SCIEX ssDNA 100-R Kit

For P/ACE™ MDQ and P/ACE™ MDQ *plus* Capillary Electrophoresis Systems

Instruction Guide

SCIEX

726412AG
June 2015

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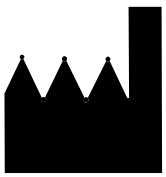

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Contents

Introduction

The SCIEX ssDNA 100-R Kit contains the supplies necessary to perform rapid separation and analysis of oligonucleotides between 10 to 100 base pairs long. This kit features a replaceable gel buffer and a coated capillary for maximum reproducibility.

Note: The system must be equipped with a UV detector and a 254 nm filter to perform this assay.

Safety

Refer to the Safety Data Sheets (SDS) information, available at sciex.com/safety-data-sheets, regarding the proper handling of materials and reagents. Always follow standard laboratory safety guidelines.

Materials and Reagents

Table 1-1 Kit Contents (PN 477480)

Component	Quantity	Reorder PN
DNA capillary, 65 cm, 100 µm ID	2	477477
ssDNA 100-R Gel (lyophilized)	1.0 g	477621
Tris-Borate Buffer	1	(see note below)
7 M Urea	1	(see note below)
pd(A) 40-60 Test Mix	0.2 O.D.	477626

Note: The buffer and urea are available for reorder as a kit, use PN 338481.

Table 1-2 Additional Required Materials

Description	Part Number	P/ACE System	
		MDQ	MDQ plus
From SCIEX			
254 nm filter	144438	✓	✓
PCR vials (100-pack)	144709	✓	✓
PCR vial springs (10-pack)	358821	✓	
2 mL glass vials (100-pack)	144980	✓	

Table 1-2 Additional Required Materials

Description	Part Number	P/ACE System	
		MDQ	MDQ plus
Red caps for 2 mL glass vials (100-pack)	144648	✓	
Gray caps for PCR vials (50-pack)	144656	✓	
PCR vial holders (50-pack)	144657	✓	
Universal plastic vials (100-pack)	A62251		✓
Blue rubber caps for universal vials (100-pack)	A62250		✓
From Other Laboratory Suppliers			
10 mL disposable syringes	Various	✓	✓
Adequate pipettes and tips	Various	✓	✓
Double-deionized (DDI) water with 16 to 18 megaohm resistance, filtered with 0.2 µm pore filter.	Various	✓	✓
Magnetic stir plate and stir bar	Various	✓	✓
Membrane syringe filters (0.2 µm and 0.45 µm pores)	Various	✓	✓
Vortex mixer	Various	✓	✓

Storing Kit Components

Upon receipt, store as follows:

- DNA capillary and lyophilized gel at 2°C to 8°C
- Test Mix at -35°C to -15°
- Unreconstituted Tris-borate and urea buffer bottles at room temperature

Cleaning the Capillary Interface

Due to the viscosity of the Gel Buffer it is necessary to clean the lever arms, electrodes and the outside of the capillary before use. Clean the system electrodes and interface block as described in the “Maintenance Procedure” section of the instrument manual. Repeat this general maintenance procedure before using the capillary.

Installing the Capillary

Note: Within 10 minutes of trimming the end of a capillary, dehydration of the coating inside the capillary begins. Any dry portion of the capillary becomes unusable. Do not cut the capillary to its final length before placing it in the cartridge.

Install the DNA capillary into a capillary cartridge using the *Capillary Cartridge Rebuild Instructions* (PN 144655). The recommended capillary length is 20 cm to the window and 30.2 cm total length. Use a 100 x 200 µm aperture.

Follow these modifications to the instructions to minimize damage to the capillary coating:

- Cut the end-caps at both sides and then install the capillary into the cartridge.
- Fill two vials with DDI water and seal them.
- Trim the capillary ends and then submerge both ends of the capillary in the water-filled vials. Do not expose the capillary ends to air for more than 3 minutes.

Conditioning a New Capillary

The DNA capillary is filled with a storage solution which must be replaced with ssDNA 100-R Gel Buffer before use. Replace the storage solution by performing a 20-minute rinse with ssDNA 100-R Gel Buffer.

Storing the Capillary

After the capillary has been used, it should be stored as follows:

Short Term Storage (<48 hours)

- Perform a 10-minute rinse at 20 psi with unused ssDNA 100-R Gel Buffer.
- Store the capillary on the instrument with both ends submerged in fresh Tris-Borate-Urea buffer.

Long Term Storage (>48 hours)

- Perform a 10-minute rinse at 20 psi rinse with unused ssDNA 100-R Gel Buffer.
- Remove the capillary from the instrument and place it in a capillary storage box with both ends immersed in vials containing fresh Tris-Borate-Urea buffer.
- Store the capillary storage box upright in the refrigerator between 2°C and 8°C.

If the capillary has not been used for more than 12 hours, perform a 10-minute rinse at 20 psi with fresh Gel Buffer, before performing a separation.

Preparing the Tris-Borate-Urea Buffer

Note: Use only SCIEX Tris-Borate buffer and SCIEX 7 M urea. The purity of the buffer raw material components is critical to the life of the buffer and the coating on the DNA capillary. Do not substitute buffer components from other vendors.

1. Add 135 mL of DDI water to the bottle containing the dry Tris-Borate buffer.
2. Clean a large magnetic stir bar with methanol and rinse with deionized water.
3. Use the magnetic stir bar to stir the solution for 20 to 30 minutes until the boric acid is completely dissolved.

   Be sure the boric acid is completely dissolved before going to the next step.
4. Slowly add the dry 7 M urea to the Tris-Borate buffer while continuing to stir the solution.
5. Stir the solution for up to 2 hours at room temperature until the urea is completely dissolved and the buffer is clear.

   The dissolution of urea is endothermic, so the bottle will get very cold.

   Do not heat the buffer solution to speed the warming process. This will shorten the usable life of the buffer.

   Tip! Some magnetic stirrers produce enough heat to degrade the urea. A small piece of corrugated cardboard can be used as an insulator between the buffer and the stirrer to minimize the heating.
6. Store the Tris-Borate-Urea buffer at 2°C to 8°C. The reconstituted Tris-Borate-Urea buffer can be used for up to 30 days after preparation.

Using the Prepared Tris-Borate-Urea Buffer

Use the correct vials and caps for your system:

- For the P/ACE MDQ system–use glass vials and red caps
- For the P/ACE MDQ *plus* system–use universal vials and blue caps

1. If the buffer solution was previously reconstituted and refrigerated, bring the entire container of buffer to ambient temperature before use while stirring slowly and continuously with a clean stirring bar.
2. Remove the required volume to be used for the day and filter through a 0.2 µm disposable syringe filter into a clean container.
3. Pipette the filtered buffer into a buffer vial:
   - For the P/ACE MDQ system–fill with 1.7 mL
   - For the P/ACE MDQ *plus* system–fill with 1.4 mL.
4. Seal the vial with the appropriate cap and sonicate the vial 5 times to degas the buffer.

Preparing the ssDNA 100-R Gel Buffer

1. Add 5.0 mL of filtered Tris-Borate-Urea buffer to the lyophilized gel.
2. Use a large clean and dry magnetic stir bar to stir the solution for 4 to 6 hours until the gel is completely dissolved.
3. Store the Gel Buffer at 2°C to 8°C. The prepared Gel Buffer can be used for up to 30 days after preparation.

For 1 to 8 Runs

Use the correct vials and caps for your system:

- For the P/ACE MDQ system–use glass vials and gray caps
- For the P/ACE MDQ *plus* system–use universal vials and blue caps

1. Transfer 200 µL of the filtered Gel Buffer into a PCR vial.
2. Centrifuge the vial for no more than 2 minutes at a maximum of 6000 rpm to remove air bubbles.
3. Follow the appropriate instructions for your system to set up the vials:
    - PCR Vial Setup–P/ACE MDQ System
    - Universal Vial Setup–P/ACE MDQ plus System

For 9 or More Runs

Use the correct vials and caps for your system:

- For the P/ACE MDQ system–use glass vials and red caps
- For the P/ACE MDQ *plus* system–use universal vials and blue caps

1. Filter the Gel Buffer through a 0.45 µm disposable syringe filter.
2. Pipette the filtered Gel Buffer into a PCR vial:
    - For the P/ACE MDQ system–fill with 1.7 mL
    - For the P/ACE MDQ *plus* system–fill with 1.4 mL.
3. Seal the vial with the appropriate cap and sonicate the vial 5 times for 30 seconds, each time.

    Allow the air bubbles to rise to the surface after each 30 second interval.

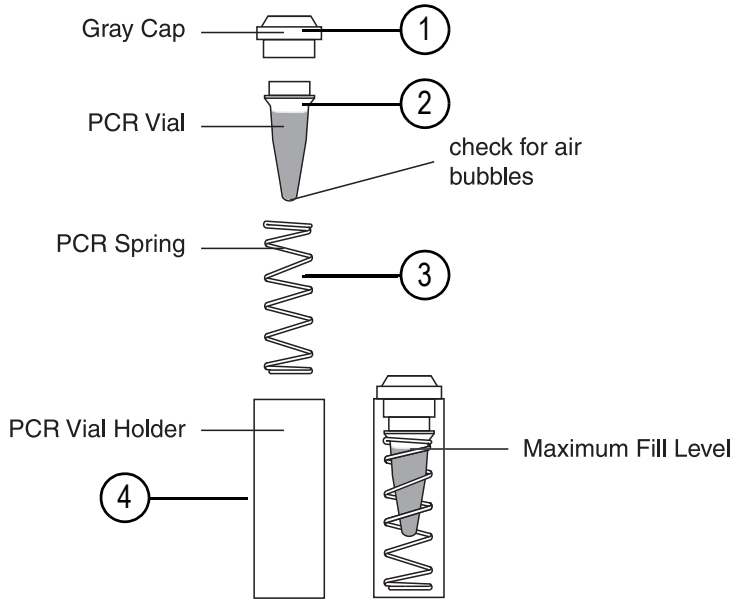
The following points are important when using ssDNA 100-R Gel Buffer:

- On-board stability for a vial filled with Gel Buffer is 24 hours.
- If using 200 µL of Gel Buffer, do not leave the Gel Buffer in the sample tray for more than 5 hours. This may result in an increased migration time due to an increase in the viscosity of Gel Buffer.
- For optimal migration time reproducibility, replace the Gel Buffer in the capillary after every 8 runs.

PCR Vial Setup–P/ACE MDQ System

Place the PCR vial in a PCR holder equipped with a vial spring (Figure 1.1). Seal the PCR vial with a clean gray cap and place it in the inlet sample tray.

Figure 1.1 PCR Vial Setup–P/ACE MDQ System

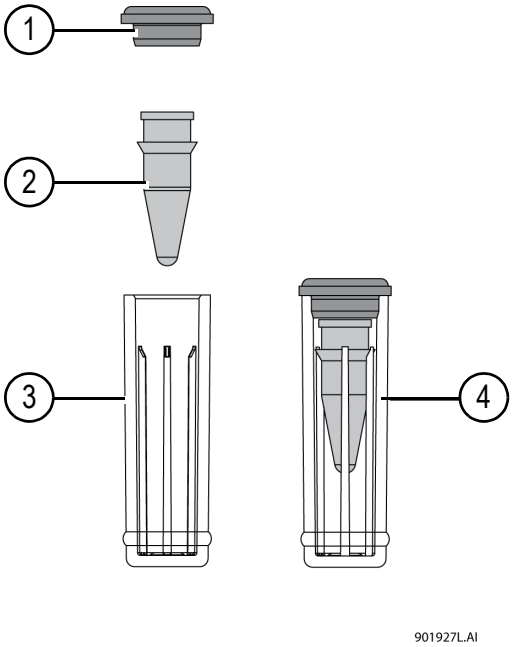


Item	Description
1	Vial cap (PN 144656)
2	PCR vial (PN 144709)
3	PCR vial spring (PN 358821)
4	PCR vial holder (PN 144657)

Universal Vial Setup–P/ACE MDQ *plus* System

Place the PCR vial into the universal vial and seal with a blue cap (Figure 1.2).

Figure 1.2 Univeral Vial Setup–P/ACE MDQ *plus* System



Item	Description
1	Universal vial cap (PN A62250)
2	PCR vial (PN 144709)
3	Universal vial (PN A62251)
4	Micro vial inside of universal vial

Preparing the Test Mix

Use the correct vials and caps for your system:

- For the P/ACE MDQ system–use glass vials and red caps
- For the P/ACE MDQ *plus* system–use universal vials and blue caps

1. Add 500 µL of filtered deionized water (filter through 0.2 µm filter) to the vial labeled “pd(A) 40-60” and mix well.
2. Pipette 100 µL of the pd(A) 40-60 Test Mix solution into a PCR vial.
3. Follow the appropriate instructions for your system to set up the vials:
   - PCR Vial Setup–P/ACE MDQ System
   - Universal Vial Setup–P/ACE MDQ plus System

4. Transfer 100 µL aliquots of the remaining prepared Test Mix to sealable 400 µL vials and store frozen until needed.

The lyophilized Test Mix has a shelf life of one year, when stored at -35°C to -15°C. However, the reconstituted Test Mix deteriorates after several days at room temperature. Therefore, the reconstituted Test Mix should be stored frozen at -35°C to -15°C, when not in use.

Preparing the Oligonucleotide Sample

The oligonucleotide sample concentration should be between 5 O.D./mL to 10 O.D./mL. Prepare the sample as in Preparing the Test Mix on page 11.

Running Methods

The SCIEX ssDNA100-R kit requires two methods:

- Gel-filling method–Fills the capillary with ssDNA-100R Gel Buffer, performs an equilibration step, injects a sample and separates it.
- Separation method–Injects the sample and separates it. This method can be repeated consecutively, up to 20 times.

Both methods have the same Initial Conditions (Figure 1-1) and UV Detector Initial Conditions (Figure 1-2), but the Time Program differs:

- Figure 1-3 shows the Time Program for the gel-filling method. Save this method as “ssDNA-100R gel filling.met”.
- Figure 1-4 shows the Time Program for the separation method. Save this method as “ssDNA-100R NO gel filling.met”.

Note: These methods can be downloaded from sciex.com/products/capillary-electrophoresis-instruments/p/ace-mdq-plus (click **Resources**).

Initial Conditions for All Methods

Figure 1-1 Gel-Filling and Separation Methods–Initial Conditions Tab

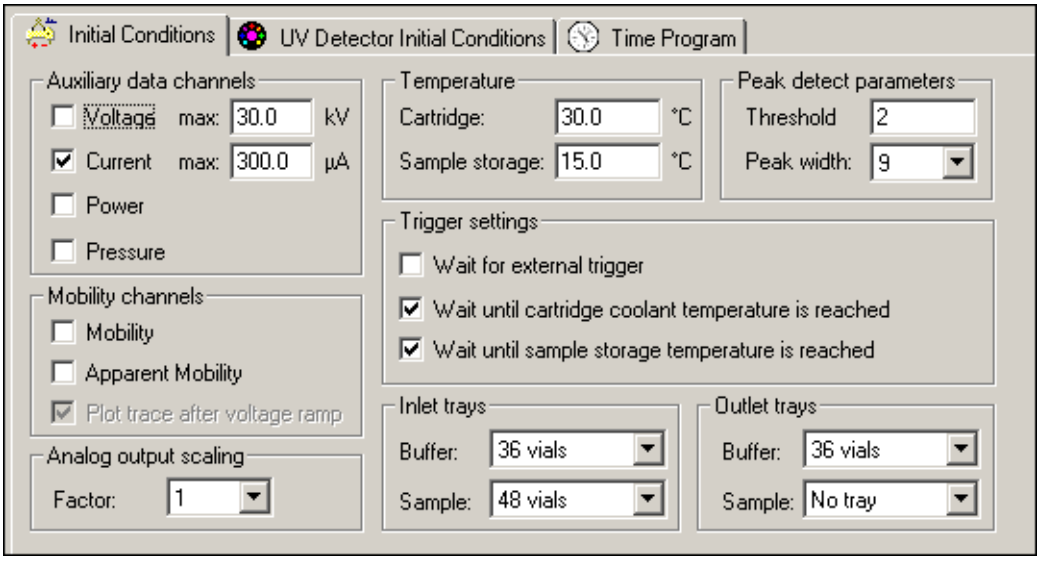
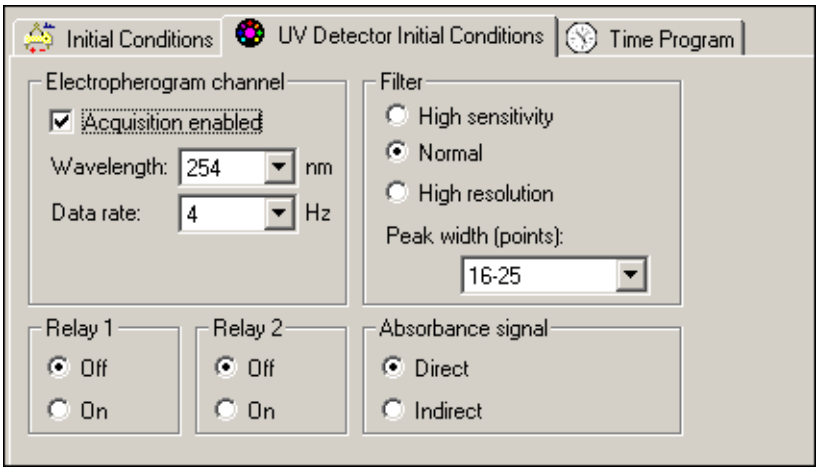


Figure 1-2 Gel-Filling and Separation Methods–UV Detector Initial Conditions Tab



Time Program for Gel-Filling Method

Figure 1-3 Gel-Filling Method–Time Program Tab

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	5.00 min	BI:B1	BO:B1	forward	fill cap with ssDNA-100 gel into vial filled with water
2		Inject - Voltage	1.0 KV	1.0 sec	BI:A2	BO:A2	No override, reverse polarity	cleaning tips w/water vials at both sides
3		Inject - Voltage	1.0 KV	1.0 sec	BI:A3	BO:A3	No override, reverse polarity	cleaning tips w/water using another pair of vials filled with water
4		Separate - Voltage	3.0 KV	2.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	preelectrophoresis between buffer vials (do not use gel vial)
5		Separate - Voltage	5.0 KV	2.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	preelectrophoresis between buffer vials (do not use gel vial)
6		Separate - Voltage	9.3 KV	10.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	pre-electrophoresis between buffer vials (do not use gel vial)
7		Inject - Voltage	3.0 KV	4.0 sec	BI:A4	BO:A4	No override, reverse polarity	cleaning tips with water using a different pair of water filled vials
8		Inject - Voltage	3.0 KV	4.0 sec	SI:A1	BO:B2	Override, reverse polarity	sample injection against buffer vial
9	0.00	Separate - Voltage	9.3 KV	40.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	use buffer vials
10	1.00	Autozero						
11	40.00	End						
12								

Time Program for Separation Method

Figure 1-4 Separation Method–Time Program Tab

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Wait		0.00 min	BI:A1	BO:A1		H20 dip
2		Inject - Voltage	3.0 KV	4.0 sec	SI:A1	BO:B2	Override, reverse polarity	sample injection against buffer vial
3		Wait		0.00 min	BI:A1	BO:A1		H20 dip
4	0.00	Separate - Voltage	9.3 KV	40.00 min	BI:B2	BO:B2	0.17 Min ramp, reverse polarity	used buffer vials
5	1.00	Autozero						
6	40.00	End						
7								

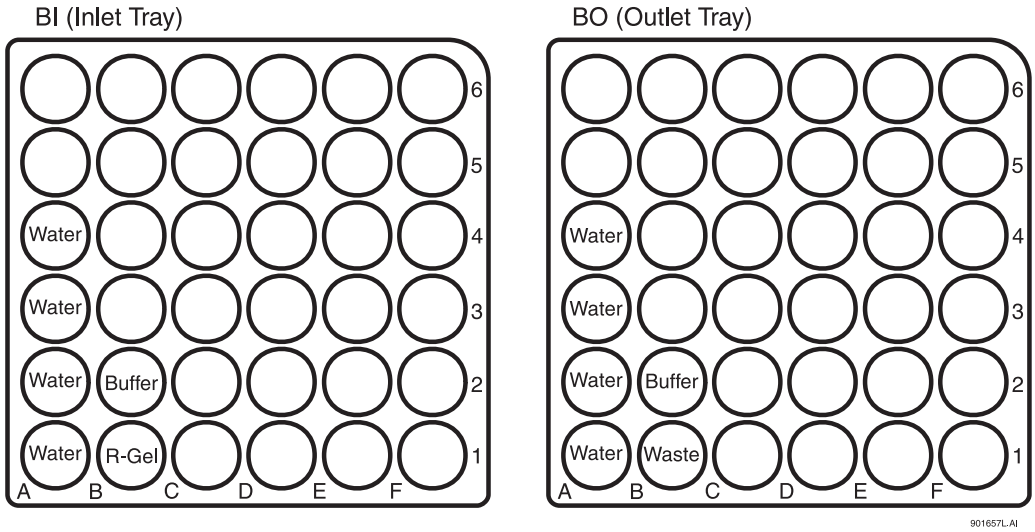
Performing a Test Run

Use the correct vials and caps for your system:

- For the P/ACE MDQ system–use glass vials and red caps
- For the P/ACE MDQ *plus* system–use universal vials and blue caps

1. Fill 8 vials with DDI water and place them the positions labeled “Water” in the buffer tray (Figure 1-5).
   - For the P/ACE MDQ system–fill with 1.7 mL
   - For the P/ACE MDQ *plus* system–fill with 1.4 mL.
2. Place the other vials in the buffer trays:
   a. Fill a vial half way with DDI water and place it in the position labeled “Waste”.
   b. Place 2 Tris-Borate-Urea buffer vials in the positions labeled “Buffer”.
   c. Place the ssDNA 100-R Gel Buffer vial in the position labeled “R-Gel”.

Figure 1-5 Buffer Tray Configuration



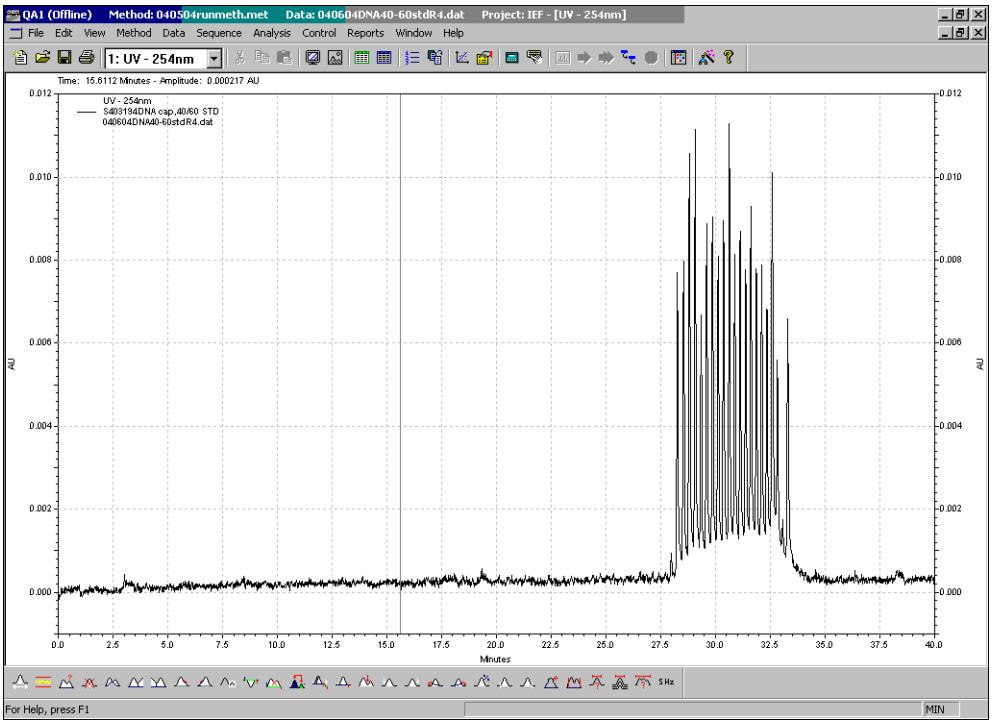
3. Place the sample vial containing the Test Mix in the sample inlet tray at position A1 (SI:AI).
4. Run the pd(A) 40-60 Text Mix using the gel-filling method created above.

Checking the Results

The pd(A) 40-60 Test Mix should give a baseline separation of all the oligonucleotides in 35 minutes when using a 30.2 cm capillary at a field strength of 300 V/cm (Figure 1-6). Peak intensity may vary due to batch-to-batch variation. Refer to the electropherogram provided with the Test Mix to check your results. The electrical current should remain fairly stable between 6 µA and 8 µA.

The high resolving power of this kit can achieve not only single-base resolution of each oligonucleotide, but also show the appearance of shoulders on the major peaks which may be the de-phosphorylated form of each oligonucleotide or other contamination. However, the intensity of these shoulders may vary due to the lot-to-lot variation in Test Mix preparation.

Figure 1-6 Example Electropherogram for Test Mix



Tips for Best Results

- If resolution decreases over time, replace the ssDNA 100-R Gel Buffer using the gel-filling method described previously. Perform a test run to make sure the resolution has improved.
- If baseline separation is not achieved, use the same field strength (V/cm) and increase the capillary length as needed.
- For optimal migration time reproducibility, replace the vials of Tris-Borate-Urea buffer after 18 runs.
- Monitor the current at all times. Changes in the average current or fluctuations in the current may indicate changed in ionic strength, Gel Buffer degradation, or the formation of bubbles.

Troubleshooting

Problem	Possible Cause	Corrective Action
Reduced resolution	Deteriorating buffer or Test Mix.	Change to a new buffer or Test Mix.
	Deteriorating Gel Buffer inside the capillary.	Replace the Gel Buffer and repeat the equilibration procedure.
	Bad capillary coating	Replace the DNA capillary.

Problem	Possible Cause	Corrective Action
Low or unsteady current	Deteriorating buffer.	Replace the buffer.
	Capillary plugged.	Replace the Gel Buffer and repeat the equilibration procedure.
	Air bubbles in the Gel Buffer.	Replace the Gel Buffer and repeat the equilibration procedure.
	Broken capillary.	Replace the capillary.
Migration time changes from run to run	Capillary not equilibrated	Run equilibration method.
	Deteriorating gel.	Replace the Gel Buffer.
	Contamination on the electrodes.	Clean the electrodes, capillary ends.
No peaks or low UV absorption	Plugged or dried capillary.	Replace the Gel Buffer and repeat the equilibration procedure.
	Broken capillary.	Replace the capillary.
	Inadequate capillary equilibration.	Replace the Gel Buffer and repeat the equilibration procedure.
Spikes	Air in the Gel Buffer.	Make sure that the Gel Buffer is at room temperature and that the air is removed.
	Contamination or microparticles in gel or buffer.	Replace the gel and buffer vials with freshly filtered solutions.
Unstable or shifting baseline	Inadequate capillary equilibration.	Replace the Gel Buffer and repeat the equilibration procedure.
	Degraded ssDNA 100-R Gel Buffer	Replace with new Gel Buffer.
	Degraded Tris-Borate-Urea buffer	Replace with fresh buffer.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