SOP 25
Short Tandem Repeat (STR) Profiling

DNeasy® Blood and Tissue Extraction:

Cultured Cell Pellets

1) Begin with a thawed 2 x 10^6 cell pellet. Re-suspend in 200 µL of PBS (without calcium and magnesium) and 20 µL proteinase K.
2) Add 200 µL AL buffer. Vortex and incubate for 10 minutes at 56°C.
3) Prepare and label spin columns during incubation.
4) Add 200 µL ethanol (100%) to the digested pellet. Vortex thoroughly.
5) Pipet the mixture into a 2 mL spin column (620 µL total). Centrifuge at 8000 rpm for 1 minute. Discard waste and old collection tube and replace with a new collection tube.
6) Add 500 µl AW1 buffer to spin column. Centrifuge at 8000 rpm for 1 minute. Discard waste and old collection tube and replace with a new collection tube.
7) Add 500 µL AW2 buffer to spin column. Centrifuge at 14,000 rpm for 3 minutes. Discard waste and old collection tube and replace with a labeled, sterile 1.5 mL or 2 mL microcentrifuge tube.
8) Finally, add 200 µL of elution buffer (AE or TE buffer) to the spin column. Incubate at room temperature for 1 minute. Centrifuge at 8000rpm for 1 minute.
9) Quantify DNA yield using NanoDrop (blank the NanoDrop with the same liquid DNA is in)

    - 260/280 ratio should be between 1.8 – 1.9
    - Store DNA at -20°C for short term and -80°C for long term

Blood

- Begin with a 50 µL of separated RBCs. Add 170 µL of PBS (without calcium and magnesium) and 20 µL proteinase K.
- Proceed with steps 2-9 above.

Tumors

- Begin with a 5-25 mg tumor piece.
- Add 180 µL ATL buffer and 20 µL proteinase K. Incubate at 56°C until visible tumor is dissolved (occasionally vortex).
- Add 200 µL AL buffer and 200 µL ethanol (100%). Vortex thoroughly.
- Proceed with steps 4-9 above.

Dilutions and Amplification

Dilute DNA

- Prepare dilutions to 0.5ng/µL into 500 µL of sterile water.

Prepare PCR strips

All kit components are stored frozen and are returned to the freezer after use.

- Promega GenePrint® 10 System (part number B9510) reaction components:
    - Prepare below mixture into a sterile 1.5mL microcentrifuge tube and vortex.
    - 5 µL per sample of Master Mix
    - 5 µL per sample of Primer Pair Mix
- Add 10 µL of prepared Master Mix with Primer to each well of a PCR tube strip.
- Add 15 µL of each diluted sample (7.5ng of DNA for each sample)
- *Control Tube:* Add 1 µL of positive DNA control to 14 µL of amplification grade water with the 10 µL of Master Mix with Primer pairs
- Vortex. Quick spin in the post amp area and place in the thermal cycler.

Amplification
25 µL Total Reaction Volume

Initial Incubation Step	28 Cycles			Final Extension	Final Hold
96°C	94°C	59°C	72°C	60°C	4°C
1 Min	10 Sec	1 Min	30 Sec	10 Min	∞

Capillary Electrophoresis using the Applied Biosystems 3130xl Genetic Analyzer

Set up

- Create a new plate under <plate manager> that corresponds to the orientation of the samples in the PCR strips.
- Each sample entry should read as follows:
    - Sample Name
    - Results Group: Results_Group1
    - Instrument Protocol: GP10

Machine Maintenance

- Add fresh 1x running buffer to the anode buffer reservoir jar (16 mL) and cathode reservoir tray, position 1 (16 mL). Add pure water to the two water reservoir trays, positions 2 and 4, (16 mL each).
- Check for bubbles in the polymer.
    - For visible bubbles, follow the bubble remove wizard
- Evaluate the POP-7™ weekly 9at least 600 µL is required per run) .
- For all other maintenance, reference the Applied Biosystems Guide(s).

Preparing Plate

- Combine 152 µL of HiDi™ Formamide with 8 µL of ILS 600 size marker for each group of 16 wells. Vortex.
    - Never refreeze the HiDi after thawing
- Add 10 µL of prepared formamide/size standard cocktail to each of the 16 wells (minimum).
- Add 1 µL of Allelic Ladder to the last well of each set of 16 wells.
- Add 1 µL of each amplified sample and each control to the well that corresponds to the prepared plate above.
- Centrifuge the 96-well plate with septa for 15-20 seconds.
- Assemble the run plate.
- Place the plate assembly in the 3130xl Genetic Analyzer, close the doors, <Link Plate> and <RUN> (green arrow).

Reading Plates

- Samples are analyzed using GeneMapper® ID v3.2.1 software

Interpreting and Recording Results

Data Organization

- Data is organized within an STR Access® database by date and cell line name
- All samples typed (including repeats) are recorded into the STR Access® database and routinely updated into the COGcell.org and TXCCR.org STR database (this includes original patient material, cell lines, xenografts, lymphoblastoid cell lines and fibroblast cell lines)
    - Duplicate profiles from repeated testing of the same samples are recorded into the database to keep a historical record of allelic patterns
- All profiles are search-capable via the cell line name, coded patient identifier, date and the specific STR profile
- The original data worksheet for each sample listing each locus repeat number(s) and date of testing are kept alphabetically in binders after electronic data entry.

Verifying Known Profiles

- All cell lines are verified as a match against the original patient material
- All subsequently tested samples are then verified as an STR match when compared to previously tested material within the STR Access® database and COGcell.org
    - Any commercial lines are compared to available data through the cell line providers website (ex. ATCC or Sigma)
- All patient derived material should match the original processed samples

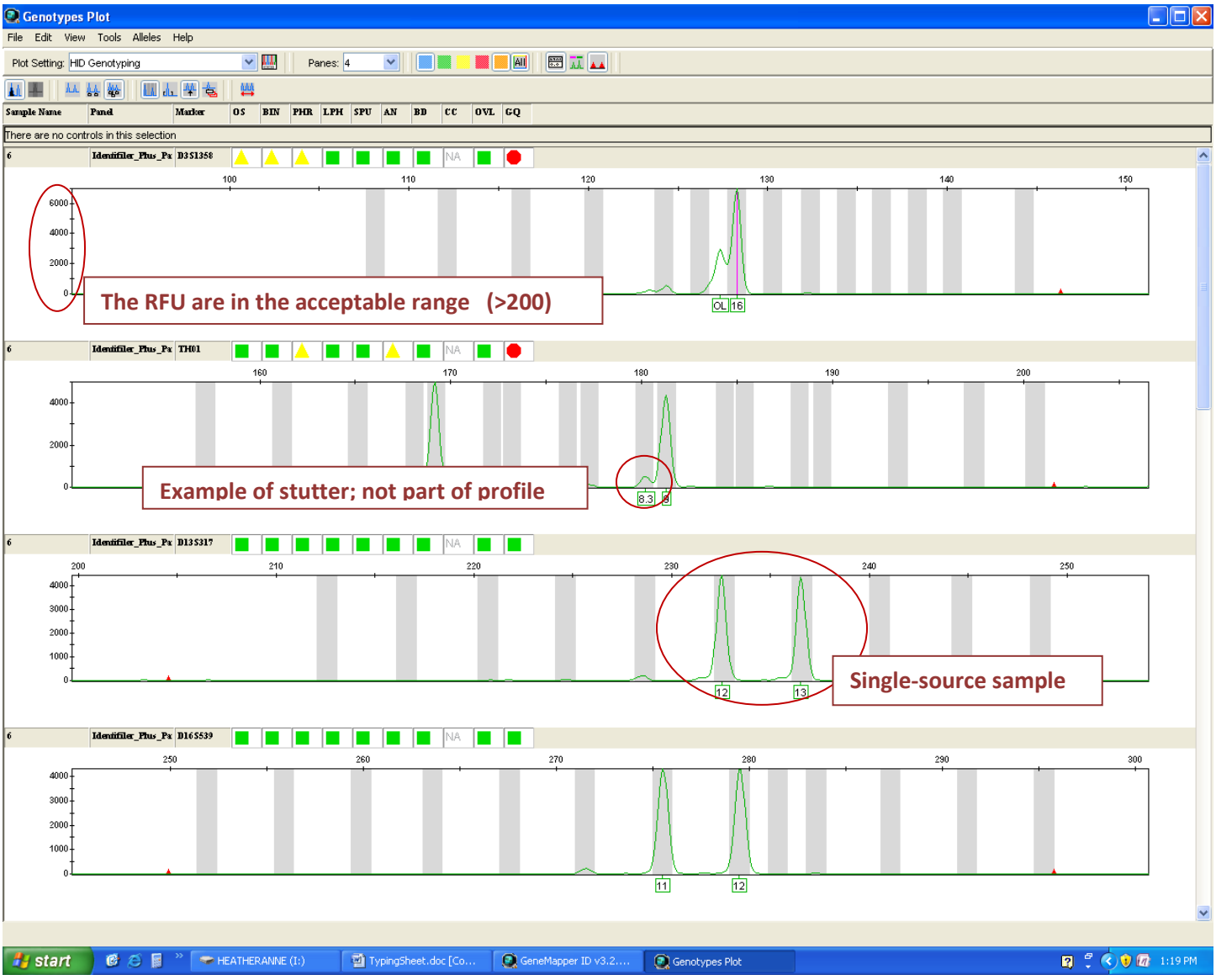
Identifying Unique Samples

- Unique samples are those that do not have any original saved patient material to test against and do not match any profiles within the STR Access® database, COGcell.org/TXCCR.org database, and available commercial search engines
- For these lines, clear notes are made that the profile is STR verified as unique, but that no original patient material is available

Cross-Contamination

- For samples that do not match their original known profiles within the STR Access® database, COGcell.org, and available commercial search engines all of the following apply:
    - Verify that the allele peaks had acceptable RFU values, if not repeat the analysis.
    - Verify that the cross-contaminate is not a laboratory staff member.
    - Compare the contaminated profile against other lines the sample submitter is working with.
    - If the contaminant remains unknown, discard all specimens associated with that sample and go back to a cell line expansion passage and re-test that previous material before continuing experiments
    - Profiles containing DNA from more than one source should be evaluated to determine possible contributors.

Example of an STR Profile within GeneMapperID



GenePrint® 10 Kit Loci

	Chromosome Location	Amplicon Sizes
THO1	11p15.5	156-195 bp
D21S11	21q11-21q21	203-259 bp
D5S818	5q23.3-32	119-155 bp
D13S317	13q22-q31	176-208 bp
D7S820	7q11.21-22	215-247 bp
D16S539	16q24-qter	264-304 bp
CSF1PO	5q33.3-34	321-357 bp
Amelogenin	Xp22.1-22.3 and Y	106 and 112 bp
vWA	12p13.31	123-171 bp
TPOX	2p23-2pter	262-290 bp