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Protocol for Oligonucleotide Analysis using Oligel® ssDNA/ssRNA Gel

Introduction

Oligel ssDNA Gel has been designed to effectively separate ssDNA and ssRNA oligos with single base resolution from 10-150 bases in length. An example separation is shown in Figure 1. Salts present in oligo preparation may affect the injection efficiency. For best results, it is recommended that samples be diluted in water.

The gel and buffer are premixed and ready to be used, reducing hands on time and inconsistencies related to gel and buffer preparation.

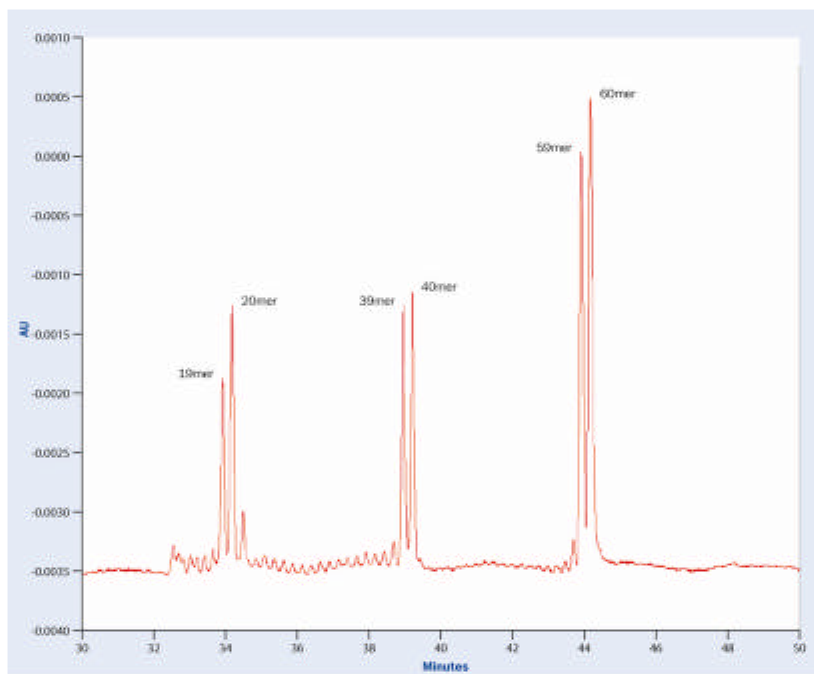

Figure 1: Electropherogram obtained from a mixture of ssDNA oligonucleotides using Oligel® gel matrix on the Beckman® MDQ.

Capillary Dimensions

The procedures are based on the use of a bare fused silica capillary with 100 µm i.d., 50 cm effective/60 cm total length.

Reagents needed

For best results, store unused portions of gel at 2-8°C, warm to room temperature prior to use

Part #	Description
scDN-415-0006	*Oligel®* ssDNA/ssRNA Gel
scDN-465-0100	ssDNA *Oligel®* Buffer
scDN-475-0050	Capillary conditioning solution
Optional:	
DN-400-0001	Oligonucleotide OQ Standard 1X Soln

*Procedure**

Instrument set up

1. Install the capillary as directed by instrument instruction manual.
2. Set cartridge temperature at 20°C
3. Set detection wavelength at 254nm

For runs with fewer than 8 samples

A. Capillary preconditioning

1. Dispense 1.5mls Capillary conditioning solution (scDN-475-0050) into a glass vial and place in inlet tray.
2. Place a glass vial in the outlet tray to collect waste.
3. Program system to rinse new capillary with Capillary conditioning solution (scDN-475-0050) at 60 psi for 15 min.

B. Gel filling and pre run

1. Place 0.2mls *Oligel®* ssDNA/ssRNA Gel (scDN-415-0006) into a PCR tube and centrifuge (6,000 rpm for 2 minutes) to remove air bubbles.
2. Place PCR tube into glass vial and seal as directed by instrument instruction manual. Place sealed vial in outlet tray.
3. Program system to fill capillary at 100 psi for 60 min.
4. After capillary if filled, perform pre-run at -12 kV for 40 min to stabilize current.

C. Sample analysis

1. Fill 2 glass vials with 1.5mls ssDNA *Oligel®* Buffer (scDN-465-0050). Place one vial in the inlet tray and one vial in the outlet tray
2. Place 50µl of the OQ standard (DN-400-0001) into a PCR tube. Place the PCR tube into a sample vial holder as directed by instrument instruction manual.
3. Place samples in individual vials, using small microfuge tubes if necessary.
4. Fill a glass vial with deionized water to perform a water dip prior to sample injection (inlet tray).
5. Complete water dip for 30 seconds.
6. Perform sample injection at -5 kV for 5-15 seconds. Injection time is dependent on sample concentration and salt content.
7. Separatate samples at -12kV for 60 minutes.
8. Detection wavelength: 254 nm.
9. Repeat step 6-9 for subsequent sample analysis.

After running 8 samples, refill capillary with fresh gel and replace the buffer vials with 1.5mls of fresh ssDNA *Oligel®* Buffer.

If separation resolution/performance decreases to an unacceptable level, repeat steps B1-B4 to refresh gel matrix in the capillary.

If refreshing gel matrix does not restore the separation performance, repeat from Step A1 through B4 to re-condition and refill the capillary.

For runs with more than 8 samples

A. Capillary preconditioning

1. Place 1.5mls Capillary conditioning solution (scDN-475-0050) in inlet glass vial.

2. Program system to rinse new capillary with Capillary conditioning solution (scDN -475-0050) at 60 psi for 15 min.

B. Gel filling and pre run
1. Place 1.5mls *Oligel®* ssDNA/ssRNA Gel (scDN-415-0006) into a PCR vial and degas for 15 minutes.
2. Place glass vial into outlet tray and seal as directed by instrument manufacturer.
3. Program system to fill capillary at 100 psi for 60 min.
4. After capillary if filled, perform pre -run at -12 kV for 40 min to stabilize current.
5. Run samples as directed in section C above.
6. Program the system to refill the capillary after every 8 samples and use separate using fresh ssDNA *Oligel®* Buffer.

Shutdown and clean up
1. If samples will be run within 1 day of last use, it is recommended that a shutdown method be established and run. Consult instrument owners manual for apporpriate shutdown sequences.
2. If no additional sample will be run, it recommended that a clean up method be established and run. Consult instrument owners manual for apporpriate shutdown method.

*procedure tested on Beckman MDQ

Notes
1. Gel can be left at room temperature for >2 days, if needed.
2. For separation of longer oligonucleotides, a capillary with longer effective length may be required.
3. Typical sample concentration is 1 μM diluted in deionized water. The injection time and voltage can be adjusted to yield a reasonable peak height without a loss in separation resolution.
4. Any anionic salts present in the sample will reduce the injection efficiency and require an increase in injection voltage and/or time.
5. A voltage pre-run is required each time the capillary is flushed with fresh gel matrix.
6. The reagents are made with room temperature stable polymers; it is recommended, however, that unused reagents be stored at 2-8°C. All reagents have a 45-day shelf life.

Need support? – contact us at tech-support-CE@aati-us.com or phone 515-294-1690