Molecular Capabilities in TRIP

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TRIP Lab Services

Wednesday, July 20, 2016

1022 WIMR

12noon-1pm

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Translational Research Initiatives in Pathology
HISTOLOGY - MOLECULAR - IMAGING

Translational Research Initiatives in Pathology (TRIP)

Molecular Capabilities

- **Special Sample Processing for Molecular Analysis**
  tissue sections, laser capture microdissection, macrodissection and TMA cores
- **Nucleic Acid Isolation (DNA/RNA/miRNA/TNA):**
  fresh/frozen tissue/cells, blood/plasma, formalin fixed paraffin embedded (FFPE) tissue, dissected/TMA core tissue, cell free DNA (plasma)
- **Molecular Project Assay Development**
  - Qualitative/Quantitative real time RT-PCR or PCR analysis
  - Genotyping
    - Fluorescent Melting Curve Analysis
    - Sanger Sequencing (Biotech, UWHC MDx)
    - Pyrosequencing (3P Lab, UWHC MDx)
    - NGS (Biotech, 3P Lab, UWHC MDx)
- **Human Cell Line Authentication**

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Promega Maxwell® CSC and the Maxwell® DNA FFPE Kit

Promega Maxwell® CSC[1]
Maxwell® CSC DNA FFPE Kit[1,2]

[1] For In vitro Diagnostic Use
[2] Intended for use with FFPE tissues collected from human breast, lung or colon

- Graphical user interface via integrated tablet touchscreen
- Self Test documented: mechanical operation, heater works, database functional
- Complete technical manual for Maxwell® CSC and protocols on tablet
- User documentation (network administrator); documents on report (audit trail)
- Use of UV light can be controlled/documented by administrator
- Reporting: electronic or hardcopy via printer
- Select (barcode), add and update protocols
- Sample tracking capabilities via barcode scanner (50 characters)
- Reagent kit lot number and expiration date tracking via barcode scanner
- Software requires user acknowledgement (3 setup steps)
- All reagents and disposables are present in the kit (no aliquoting or preparation needed)
- No organic reagents required for deparaffinization
- No manual liquid pipetting in Maxwell® CSC processing
- Throughput up to 16 samples in <40 minutes hands on time; walk away run time
- Maxwell® CSC DNA FFPE kit requires 1 x 5µM tissue section

Benefits of Maxwell® CSC FFPE DNA Protocol

- **Safety**: benefits of removal of paraffin by mineral oil
- **Efficiency**: no repeated deparaffinization/ETOH/water steps
- **DNA yield**: on average provides an overall greater DNA yield
- **DNA performance**: greater functionality (amplification(s), library(s))
- **Conservation of tissue**: reduced our FFPE/slide needs (40-60%)

Analyte	Analytical Sensitivity	% Tumor Sensitivity	> %Tumor Sensitivity	≤ %Tumor Sensitivity
KRAS Testing	10%	**20%**	5 x 5uM tissue curls in tube	5 x 5uM slides (*3rd H&E stained)
BRAF Testing	5%	**10%**	5 x 5uM tissue curls in tube	5 x 5uM slides (*3rd H&E stained)
EGFR Testing	15%	**30%**	5 x 5uM tissue curls in tube	5 x 5uM slides (*3rd H&E stained)
Cancer Gene Mutation Panel	5-10% (Coverage)	**Macrodissection of Tumor**		3 x 5uM slides (*2nd H&E stained)

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Qualitative real time PCR analysis

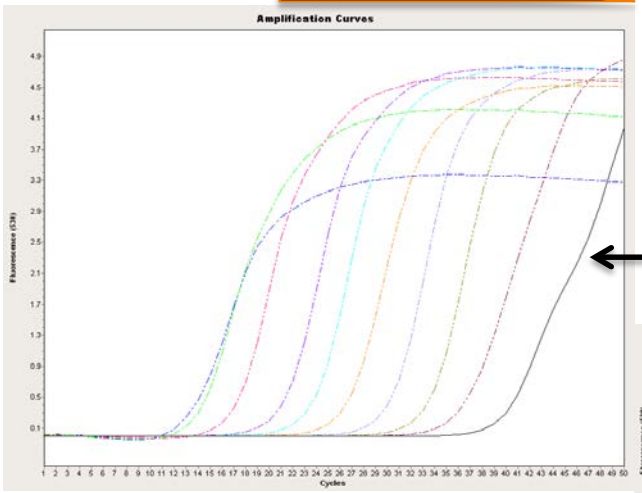
Roche
LightCycler 2.0

Dr. Amish Raval/Dr. Derek Hei
Cardiovascular Medicine/WCBF

Evaluating human stem cells
in reducing inflammation in various
animal models of heart damage.

Molecular marker differentiating human vs rat cells?

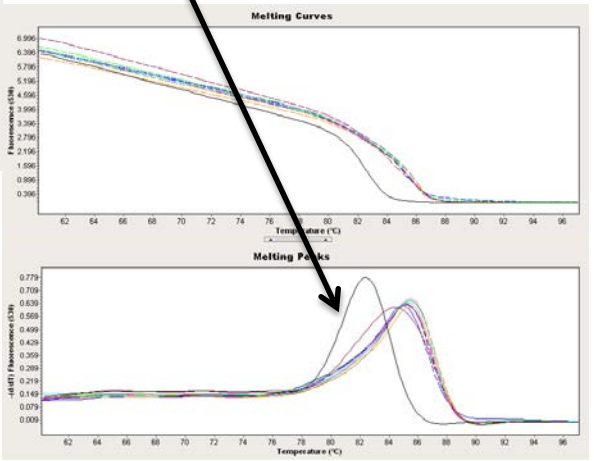
500ng human 0



Differentially amplifying human Alu repeats in the presence of 500ng of rat DNA

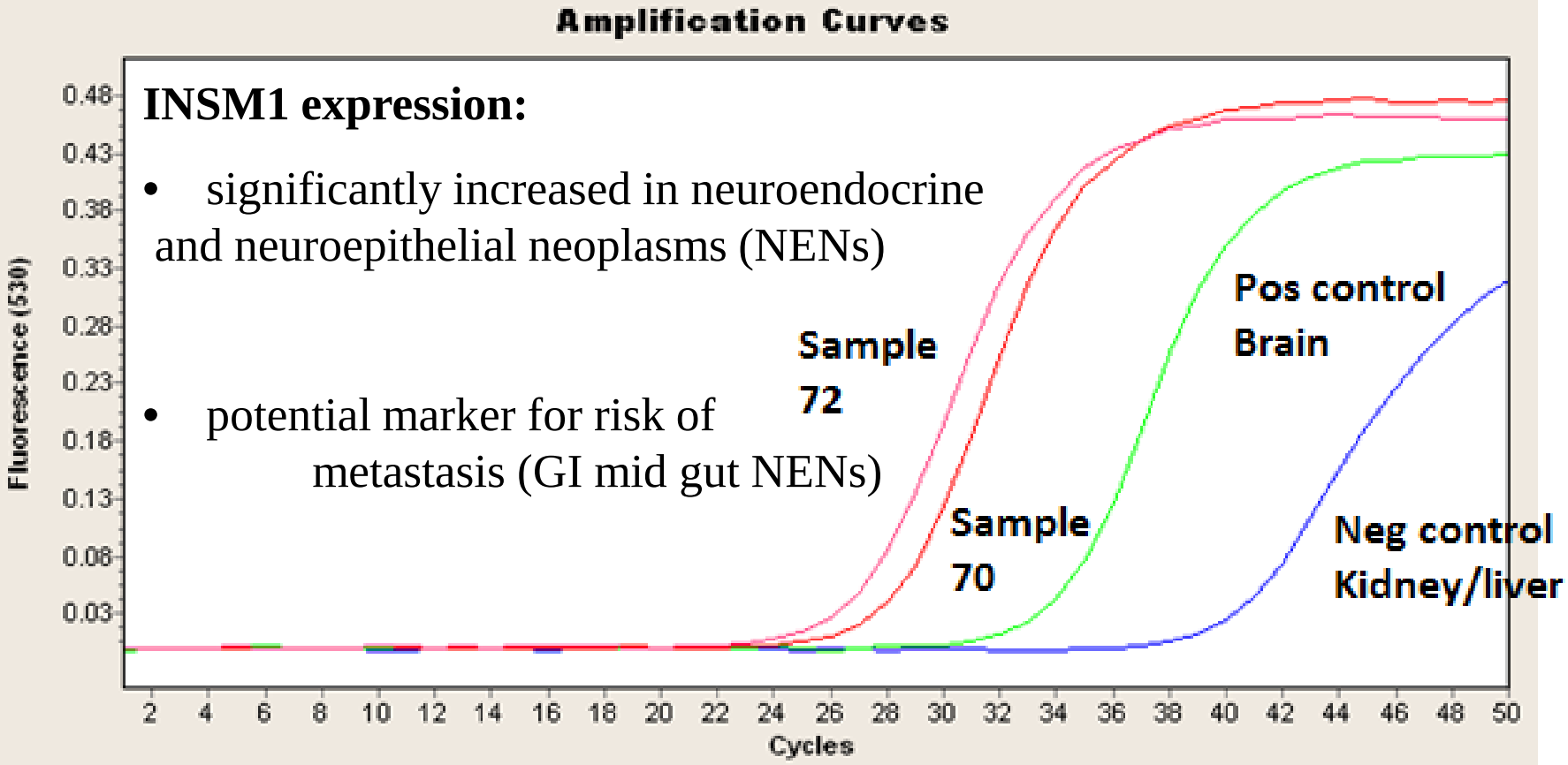
500ng rat

Sybr Green



Quantitative real time RT-PCR analysis

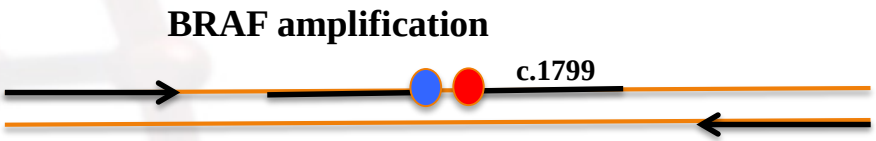
Relative INSM1 Gene Expression

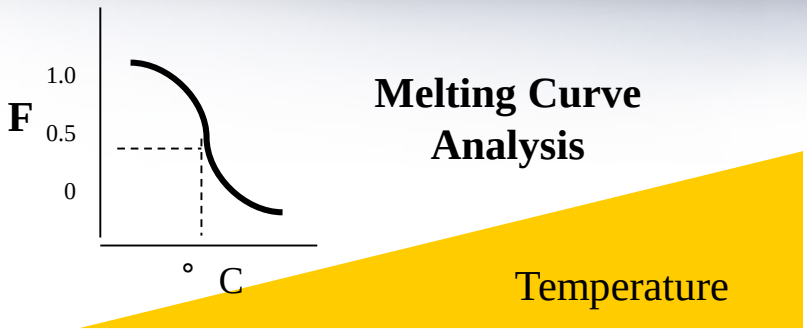


INSM1: A Novel Immunohistochemical and Molecular Marker for Neuroendocrine and Neuroepithelial Neoplasms. Rosenbaum JN, Guo Z, Baus RM, Werner H, Rehrauer WM, Lloyd RV. Am J Clin Pathol. 2015 Oct;144(4):579-591. PMID: 26386079

Genotyping: Real Time PCR and Flourescent Melting Curve Analysis

BRAF V600 Genotyping



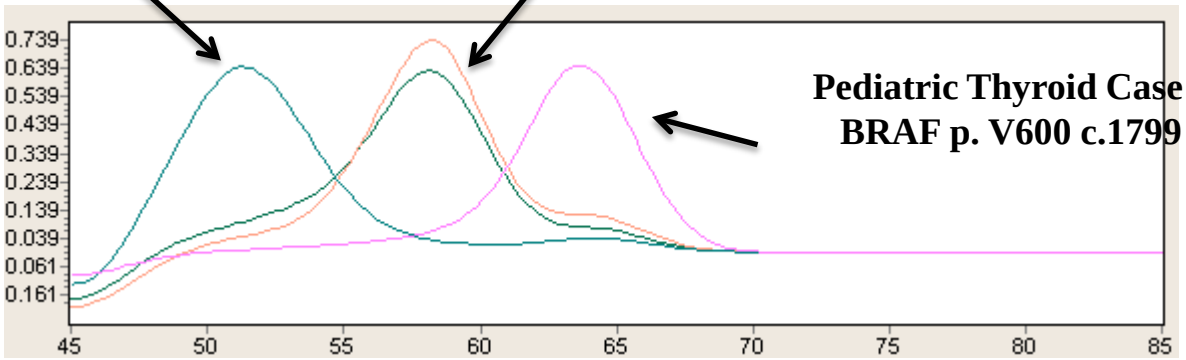


Pediatric Thyroid Case #8
BRAF p. V600_K601>E c.1799_1801delTGA

Pediatric Thyroid Cases #2 and #5
BRAF p. V600E c.1799 G>A

Pediatric Thyroid Case #11
BRAF p. V600 c.1799 G

Rate of Change Fluorescence



Temperature (° C)

Mutation in BRAF and Other Members of the MAPK Pathway in Papillary Thyroid Carcinoma in the Pediatric Population. Gertz RJ, Nikiforov Y, Rehrauer W, McDaniel L, Lloyd RV. Arch Pathol Lab Med. 2016 Feb;140(2):134-139.PMID: 26910217

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The Need for Human Cell Line Authentication

Consequences of Misidentified/Contaminated Cell Lines

- Possible (inevitable) loss of the cell line
- Costs: effort, time and money
- Misinformation within the published/private archive
- Discordant and/or irreproducible results
- Embarassment, humiliation and data retraction

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Requirement for Cell Line Authentication

-editorial policy prior to publication

AACR Journals

- Cancer Discovery
- Cancer Research
- Clinical Cancer Research
- Cancer Epidemiology, Biomarkers & Prevention
- Molecular Cancer Research
- Molecular Cancer Therapeutics
- Cancer Prevention Research

Endocrine Society journals

- Endocrinology
- Endocrine Reviews
- Journal of Clincal Endocrinology & Metabolism
- Molecular Endocrinology
- Hormones and Cancer

Society for Endocrinology journals

- Journal of Endocrinology
- Journal of Molecular Endocrinology
- Endocrine-Related Cancer

Nature Publishing Group

- Nature Reviews Molecular Cell Biology
- Nature
- Nature Genetics
- Nature Reviews Immunology
- Nature Reviews Cancer
- Nature Reviews Neuroscience
- Nature Biotechnology
- Nature Methods

and a growing number of others!

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TRIP: Human Cell Line Authentication Service

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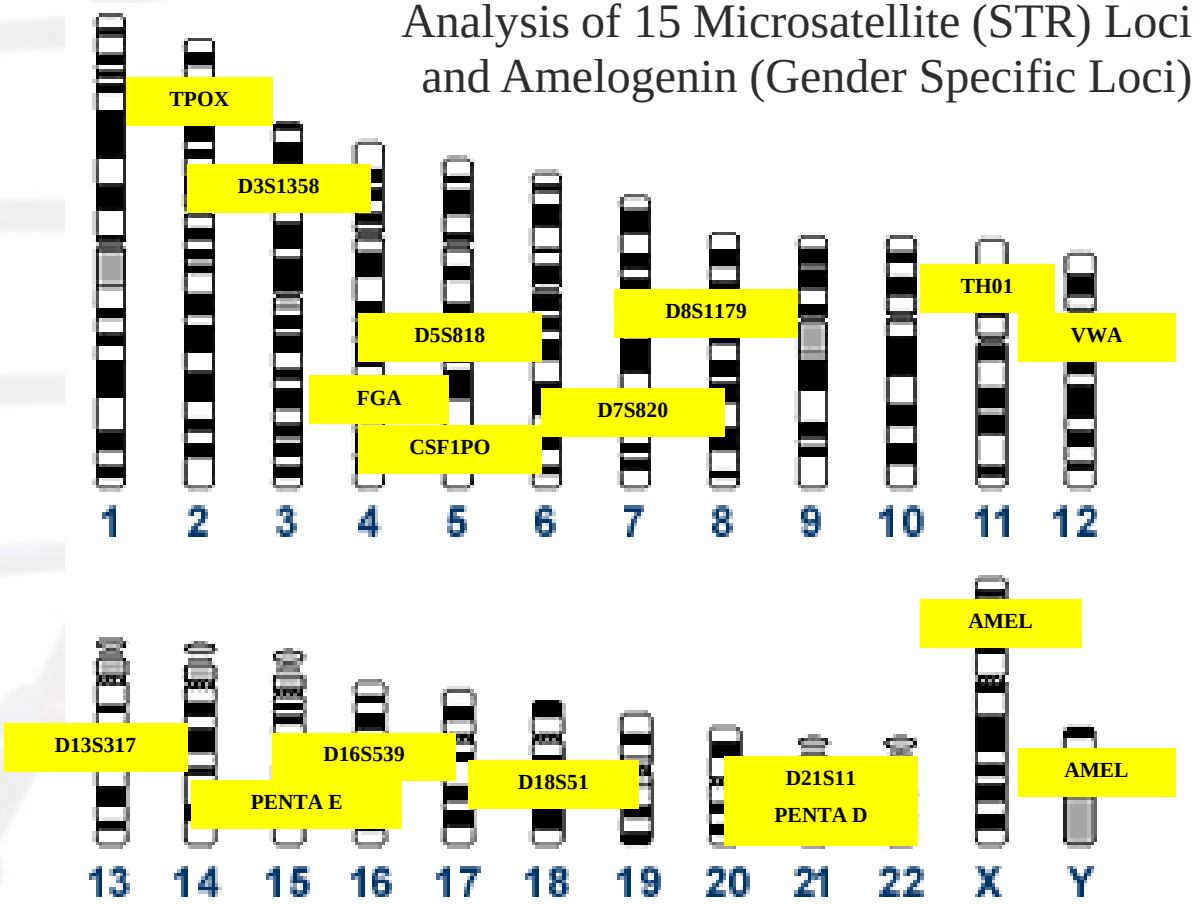
Analysis of 15 Microsatellite (STR) Loci and Amelogenin (Gender Specific Loci)



Sample Types Analyzed
- **DNA**
- **Cells**

Maximum Turnaround Time
- **10 working days**

Cost
- **$125 DNA sample**
- **$135 Cell sample**

Interpretative Report
- **Identification**
- **Authentication**
- **Contamination (2% Sensitivity)**

Analysis relative to:
- **Publication(s)**
- **Repository(s)**
- **Baseline**

TRIP: Human Cell Line Authentication Service

Since the fall of 2014, over 900 samples analyzed thus far …..

UW Investigators

David Beebe Lab
Nihal Ahmad Lab
Amy Fowler Lab
Randall Kimple Lab
Paul Lambert Lab
Igor Slukvin Lab
Ruth O'Regan Lab
Small Molecule Synthesis Facility (SMSF)
Pam Kreeger Lab
Elaine Alarid Lab
David Jarrard Lab
Shannon Kenney Lab
Beth Weaver Lab

Clients Outside of UW

WiCell QA Dept
WiCell Cytogenetics Dept
National Instutes of Health (NIH)
National Heart, Lung, and Blood Institute (NIH-NHLBI)
ALS Therapy Development Institute (ALS-TDI)
Vertex Pharmaceuticals
Gamm Lab (NIH)
Salk Institute
Stanford University
National Center for Advancing Translational Sciences
Mount Sinai School of Medicine
Huang Lab (NIH)
Thermo Fisher Scientific
Cellectar Biosciences

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Acknowledgements

Chair of Pathology

Dr. Michael Hart
Dr. Andreas Friedl

Director of TRIP /UWCCC TSB

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