Clinical Genomics
CGAT
Advanced Technology

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GEISEL SCHOOL OF
MEDICINE

Empowering STAT DNA Testing for Molecular Oncology Applications

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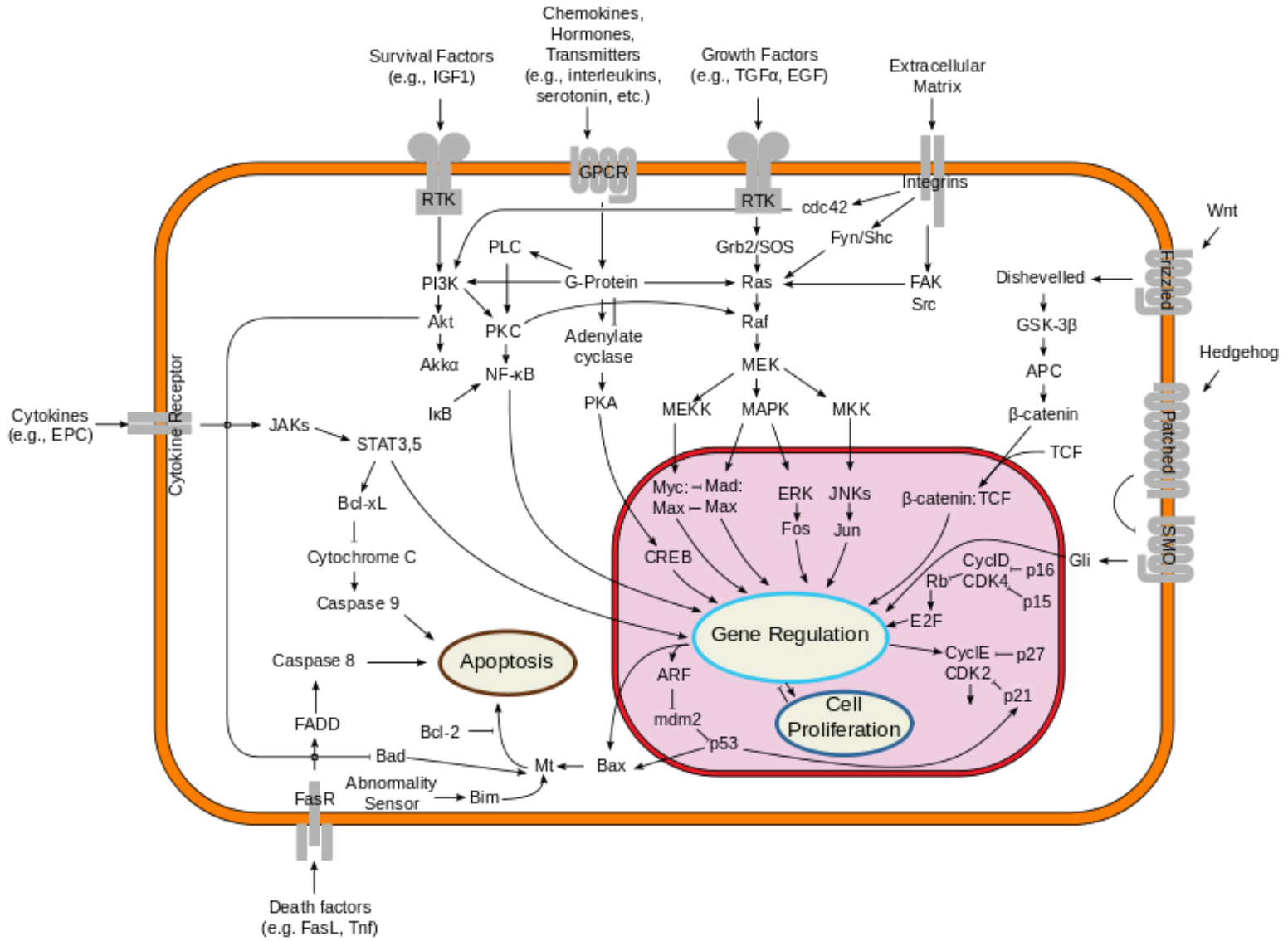
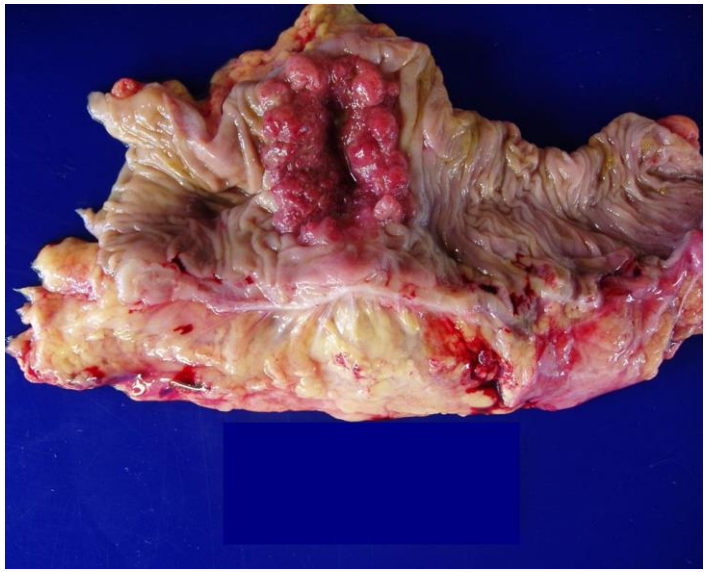
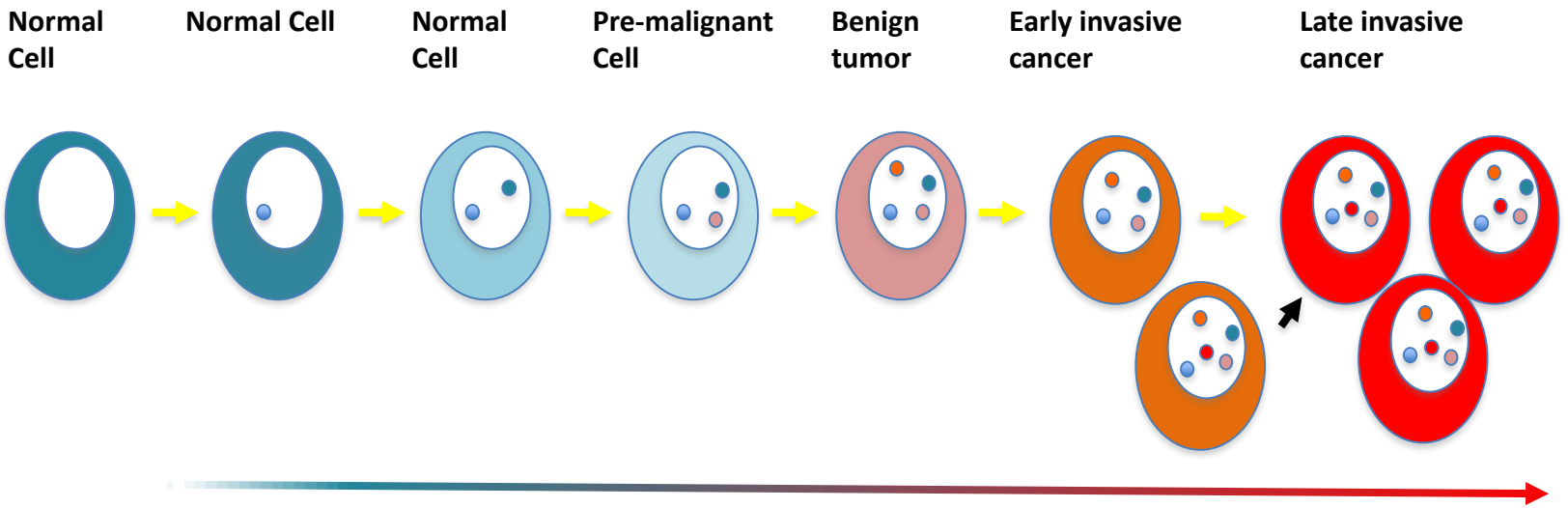
Precision Medicine and Human Cancers





- Diagnosis?
- Prognosis?
- ***Therapeutic selection***?

Precision Medicine and Human Cancers

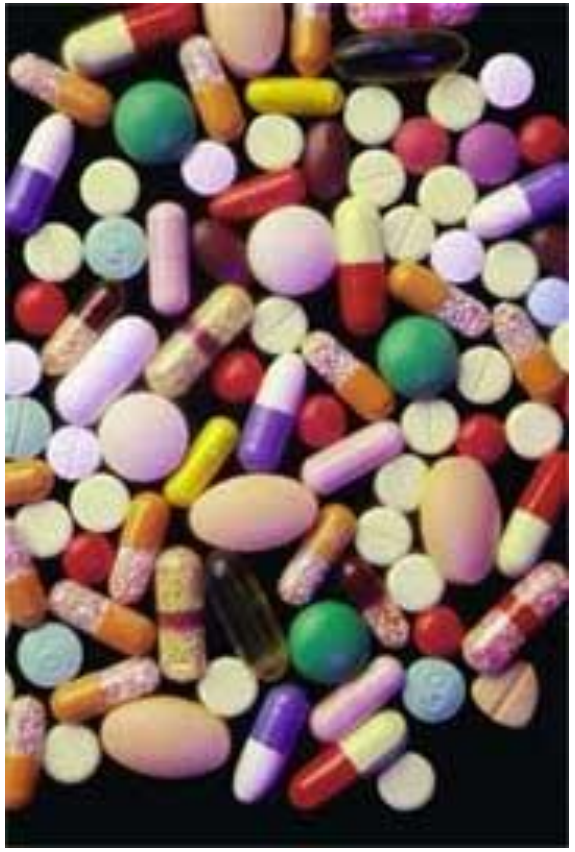


Precision Medicine (Pharmacogenomics)

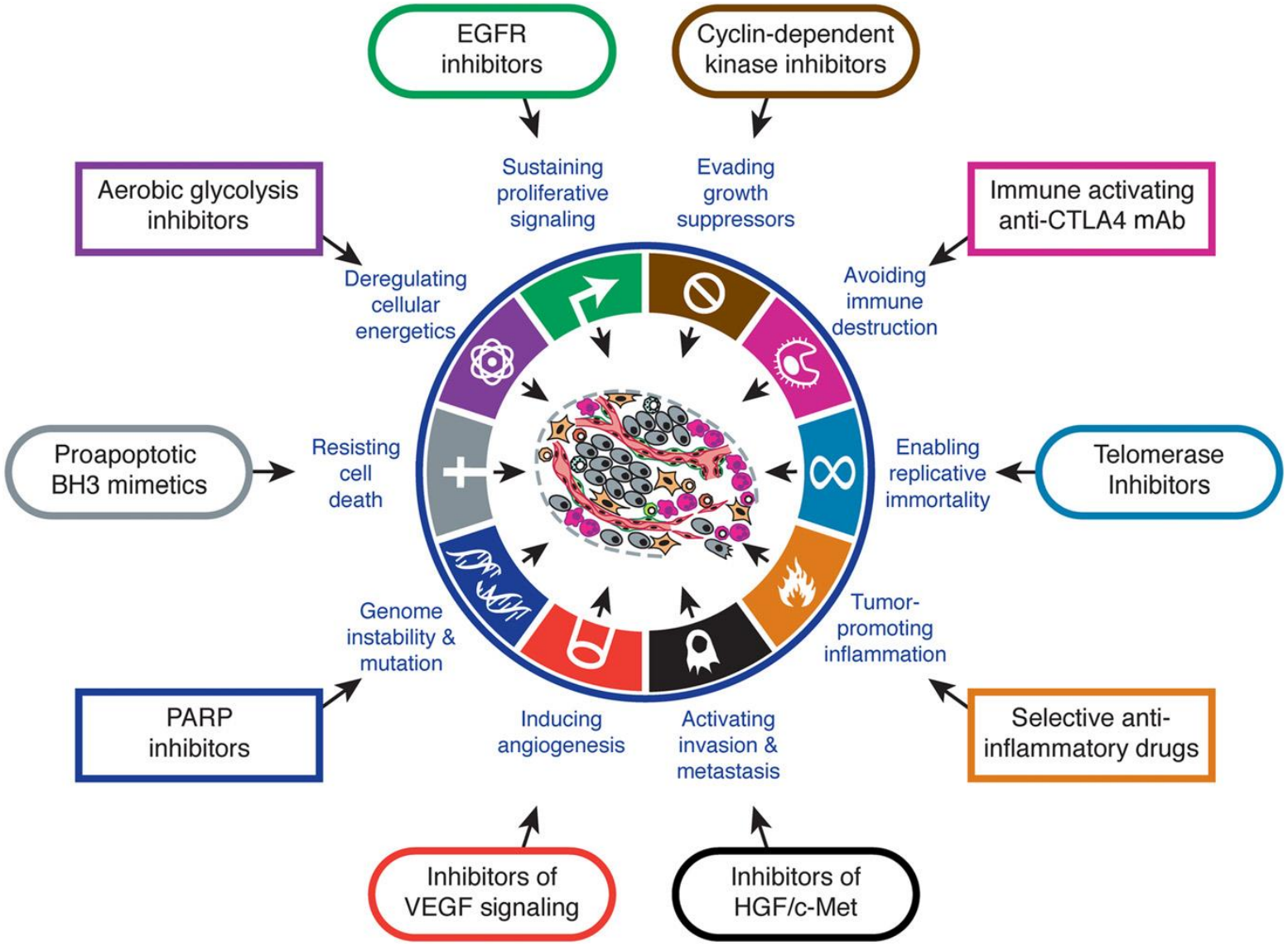
- PGx_m: pharmacokinetic
  - What the body does to the drug
    - Absorption
    - Distribution
    - Metabolism
    - Excretion
- PGx_t: targeted therapy
  - Presence/absence of therapeutic target
  - Response or lack of response
  - Resistance
  - Local or distant recurrence

Precision Medicine in Oncology

- BCR-ABL1
  - Imatinib (Gleevec) for CML
- HER2 amplification
  - Trastuzumab (Herceptin) for breast cancer
- KRAS point mutation
  - Cetuximab and Panitumimab for colon cancer
- EGFR point mutation and/or amplification
  - Iressa, Tarceva for lung cancer

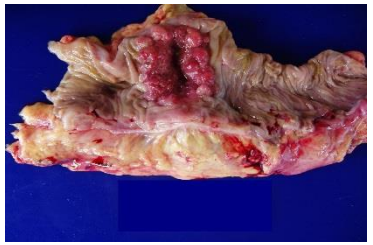


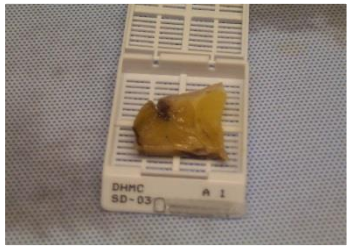
Precision Medicine and Somatic Mutation Analysis



Hanahan et al. *Cell*, 144:646–74, 2011.

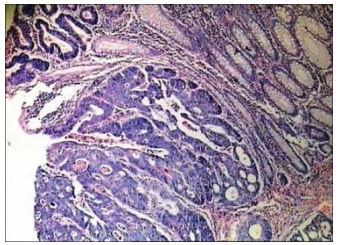
Assessment of Human Cancer





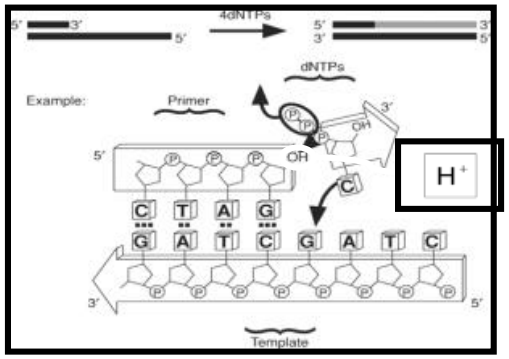


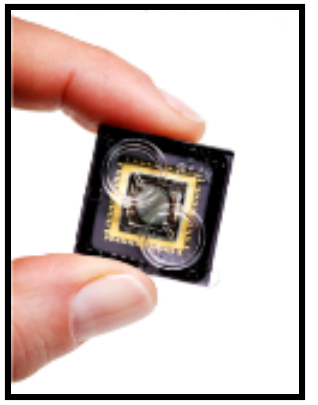


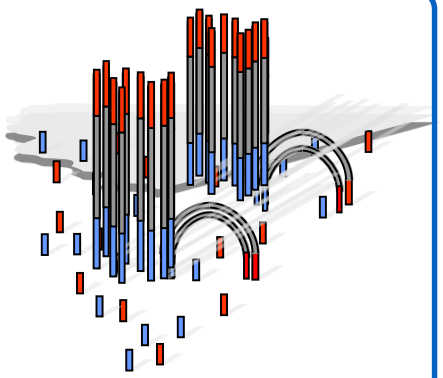


Companion/Complementary testing

Precision Medicine and Somatic Mutation Analysis

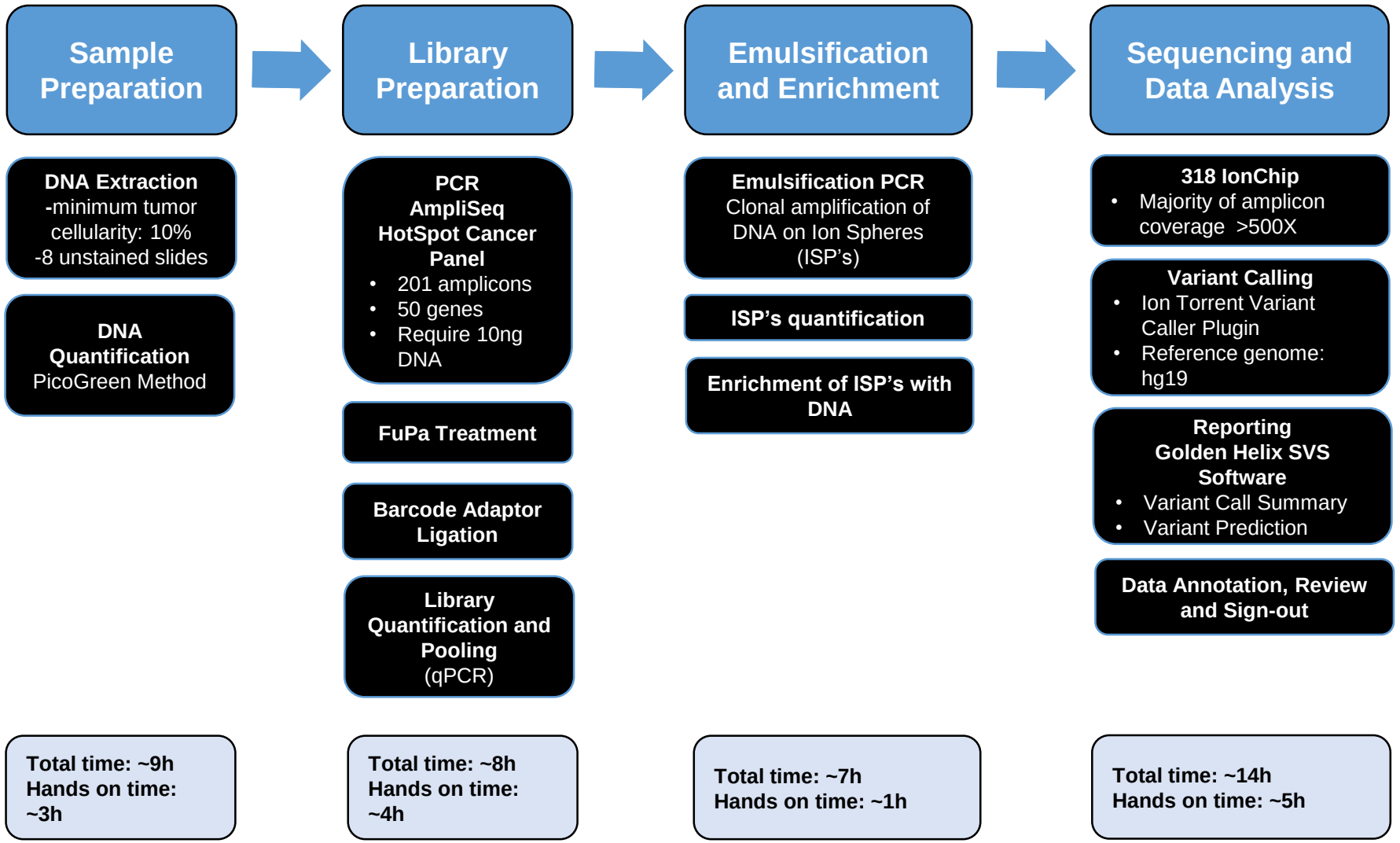






Single molecule array

Precision Medicine and Somatic Mutation Analysis
(Ion Torrent Cancer Hotpsot Panel)



ABSTRACT (1998)

DNA STAT

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Introduction. Rapid advances in molecular biology techniques over the past few years have resulted in a transition of these technologies from the research laboratory to the clinical laboratory and in the near future to the bedside. Following in the footsteps of other more established clinical diagnostic technologies, nucleic acid testing is becoming automated and very routine for the evaluation of hematologic, infectious, and genetic diseases. One disadvantage of these new technologies has been the inability for rapid turn around times, a clinical assay attribute crucial for the critically ill patient. While a STAT designation is unbecoming of nucleic acid based tests, new methods for performing DNA/RNA extraction, amplification and detection have reduced the turn around times for these assays dramatically. The aim of this study is to demonstrate some of the time savings in performing nucleic acid tests based on currently available technologies with respect to assays suitable for the critical care patient.

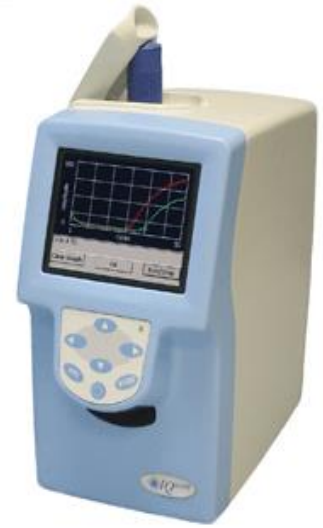
Methods. Random whole blood specimens which were submitted for CBCs were received from Hematology. DNA extraction was performed using the Puregene Kit (Gentra Systems, Minneapolis, MN) according to the recommendations of the manufacturer. Multiple PCR assays were evaluated for different target sequences, including human genomic targets and microbial targets in a time study to optimize amplification efficiency and turn around times. Detection methods included agarose and polyacrylamide gel electrophoresis, liquid hybridization assays, and fragment size analysis using an automated DNA sequencing system (OpenGene, Visible Genetics, Toronto, Canada).

Results. Using rapid column extraction protocols, DNA suitable for PCR amplification can be isolated from whole blood specimens in less than 30 minutes. While PCR amplification times are most often target dependent, newer thermal cyclers can speed this process to less than two hours. Detection by gel electrophoresis, liquid hybridization and/or automated DNA sequencer analysis can also be accomplished within two to three hours. Thus, a completed molecular diagnostic assay for the qualitative detection of a target sequence can be accomplished with an approximately five hour turn around time.

Conclusions. In this study, we demonstrate the feasibility of a STAT nucleic acid based test. Using modified protocols and newer technologies, we are able to detect the presence of a target sequence within five hours. While five hours may not seem appropriate for a STAT designation with respect to more traditional automated clinical diagnostic assays, this is extremely rapid for a molecular based assay. However, with respect to the critical care patient, our ability to detect the presence of a microbial pathogen within a few hours versus a few days may prove crucial to decreasing morbidity and mortality of these patients. In addition, continued advances in these technologies such as DNA chip based assays and highly automated instrumentation will continue to drive turn around times downward while maintaining extraordinarily high sensitivities and specificities.

Many Opportunities for Molecular Infectious Disease Testing

- Low complexity
- Medium-low throughput
- Low hands on time
- Low TAT



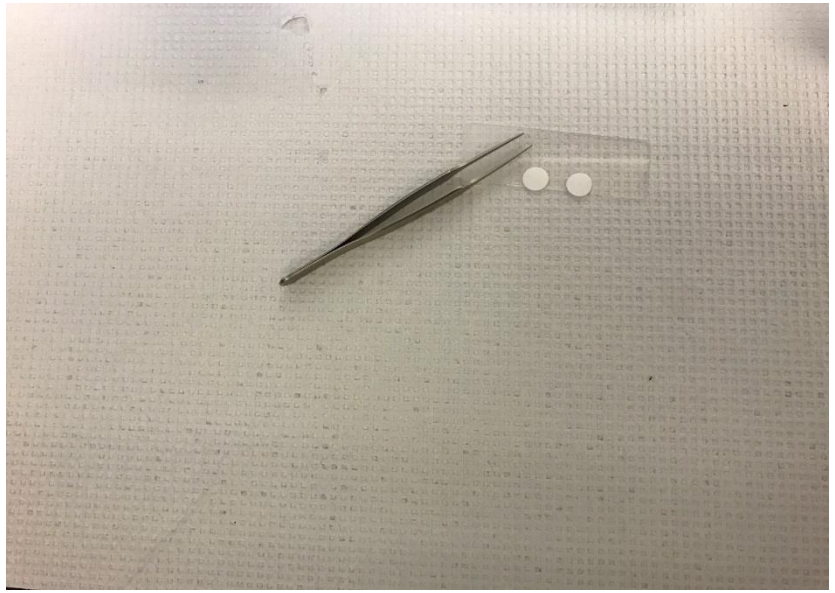
STAT DNA Testing for Oncology?

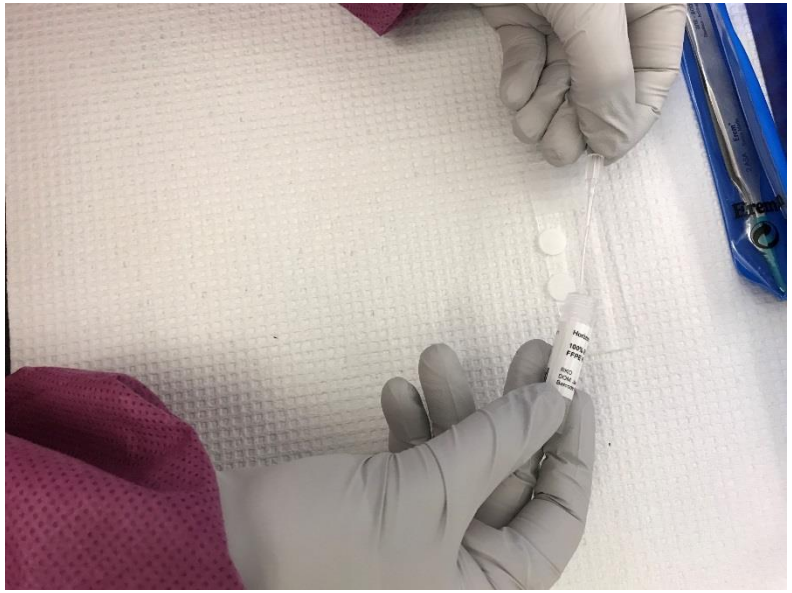
- Clinical utility
- Complex specimen (FFPE tissue)
- Assay performance
- TAT
- Data analysis

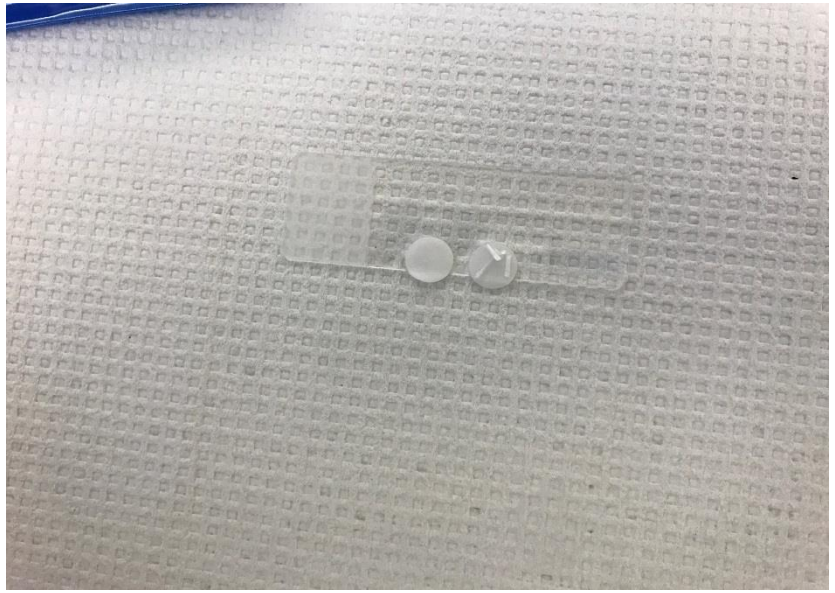
STAT DNA Testing for Oncology

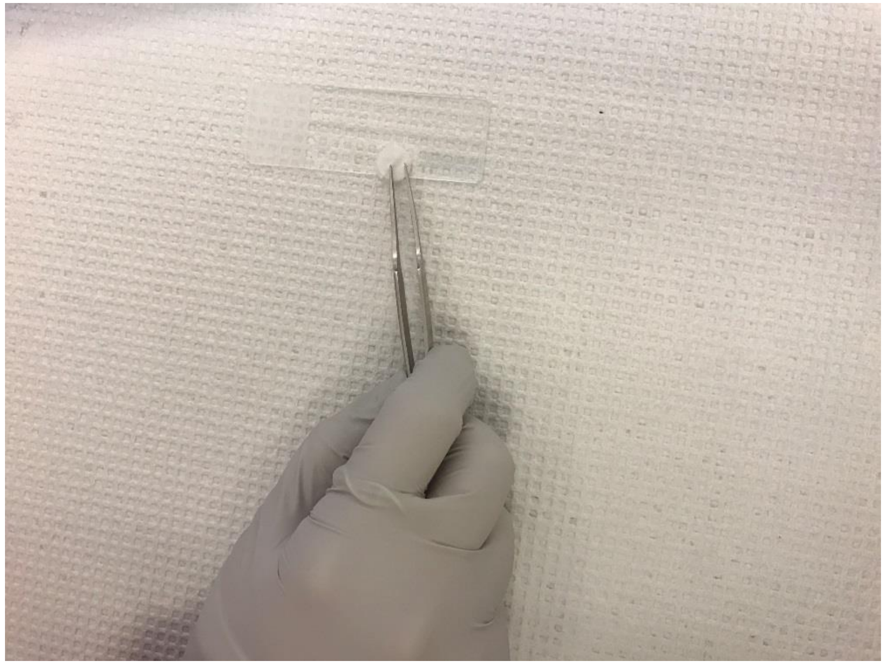
Simplifying FFPE Somatic Mutation Testing

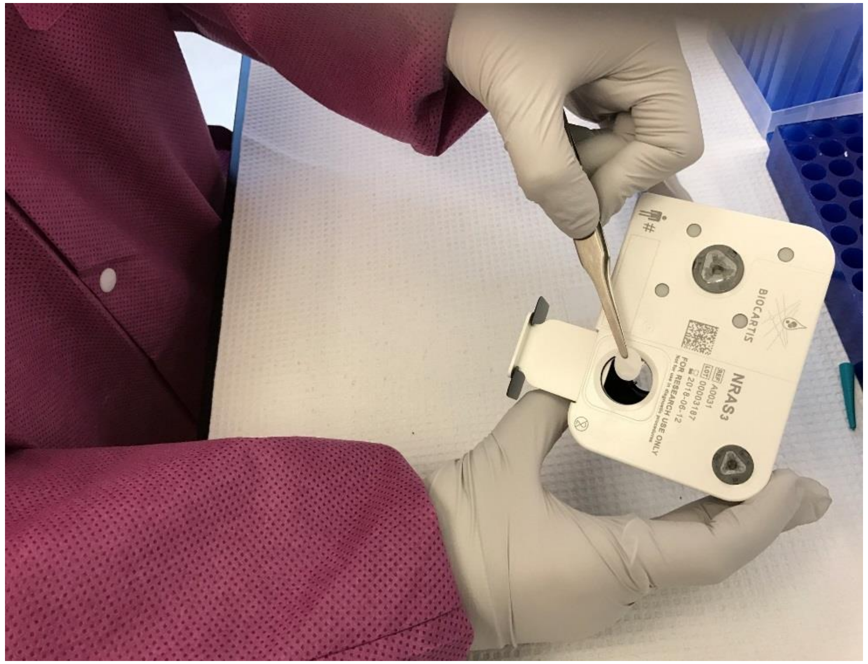
M. Rabie Al-Turkmani, PhD and Kelley Godwin, BS









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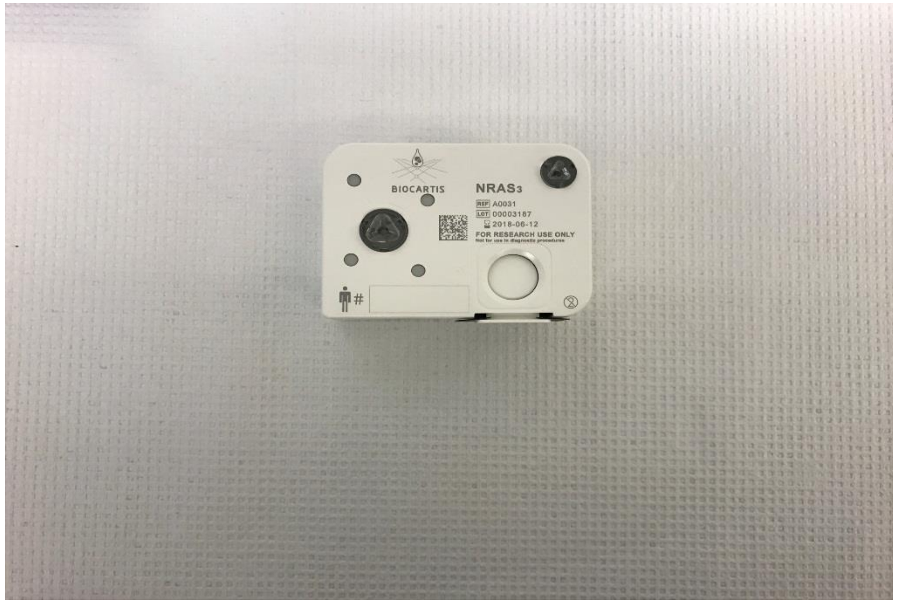
FOR RESEARCH USE ONLY
Not for use in diagnostic procedures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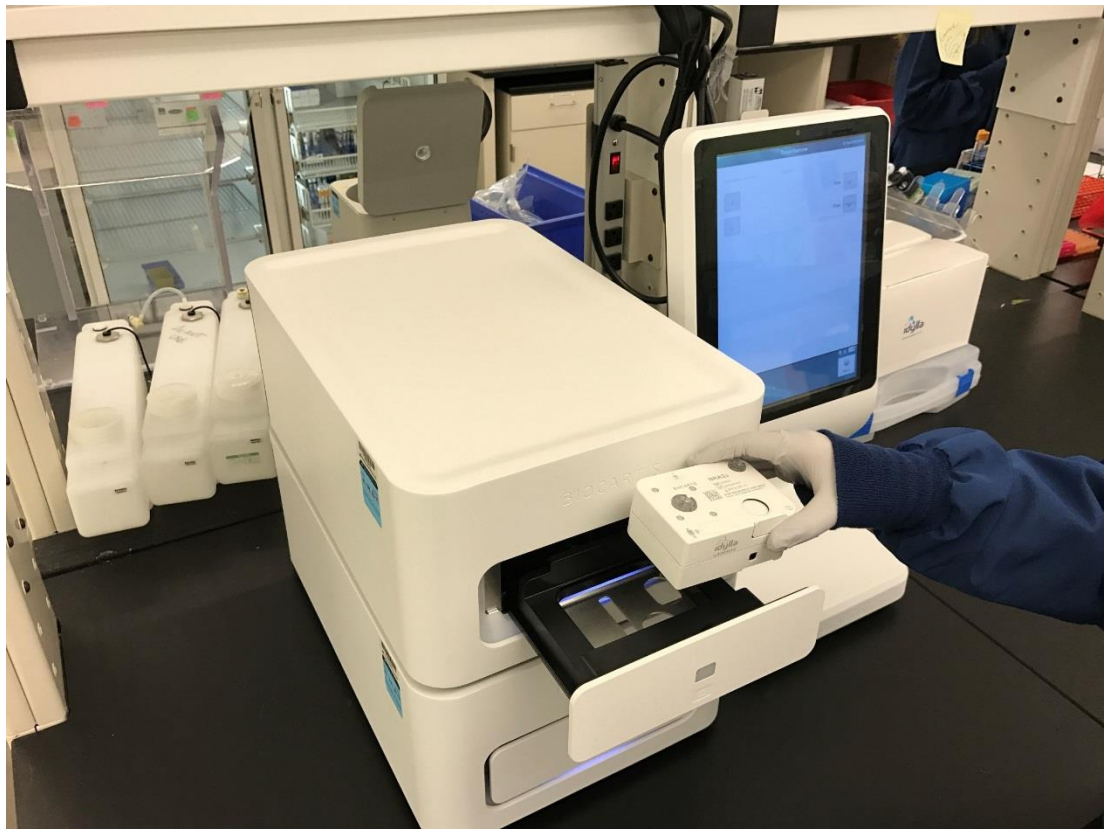

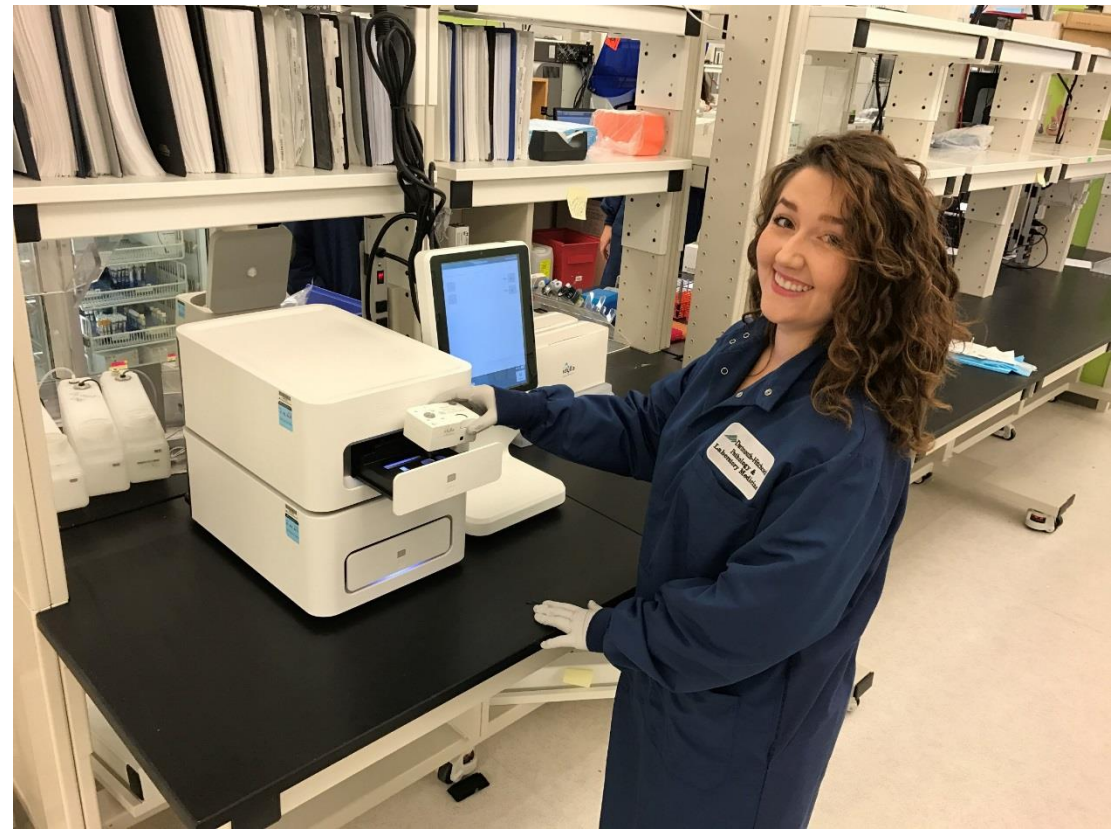

Assay Evaluated

- Idylla KRAS Mutation Assay
- Idylla NRAS-BRAF-EGFR S492R Mutation Assay ($NRAS_3$)
  - 21 mutations in KRAS exon 2, 3, and 4
  - 25 mutations in NRAS exon 2, 3, 4, BRAF exon 15, and EGFR exon 12

Samples Analyzed

- Colorectal cancer FFPE tissue samples with mutation in *KRAS* (n=17), *NRAS* (n=5), or *BRAF* (n=12) were analyzed (total = 34).
- 10 colorectal cancer tissue samples with no mutation.
- 9 horizon control samples in triplicate (27).
- A single 10 µm FFPE tissue section was used (total of 4 sections and 2 H&E slides obtained from each sample).
- Results were compared against those previously obtained by NGS using the AmpliSeq 50-gene Cancer Hotspot Panel.

KRAS Results

Sample	Tumor Content (%)	NGS	Idylla
1	10	c.34G>T, p.G12C	c.34G>T, p.G12C
2	25	c.34G>T, p.G12C	c.34G>T, p.G12C
3	75	c.35G>A, p.G12D	c.35G>A, p.G12D
4	70	c.35G>A, p.G12D	c.35G>A, p.G12D
5	40	c.35G>A, p.G12D	c.35G>A, p.G12D
6	30	c.35G>T, p.G12V	c.35G>T, p.G12V
7	60	c.35G>T, p.G12V	c.35G>T, p.G12V
8	80	c.35G>T, p.G12V	c.35G>T, p.G12V
9	25	c.38G>A, p.G13D	c.38G>A, p.G13D
10	40	c.38G>A, p.G13D	c.38G>A, p.G13D
11	50	c.38G>A, p.G13D	c.38G>A, p.G13D
12	50	c.38G>A, p.G13D	c.38G>A, p.G13D
13	40	c.181C>A, p.Q61K	**c.181C>A** / c.180_181 delinsAA, **p.Q61K**
14	50	c.182A>G, p.Q61R	**c.182A>G** / c.182A>T, **p.Q61R**/L
15	75	c.182A>G, p.Q61R	**c.182A>G** / c.182A>T, **p.Q61R**/L
16	40	c.436G>A, p.A146T	c.436G>C/ **c.436G>A**/ c.437 C>T, **p.A146**P/**T**/V
17	50	c.436G>A, p.A146T	c.436G>C/ **c.436G>A**/ c.437 C>T, **p.A146**P/**T**/V

NRAS Results

Sample	Tumor Content (%)	NGS	Idylla
1	85	c.35G>T, p.G12V	**c.35G>T**, c.35G>T**, p.G12**A/**V**
2	70	c.37G>C, p.G13R	**c.37G>C**/ c.38G>T, **p.G13R**/V
3	50	c.183A>C, p.Q61H	**c.183A>C**; c.183A>T , **p.Q61H**
4	40	c.183A>T, p.Q61H	c.183A>C; **c.183A>T , p.Q61H**
5	80	c.183A>T, p.Q61H	c.183A>C; **c.183A>T , p.Q61H**

BRAF Results

Sample	Tumor Content (%)	NGS	Idylla
1	60	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
2	50	c.1799T>C, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
3	50	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
4	60	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
5	50	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
6	50	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
7	60	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
8	20	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
9	50	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
10	70	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
11	75	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D
12	30	c.1799T>A, p.V600E	**c.1799T>A**; c.1799_1800delinsAA/c.1799_1800delinsAC, **p.V600E**/D

Horizon Control Results

Mutation	Tumor Content (%)	Repeats	Idylla
KRAS G12V	50	3	c.35G>T, p.G12V
KRAS G13D	50	3	c.38G>A, p.G13D
KRAS Q61H	50	3	c.183A>C / c.183A>T, p.Q61H
KRAS A146 T	50	3	c.436G>C/ c.436G>A/ c.437 C>T, p.A146P/T/V
NRAS Q61H	50	3	c.183A>C, p.Q61H
NRAS Q61L	50	3	c.182A>T, p.Q61L
NRAS Q61R	50	3	c.182A>G, p.Q61R
BRAF V600E	50	3	c.1798T>A; c.1799_1800delinsAA/c.1799_1800delinsAC, p.V600E/D
BRAF V600R	50	3	c.1798_1799 delinsAA/c.1798_1799delinsAG, p.V600K/R

STAT DNA Testing for Oncology

- Robust performance
- Rapid TAT
- Ease of use
- Targeted mutations but ALL are actionable
- Potential for liquid bx analysis

Clinical Genomics and Advanced Technology (CGAT)

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UCONN Interns
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