Detection of mutations in single circulating tumor cells using MALDI-TOF mass spectrometry

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Abstract 750

Table of mutations targeted for SNP genotyping

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mutation</th>
<th>Allelity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-231</td>
<td>c.205G&gt;T</td>
<td>Pro</td>
</tr>
<tr>
<td></td>
<td>c.2369C&gt;T</td>
<td>Mut</td>
</tr>
<tr>
<td></td>
<td>c.353G&gt;A</td>
<td>HET</td>
</tr>
<tr>
<td></td>
<td>c.38G&gt;A</td>
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</tr>
<tr>
<td></td>
<td>c.691A&gt;G</td>
<td>Mut</td>
</tr>
<tr>
<td></td>
<td>c.818C&gt;T</td>
<td>Mut</td>
</tr>
</tbody>
</table>

MDA-MB-231 cells identified from spike-in

- Composite
- DAP
- CytoFasten
- CytoFasten DH
- CytoFasten DH/EpCAM

NCl-1975 cells identified from spike-in

- Composite
- DAP
- CytoFasten
- CytoFasten DH
- CytoFasten DH/EpCAM

Table: samples targeted for SNP genotyping

<table>
<thead>
<tr>
<th>10ng of input DNA</th>
<th>Single protocol with spike-in (PlexiLUX Pro)</th>
<th>Automated analysis (Pro)</th>
<th>User-friendly mutation reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA to data in 8-hours with less than 30 minutes of manual processing</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Schematic workflow for somatic mutation detection using PlexiLux® chemistry and the MassARRAY® System

MDA-MB-231, Breast

- c.205G>T, Pro
- c.2369C>T, Mut
- c.353G>A, HET
- c.38G>A, HET
- c.691A>G, Mut
- c.818C>T, Mut

NCl-1975, Breast

- c.205G>T, Pro
- c.2369C>T, Mut
- c.353G>A, HET
- c.38G>A, HET
- c.691A>G, Mut
- c.818C>T, Mut

Examples of mutations found in single circulating tumor cells

- MDA-MB-231
  - Whole-genome Amplification: No Amplification
  - No Amplification

- NCl-1975
  - No Amplification

Comparison of Mutation TP53-616 from NCl-1975 found in single cell (Direct enrichment on pooling of cells) or whole (WGA).

*Conclusions: MassARRAY® technology successfully detected mutations in single model CTCs that were individually picked from a blood sample processed by the AccuCyte® - CytoFasten system both with and without whole genome amplification. Integrating CTC isolation with MassARRAY® technology may be a practical way to identify and monitor known cancer mutations non-invasively.

**Significance:** This is a simple and rapid way to identify multiple mutations in single cells, with the potential for diagnosis without introduction of sequence error by workflow whole genome amplification.