DNA: Typing

Procedural safeguards

The following is a list of procedural safeguards for DNA typing.

- Access to the amplification/typing room is restricted to authorized personnel only.
- Gloves and a dedicated lab coat must be worn at all times.
- All tubes containing amplified DNA are to be pulse-centrifuged before being opened. Do not attempt to open tubes with just one hand.
- Pipette all amplified samples slowly to avoid creating an aerosol. Do not "blow out" the last bit of liquid in a pipette tip.
- All amplified DNA is to remain in the amplification/typing room.
- No equipment used in the amplification/typing room is to be removed from this area of the laboratory.
- Wash down all surfaces with ethanol followed by water.
  - Wash the tube openers, thermal cycler, and pipette barrels with ethanol and dry with a clean lab wipe.

Principle

The PCR products from the amplification process are analyzed by electrophoresis to separate the STR alleles according to size. The alleles are detected using fluorescent dye labeling.

Applied Biosystems fluorescent multicolor dye technology allows multiple loci, including loci that have alleles with overlapping size ranges, to be analyzed in a single capillary injection. Alleles for overlapping loci are distinguished by labeling locus-specific primers with different color dyes.

Equipment

This procedure uses the following equipment:

- ABI PRISM® 3130 Genetic Analyzers
- heat block
- pipettes and barrier tips
- centrifuge

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Chemicals and supplies

This procedure uses the following chemicals and supplies.

- PCR amplification kit containing:
  - *AmpFℓSTR Identifiler Plus* Allelic Ladder
  - *AmpFℓSTR Yfiler* Allelic Ladder
- From Applied Biosystems:
  - 96-well plate septa
  - reservoir septa
  - 3130 Capillary Array, 36 cm
  - *MicroAmp* Optical 96-Well Reaction Plate
  - 10X Genetic Analyzer Buffer with EDTA
  - Matrix Standard Set DS-33
  - *3130 POP-4* Polymer
  - *GeneScan*-500 [LIZ] Size Standard
  - *Hi-Di* Formamide
- 1.5 mL microcentifuge tubes
- PCR product

WARNING – Formamide is an irritant and known teratogen. Avoid skin contact and inhalation; use in a well ventilated area. Wear lab coat, gloves and protective eyewear when handling.

Standards

This procedure uses the following standards for each run of samples:

- ladder
- internal size standard

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Running samples

This is the procedure for preparing and running samples on the 3130 genetic analyzers.

Step	Action
1	Prepare sample sheet for run by going to *DNA Manager*, selecting appropriate 3130 tab, and following the directions in the instruction box.
2	Prepare a mixture of size standard/formamide. 0.3 µL of LIZ GS500 Size Standard X (# samples + 4) 8.7 µL of Hi-Di Formamide X (# samples + 4) Vortex and spin.
3	Dispense 9 µL of the mixture into each sample well.
4	Add l uL of amplified product or allelic ladder into appropriate wells.
5	Add 9 µL of formamide and 1 µL of size standard/formamide mixture into "Blank" wells.
6	Cover the plate with a Plate Septa.
7	Vortex and spin (Centrifuge at 2000 rpm for 1 minute).
8	Denature the plate at 95°C for 3 minutes.
9	Snap cool the plate for 3 minutes at 0°C.
10	Secure the plate into the base and lock with the plate cover. Load plate assembly onto the 3130 autosampler.
11	Create a plate record or import the plate record from *DNA Manager*. NOTE: The Identifiler or Yfiler Instrument Protocol should be used depending on the types of samples being run.
12	Click “Start run” to run the plate.

Instrument operating parameters
4.13.2.5.2

The instrument operating parameters are stored in the Identifiler and Yfiler instrument protocols and are recorded electronically with the sample data files.

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Genetic analyzer maintenance

These routine maintenance tasks may need to be performed on the 3130 genetic analyzer.

- Changing the water and buffer in the instrument reservoir.
  - Performed daily when the instrument is in use.
  - The buffer used on the instrument is a 1:10 dilution of the 10X *AB Genetic Analyzer Buffer*.
- Changing the polymer on the instrument.
  - Performed weekly when the instrument is in use.
  - The procedure can be found in the *Wizards* menu of the *Data Collection* software.
- Changing the array on the instrument
  - Performed as needed.
  - The procedure can be found in the *Wizards* menu of the *Data Collection* software.
- Performing a Spatial Calibration
  - A Spatial Calibration is required when
    - the capillary array is installed or replaced
    - when the capillary array is temporarily removed from the detection block
    - when the instrument is moved.
  - Refer to *AB Getting Started Guide* and *AB Maintenance, Troubleshooting, and Reference Guide* for procedures.
- Performing a Spectral Calibration
  - A Spectral Calibration should be performed when
    - a new dye set is used on the instrument
    - the capillary array length or polymer type is changed
    - after the laser or CCD camera has been realigned or replaced by a service engineer.
  - Refer to *AB Getting Started Guide* and *AB Maintenance, Troubleshooting, and Reference Guide* for procedures.
- Additional, non-routine tasks, such as flushing the water trap or instrument shutdown, may be performed as needed. Refer to the *Wizards* menu on the *Data Collection* software, the *AB Getting Started Guide*, and the *AB Maintenance, Troubleshooting, and Reference Guide* for specific tasks and procedures.