Multiple gene expression using the HyCEAD assay in CTCs isolated with the Parsortix™ system

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ABSTRACT

Methods: Parsortix™ (Parsortix, ThermoFisher) is a system to isolate CTCs from whole blood. When blood sampled into the Parsortix cartridge, red blood cells sediment down and CTCs are isolated from the supernatant. After harvest, the CTCs are lysed and RNA can be prepared for downstream analysis.

Results: Gene expression analysis was performed for three distinct assays using two classes of samples (Parsortix harvests and unspiked controls). Automated analysis was used to screen probes and probe sets. Reproducibility of expression measurements between sets was calculated from technical replicates along with linearity of signal over the dynamic range of the assay.

Conclusions: HyCEAD combined with hybridization on flow-through microarrays enables multiplex gene expression directly from a single cellular lysate without prior RNA extraction. Probe sets may be screened with representative sample sets to select probes that provide relatively strong signal intensities and that are likely to accurately target the intended targets (based on correlations between probe sets).

Analytical Sensitivity and Dynamic Range

**HyCEAD Sensitivity and Dynamic Range**

<table>
<thead>
<tr>
<th>Species</th>
<th>PCR cycles</th>
<th>ddPCR 31 cycles</th>
<th>ddPCR 27 cycles</th>
<th>ddPCR 27 cycles</th>
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</tr>
<tr>
<td>Human</td>
<td>2</td>
<td>75%</td>
<td>100%</td>
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</tr>
</tbody>
</table>

**CTCs**

- **Droplet digital PCR (ddPCR)**: The number of molecules is estimated by counting the droplets containing amplified target DNA.
- **HyCEAD**: The number of molecules is estimated by counting the droplets containing amplified target DNA.

**Background**: Differences between baseline and replicated probe data are identified as probes with extreme CVs. The HyCEAD™ system was designed to evaluate the performance of PCR-based assays for the detection of circulating tumor cells (CTCs). The sensitivity and dynamic range of the HyCEAD™ system were characterized using unspiked controls and CTCs isolated from human blood samples.

**Objectives**: To evaluate the sensitivity and dynamic range of the HyCEAD™ system for the detection of CTCs.

**Methods**: Three distinct assay formats were used: ddPCR, HyCEAD™, and Parsortix™. The ddPCR assays were performed on a Bio-Rad QX200 Droplet Digital PCR System. The HyCEAD™ assays were performed on a HyCEAD™ system.

**Results**: The sensitivity and dynamic range of the HyCEAD™ system were characterized using unspiked controls and CTCs isolated from human blood samples. The sensitivity of the HyCEAD™ system was determined to be 30 femtomoles per reaction, and the dynamic range was estimated to be 100% to 1000%.

**Conclusions**: The HyCEAD™ system is sensitive and flexible for the detection and quantification of CTCs in human blood samples.