Targeted Next Generation Sequencing-based Preimplantation Genetic Screening Enables Calling of Uniparental Isodisomy, Triploidy, and Familial Relationships

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Introduction

We have previously validated the FAST-SeqS technology for targeted next generation DNA sequencing-based detection of full chromosome and segmental aneuploidy in human embryos. While not leveraged in those validations, we have observed that the regions captured by FAST-SeqS include thousands of polymorphic sites. The representation of alleles at such sites can in theory be used to derive information beyond segmental and full chromosome aneuploidy, including enabling the calling of additional classes of chromosomal abnormalities that are correlated with adverse reproductive outcomes. Along those lines, we have developed new analytical strategies that utilize the quantitative single nucleotide polymorphism (SNP) information captured by FAST-SeqS to facilitate the calling of uniparental isodisomy (UPiD) and triploidy and to infer familial relationships amongst samples.

Here, we evaluate the analytical performance of our new SNP-based analysis strategies. First, to assess the performance with respect to uniparental isodisomy and triploidy, we ran a cohort of known diploid, triploid, and uniparental isodisomic cell line derived samples through FAST-SeqS and subsequently processed the data utilizing our refined analytical strategies. Next, to determine if FAST-SeqS can recapitulate expected familial relationships, samples from a 17-member multi-generation family were tested in replicate and per-sample SNP genotypes were called and subsequently used to cluster the samples. The resulting similarity matrix was then compared to the expected family tree.

Methods

I. FAST-SeqS: Targeted Amplification and Sequencing of Repetitive Elements

II. FAST-SeqS analyses leveraging captured polymorphic sites

III. FAST-SeqS SNP analysis validation study design

Results

I. Example FAST-SeqS data for correctly called diploid and triploid samples

Cell Line | Count-based Analysis | SNP-based Analysis
---|---|---
GM00321 & 46,XX | Diploid & Triploid
GM15603 & 46,XY | Diploid & Triploid
GM10013 & 69,XXX | Triploid & Triploid

II. Example FAST-SeqS data for correctly called UPiD chromosomes/samples

GM11496 & UPiD(7) | GM15603 & UPiD(8)
GM07489 & Complete Genome UPiD

III. FAST-SeqS with SNP analysis enables accurate calling of triploidy and UPiD

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Type</th>
<th># True Positives</th>
<th># False Negatives</th>
<th># True Negatives</th>
<th># False Positive</th>
<th>Specificity 95% CI</th>
<th>Sensitivity 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triploidy</td>
<td></td>
<td>48</td>
<td>0</td>
<td>183</td>
<td>0</td>
<td>100%</td>
<td>(97.9%, 100%)</td>
</tr>
<tr>
<td>UPiD</td>
<td></td>
<td>228</td>
<td>0</td>
<td>208</td>
<td>0</td>
<td>100%</td>
<td>(98.2%, 100%)</td>
</tr>
</tbody>
</table>

IV. FAST-SeqS SNP genotype profiles recapitulate established familial relationships

Conclusions

- FAST-SeqS with SNP analysis can accurately call triploidy and UPiD, abnormalities incompletely called by other NGS-based PGS technologies.
- FAST-SeqS SNP genotype profiles can be used to infer familial relationships and represent a promising tool for sample quality control.

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