Laboratory Training Manual

# *Subject 5: Amplified DNA Product Separation*

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# Laboratory Training Manual

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## Purpose

To instruct the trainee how to perform amplified product separation using electrophoresis methods. This laboratory manual component incorporates exercises on sample setup and operation and maintenance on the laboratory's platform(s).

The trainee will also be introduced to the laboratory's procedures for quality control, to include procedures for limiting the risk of contamination.

## Objectives

Upon successful completion of these exercises, the trainee will be able to:

- Describe the theories and procedures for amplified DNA product separation.
- List the manufacturer(s), corresponding model numbers and unique identifiers for the laboratory's electrophoresis instrumentation.
- Describe the functions of each major component of the electrophoresis instrument(s).
- List and demonstrate the steps involved in the maintenance of the instrument(s), per the laboratory's SOPs.
- List the types and versions of the instrumentation software.
- Demonstrate the use of relevant software for each type of laboratory instrument.
- Describe the controls used in the electrophoresis process.
- Describe the reagents used in the electrophoresis process.
- Describe the sensitivity and limitations of the electrophoresis system(s).
- Perform amplified product sample preparation, per the laboratory's SOPs.
- Conduct preliminary evaluation of data, per the laboratory's SOPs.
- Describe the quality system procedures employed by the laboratory to avoid, detect, and document contamination.

## Preparation for Exercises

### Technical Leader Responsibilities

1. Provide documented safety practices specific to chemicals used in the electrophoresis process, to include the pertinent MSDSs.

### Trainer Responsibilities

1. Assign required samples to be electrophoresed, as outlined in the Individual Training Plan.
   *Note:* It is recommended that samples run in this section of the Laboratory Training Manual be used for all exercises. Ensure that the trainee saves samples and data from each exercise for subsequent exercises.
2. Demonstrate each step of the electrophoresis process, including sample setup and instrument operation and maintenance.
3. Observe the trainee performing the electrophoresis processes.
4. Determine the assessment criteria.
5. Review, verify, and document exercise completion.

## Trainee Responsibilities

1. Review documented safety practices specific to chemicals used in the electrophoresis process, to include pertinent MSDSs.
2. Observe each step of the electrophoresis process, including sample setup and instrument operation and maintenance.
3. Perform the electrophoresis process, including sample setup and instrument operation and maintenance.
4. Document and submit exercise completion, as required by the trainer.

## Exercise 1: Instrument Maintenance

### *Purpose*

To observe and perform the proper maintenance of the Applied Biosystems 3500 Genetic Analyzer per the laboratory's SOP's. This demonstration should include the use of the software as well as the proper documentation of the maintenance performed on the instrument(s). (Proper documentation may include logbooks and/or worksheets.)

### *Tasks*

- View Applied Biosystems Training CD/DVD for the Applied Biosystems 3500 (obtain from Technical Leader)
- Set-up and clean the instrument(s).
- Identify and explain the functions of the various components of the instrument.
- Demonstrate the proper use of the software as it pertains to instrument maintenance, to include the following:
    - Manual control and/or wizards

### *Resources*

**Laboratory Manuals**

**User Manuals: Applied Biosystems**

# Exercise 2: Sample and Run Set-up

## *Purpose*

To observe and perform the proper set up of matrix standards and previously amplified samples to run on the Applied Biosystems 3500 Genetic Analyzer.

## *Tasks*

- Set up samples to run on the instrument, to include:
  - Sample sheets
  - Injection lists
  - Plate records.
- Verify the run parameters per the laboratory SOPs
  (Data should be saved for further analysis.)
  - Run and test modules
  - Voltage
  - Current
  - Run time
  - Injection time
  - Run temperature

## *Resources*

**Laboratory Manuals**

**User Manuals: Applied Biosystems**

# Exercise 3: Initial Data Evaluation

## *Purpose*

To evaluate the data during the run or prior to analysis to ensure that data meets the established criteria.

## *Tasks*

Using a set of practice samples, perform initial data evaluation.

- Elements of the data that should be evaluated, to include:
  - Internal lane size standard
  - Ladder
  - Baseline
  - Presence of the primer peak(s)
  - Injection quality, if applicable
  - Spikes
  - Pull-up
  - Minus A
  - Low peak heights
- Reinject samples for which data does not meet the established criteria; adjust parameters as needed

## *Resources*

**Laboratory Manuals**

**User Manuals: Applied Biosystems**

## Exercise 4: Known Sample Sets

### *Purpose*

To set up, run, and evaluate previously extracted previously extracted known samples and run on the Applied Biosystems 3500 Genetic Analyzer.

### *Tasks*

- Set up and run all assigned samples (and matrices, if necessary) on Applied Biosystems 3500 Genetic Analyzer.
- Evaluate the data and determine which samples, if any, need to be re-run (re-injected). (See Exercise 3: Initial Data Evaluation). Data should be saved for further analysis.

### *Resources*

**Laboratory Manuals**

**User Manuals: Applied Biosystems**

## Exercise 5: Variations in Injection Times

### *Purpose*

To produce data that illustrates the cause and effect of varying the injection times.

### *Tasks*

Perform the following and write a summary for each:

- Vary the injection time on 2 samples:
  - 3 seconds
  - 5 seconds
  - 7 seconds
  - 10 seconds

### *Resources*

**Laboratory Manuals**

**User Manuals: Applied Biosystems**

## Subject Review

After completion of the laboratory manual exercises and having previously completed the corresponding theory modules, the trainee should be able to answer the following questions:

- How is temperature controlled on the instrument platform(s)?
- How is data collected on the instrument platform(s)? What are the steps involved?
- What is a cathode? An anode?
- What is the composition of a capillary? An electrode?
- What are the consequences of a dirty capillary window?
- What are the consequences of the presence of bubbles in the polymer?
- How is a capillary stored between runs?
- What is the effect of an improperly positioned capillary window? How is this determined?
- How often should an instrument be cleaned? Why?
- What is the purpose of formamide and how is its quality verified?
- When does the buffer need to be replaced? Why?
- What is the length of a capillary? How does capillary length affect separation?
- What type of polymer and/or gel is used in the laboratory?
- What are the conditions under which a sample is re-injected? How is this performed?
- What are the effects of an increased or decreased injection time?
- What are the run parameters used in the laboratory? How is this verified during and after a run?
- What is the effect of varying the following settings?
    - Voltage
    - Current
    - Run time
- What controls are used in electrophoresis? What is the purpose of each control?
- What is the purpose of a matrix, a spectral, and a spatial?
- What are the criteria outlined by laboratory SOPs when a capillary doesn't pass the spatial calibration?
- What is a virtual filter?
- How can matrix quality affect the data?
- How does varying the following settings affect the data?
    - Voltage?
    - Current?
    - Run time?
- What are the effects of an increased or decreased injection time?
- What are the advantages and disadvantages of varying the amount of internal size standard?
- What are the advantages and disadvantages of using formamide versus water?
- What are the effects of varying the concentrations of buffer?
- What are the effects of varying the amount of amplified product?