutes at maximum speed.
5	Carefully aspirate the supernatant with a pipette tip and discard. **IMPORTANT** The supernatants must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the tubes, the more unincorporated dye terminators will remain in the samples.
6	Rinse the pellet with 250 µL of 70% ethanol.
7	Vortex briefly.
8	Spin for 5 minutes in a microcentrifuge at maximum speed. Again, carefully aspirate the supernatant and discard.
9	Dry the pellet in a vacuum centrifuge for 10–15 minutes, or until dry. Do not over-dry. (Alternatively, place the tubes with the lids open in a heat block or thermal cycler at 90 °C for 1 minute.)