Quantification of donor/recipient chimerism in bone marrow transplants of leukemia samples – Dr. Jiménez-Velasco – Carlos Haya Hospital

Digital PCR Applications Grant Program
Agenda

- Introduction to Digital PCR
- Objectives of Study & Experiment Details
- Results
Digital PCR Workflow

Digital PCR is an analytical technique for quantification of nucleic acid samples based on PCR amplification of single template molecules, without reference to a standard curve.

Use the number of **positive** and **negative** PCR reactions to count the number of target molecules.

[www.lifetechnologies.com/digitalpcr](http://www.lifetechnologies.com/digitalpcr)
QuantStudio™ 3D Digital PCR 20K Chip
QuantStudio™ 3D Digital PCR 20K Chip
Simple Workflow With Minimal Sample Handling

Mix → Load → Amplify → Read

Sealed System
Introduction

- Mixed chimerism is a state in which both recipient and donor cells are present in the bone marrow or peripheral blood after transplantation.
- Chimerism analysis is performed to monitor peripheral blood or bone marrow in the recipient after allogenic stem cell transplantation to avoid leukemic relapse.
- Increasing mixed chimerism after transplantation is associated with a high risk of relapse in acute leukemia.
Objective

- A qPCR technique developed in the laboratory of Dr. Jiménez-Velasco, the grant recipient, was found to predict relapse in 88.2% vs. 44.4% of individuals analyzed by VNTR markers with a median anticipation period of 58 days and a sensitivity of 0.01% vs. 3%.
  - Jiménez-Velasco et al., (Leukemia (2005) 19, 336–343)

- The goal of this project is to compare the QuantStudio™ 3D Digital PCR System to qPCR to determine if relapse can be predicted earlier and with greater accuracy.
Experiment Details

- To increase sensitivity of detection by qPCR Dr. Jiménez-Velasco selected assays targeting insertion/deletion polymorphisms.
- Assays were selected where donors possessed the null allele (deletion) and the recipients possessed the insertion polymorphism.
- By using large polymorphisms instead of single nucleotide polymorphisms (SNPs) greater sensitivity is achieved.
- Because donors do not possess alleles detected by the target assays a reference assay is used to quantify the amount of genomic DNA from both the donor and recipient within the post-transplantation samples.
- The normalized ratio of signal from the target vs. the reference assay is used to calculate the fraction of DNA from the recipient.
  - If the recipient is homozygous for the insertion then the target/reference ratio is equal to the relative amount of recipient to total DNA.
  - If the recipient is heterozygous for the insertion then the target/reference ratio is multiplied by 2.
DNA Samples

- Total samples: 53

- 8 Recipient/donor sets
  - Donor sample
  - Recipient sample before stem cell transplantation
  - Samples were also recovered from each recipient at various intervals after transplantation

- Genomic DNA was purified by the grant applicant from fresh whole peripheral blood (PB) or bone marrow (BM) using standard procedures.

- Some recipients were known to have relapsed after transplantation while others had not.
### Recipient-Assay Association

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Assay</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MID-1039</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>MID-2113</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>MID-R271</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>GSTT1</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>MID-2113</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>SRY</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>GSTM1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>MID-1732</td>
<td>10</td>
</tr>
<tr>
<td><strong>Reference Assay</strong></td>
<td><strong>Beta-Globin</strong></td>
<td><strong>11</strong></td>
</tr>
</tbody>
</table>

- Assays targeting recipient DNA were labeled with **FAM**.

- A β-Globin reference assay targeting DNA was labeled with **VIC** and run in duplex with each target assay on the same dPCR chip.

- The reference assay was used for quantification of the total number of copies of the genome on each chip regardless of whether the DNA originated from the donor or the recipient. The percent chimerism was then calculated by dividing the number of DNA molecules from the recipient by the total.
Procedure

- One chip was run per sample

- Samples were diluted approximately 2-fold by the addition of master mix and 20x assay.
  - 8 ul DNA (undiluted)
  - 1 ul 20x Beta Globin VIC assay
  - 1 ul 20x Target FAM assay
  - 10 ul 2x QuantStudio™ 3D Digital PCR Master Mix
  - 20 ul total

- A total of 40 cycles were performed for dPCR.
Recipient Pre-SCT Data

Reactions containing target DNA

Reactions containing both target and β-globin DNA

Reactions containing master mix with ROX but no target or reference β-globin DNA

Reactions containing β-globin reference DNA
Example dPCR Data: Relapse Post-Transplantation

- dPCR reactions containing recipient DNA appear in blue (reactions with target alone) or green (reactions with both target and reference DNA molecules).
- At day 192 the presence of recipient DNA is readily apparent.
- Recipient DNA was detected as early as day 62.
dPCR Can Predict Leukemia Relapse

- This is an example of leukemia relapse.
- An increase in % recipient chimerism was detected by dPCR and by qPCR.
- The % chimerism values from dPCR and qPCR are similar.
- With dPCR a slight increase is even visible between day 55 and day 61, 2 months earlier than qPCR (day 61 vs. day 127).

<table>
<thead>
<tr>
<th>Description</th>
<th>% Recipient Chimerism</th>
<th>ΔΔCT Run 1</th>
<th>ΔΔCT Run 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-SCT</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>0.05</td>
<td>0.0804</td>
<td>0.1114</td>
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<td>61</td>
<td>0.10</td>
<td>0.1298</td>
<td>0.0368</td>
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<td>91</td>
<td>4.08</td>
<td>0.8254</td>
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<tr>
<td>127</td>
<td>90.00</td>
<td>72.94</td>
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</tbody>
</table>

Days Post=SCT

% Recipient Chimerism

qPCR results
dPCR is More Sensitive than qPCR

- This is another example of leukemia relapse.
- dPCR detects recipient DNA in all post-SCT samples.
- Relapse was also detected by qPCR, however no recipient DNA was detected until day 192.
- An additional sample beyond 192 days was required to confirm relapse by qPCR.
dPCR Can Detect Absence of Relapse

- This is an example of no relapse.
- dPCR and qPCR results are in agreement showing a progressive decrease in % recipient chimerism over time.
- dPCR was in agreement with the qPCR results demonstrating that this technique does not produce false positive results.
Conclusions

- The QuantStudio™ 3D Digital PCR System produced similar percent recipient chimerism values when recipient DNA is present above the 1% level.

- The QuantStudio™ 3D Digital PCR System is more sensitive than qPCR and was able to detect the presence of recipient DNA in a relapsed recipient about 2 months earlier than qPCR where the percent recipient chimerism was 0.2% or less.

- The false positive rate was close to the complete chimerism value of 0.01% for peripheral blood samples.

- Increasing mixed chimerism was detected in recipients 1-3 and 5-7 and not in recipient 4 consistent with known outcomes.
The QuantStudio™ 3D Digital PCR System is For Research Use Only. Not for use in diagnostic procedures.

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