Clinically validating automation of library preparation for NGS-based detection of cancer susceptibility and MSI in endometrial cancer patients

Natalie Bir, Patrick Brennan, Andrei Rajkovic, Jeffrey Gaither, Benjamin Kelly, Greg Wheeler, Joseph McElroy, Peter White, Catherine E. Cottrell, Vincent Magrini, Richard K. Wilson, Elaine R. Mardis, Paul Goodfellow

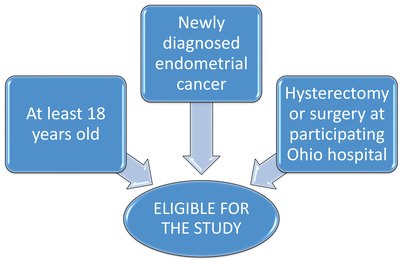
The Steve and Cindy Rasmussen Institute for Genomic Medicine at The Abigail Wexner Research Institute at Nationwide Children's Hospital; Departments of Pathology, Pediatrics, Internal Medicine and the Comprehensive Cancer Center at The Ohio State University; Department of Obstetrics and Gynecology at The Ohio State University Wexner Medical Center

Nationwide Children's — When your child needs a hospital, everything matters.

For more information on the informatics validation of this assay:
Benjamin Kelly – Poster 4004

OPTEC

Ohio Prevention and Treatment of Endometrial Cancer (OPTEC) Study is a statewide initiative to help identify women with endometrial cancer who may be at risk for other types of cancers due to their genetic makeup, and to help match women with endometrial cancer to the best treatment options for their particular cancer.



Enrollment will provide:

Free screening	• Screening for Lynch Syndrome
Tumor Testing	• Identify MMR mutations and MSI status
Therapies	• Identify best therapy or clinical trial for patient

What is Lynch Syndrome?

Germline mutation in one of the Mismatch Repair (MMR) genes → High risk for developing colon and endometrial cancer, along with other types of cancer.

5 in 100 people with endometrial cancer have Lynch syndrome

Most common approach to MSI detection is using a PCR-based assay to compare the length of microsatellite (MS) regions in tumor tissue to normal tissue. We developed a novel machine learning approach to analyze the 21 MS loci captured and sequenced by NGS from tumor only. This assay and analytic pipeline were validated in our CAP-CLIA laboratory

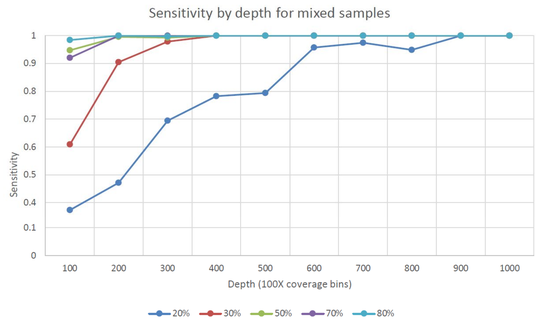
Region of Interest	Target
PMS2	All coding variants; excluding pseudogene region
MSH2	All coding variants
MSH6	All coding variants
MLH1	All coding variants
POLE	All coding variants
MS Status	21 MS regions MSS: microsatellite stable MSI: microsatellite instable

Validation Setup and Results

Sensitivity and Precision
- Combining DNA from Ashkenazim trio to mimic percentages of tumor nuclei
  - **Limit of Detection** = 15 % Variant Allele Frequency (VAF) (30% Tumor Nuclei)
- Examined 637 known variants in NA12878
  - **Results**: Sensitivity = 98.6%; Precision = 96.6%

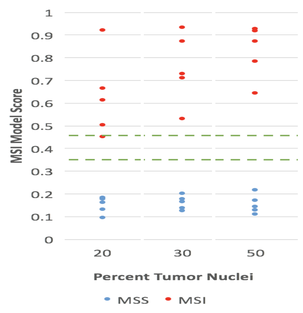
Accuracy
- Samples Tested – Total of 52 Samples
  - 52 Patient Samples - MS status
  - 32 Patient Samples - POLE status
  - 19 Patient Samples - MMR status
  - **Results**: MSI = >99%; MMR/POLE = 93.8%

Reproducibility
- Between Runs
  - Samples processed by same technologist on two different dates
  - **Results**: MSI = 100%; MMR/POLE = 87.5%
- Within Runs
  - Samples processed by two different technologists
  - **Results**: MSI = 96.2%; MMR/POLE = 96.9%



- Genome in a bottle Ashkenazim paternal DNA sample was mixed with the child DNA sample at 20%, 30%, 50%, 70%, and 80% to mimic tumor content.
- Sensitivity at 15% VAF (30% tumor nuclei) is 90.4% at 200X coverage and increases to 97.9% at 300X coverage.
- Minimum average sequencing depth is set to 1000X to achieve optimal sensitivity to call low VAF alterations across the MMR gene loci.

We computationally performed a random sample from five MSS and five MSI sample NGS read data sets to simulate 20%, 30%, and 50% tumor content. The graph below displays samples greater than 30% tumor nuclei were called at 100% concordance with prior testing.



Workflow and Automation

FFPE Tumor Tissue
- Greater than 30% tumor nuclei
- DNA extraction using AllPrep FFPE kit

Library Prep
- NEBNext Ultra II FS Kit
- IDT dual index adaptors
- Performed on Agilent Bravo Automation

Hybrid Capture
- IDT Custom Endometrial Panel (3536 probes) with the addition of the IDT CNV Plus spike-in
- IDT xGen Hybridization and Wash Kit
- Total size 1.77 Mb

The Agilent Bravo liquid handler only has 9 positions on the deck, so the protocol is split into six different modules to accommodate all the labware.

Module 1 – Fragmentation/End Repair
- Bravo adds master mix to the samples
- Incubation occurs off the deck

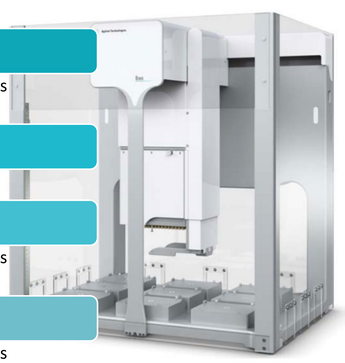
Module 2 – Adaptor Ligation
- Bravo adds master mix to the samples
- Incubation occurs ON the Bravo deck

Module 3 – Cleanup
- Bravo performs SPRI cleanup

Module 4 – Cycle Optimization
- Bravo adds master mix to the samples
- Incubation occurs off the deck

Module 5 - PCR
- Bravo adds master mix to the samples
- Incubation occurs off the deck

Module 6 - Cleanup
- Bravo performs SPRI cleanup



Sample Patient Data

Sample	Tumor Nuclei	MMR Results	MSI Results
1110-tumor	60%	NM_000251.2(MSH2):c.2089T>C:p.Cys697Arg (10% VAF)	MS-Instable

MMR Validation Results: We observed this variant at 10% VAF where the tumor nuclei was reported at 60%. Based on this observation we are increasing our target depth of 1000X average coverage in order to ensure detection low frequency variants.

MS Validation Status:
- To the right is a graphical illustration from our machine learning algorithm evaluation of an endometrial patient sample.
- The orange box and whiskers plot illustrates results from MSI high samples that were previously evaluated by a PCR-based clinical assay.
- The blue box and whiskers plot shows similarly evaluated MSS samples.
- The pink star shows the result for the patient sample, indicating a status of MSI high.