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


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Validation Setup and Results

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.

Sample Patient Data

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tumor Nuclei</th>
<th>MMR Results</th>
<th>MSI Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1110-tumor</td>
<td>60%</td>
<td>NM_000251.2(MSH2):c.2089T&gt;C:p.Cys697Arg (10% VAF)</td>
<td>MSI-Instable</td>
</tr>
</tbody>
</table>

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 evaluates 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.

Workflow and Automation

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

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

Library Prep
- NEBNext Ultra II F5 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

Region of Interest | Target
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>PMS2</td>
<td>All coding variants, excluding pseudogene region</td>
</tr>
<tr>
<td>MSH2</td>
<td>All coding variants</td>
</tr>
<tr>
<td>MSH6</td>
<td>All coding variants</td>
</tr>
<tr>
<td>MLH1</td>
<td>All coding variants</td>
</tr>
<tr>
<td>POLE</td>
<td>All coding variants</td>
</tr>
<tr>
<td>MSS</td>
<td>21 MS regions: microsatellite stable</td>
</tr>
<tr>
<td>MSI</td>
<td>microsatellite unstable</td>
</tr>
</tbody>
</table>

What is Lynch Syndrome?

Lynch Syndrome is a hereditary cancer syndrome characterized by an increased risk of developing colon and endometrial cancer, as well as other types due to their genetic makeup, and to help match women with endometrial cancer to the best treatment options for their particular cancer.

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.

At least 18 years old

OPTEC

Enrollment will provide:

- Screening for Lynch Syndrome
- Identify MMR mutations and MSI status
- Identify best therapy or clinical trial for patient

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.