Droplet digital PCR core

Researcher meeting
21/05/2019
Droplet digital PCR (ddPCR)

- Uses microfluidics to form water-oil emulsion droplets
  - A sample is fractionated into 20,000 droplets
  - PCR amplification is carried out within each droplet

- Each droplet is read to determine the fraction of PCR-positive droplets in the original sample
  - Absolute count of target DNA copies per input sample
Workflow

Sample preparation

DNA + primers/probes + supermix

Droplet generation (~1 min/sample)

PCR

Droplet reading (1.5 min/sample)

Analysis
ddPCR vs. qPCR

**Pros**

- Higher performance at low copy level → better accuracy
  - Liquid biopsies
- No technical replicates needed
- Standard curves or calibrator samples not needed
- Reaction efficiency, PCR inhibitors and fluorescence noise have no impact for results
- Absolute quantification, no ΔΔCt analysis

**Cons**

- Price: roughly 1.5-2x more expensive than qPCR
  - 3.9 – 4.8 €/sample
- Takes more time
- No. of samples must be divisible by 8
  - 1 NTC for each 8 samples
- All reagents and consumables must to be bought from Bio-Rad
Applications

• Gene Expression and Single Cell Analysis
  • Measure absolute quantities of a gene transcript and its abundance in response to stimuli or disease.

• Rare Sequence or Mutation Detection
  • Need to amplify single genes or detect mutations (SNP) in a complex sample or high background.

• Copy Number Variation (CNV) / Copy Number Alteration (CNA)
  • Changes in the number of copies of a gene responsible for complex behavioral traits or disease.

• Next Generation Sequencing (NGS) Validation
  • Higher sensitivity, increased throughput, faster time to results and reduced costs.

• Metrology / Validation of Standards for qPCR
  • Exact measurements of reference materials and standards. Can be applied to qPCR applications.

• Genome Editing Technologies (CRISP/Cas9)
  • Detect and quantify low-frequency mutation events in a site-specific nuclease (ZFN, TALEN, CRISPR).

• Environmental DNA Detection
  • Detect and quantify presence of rare, endangered or invasive species.
Instruments

QX200™ Droplet Generator
PX1™ PCR Plate Sealer
QX200™ Droplet Reader

Booking in Agendo
Materials and Reagents

• Provided:
  • Supermix
    • For Probes (TaqMan)
    • EvaGreen, no SYBR Green
  • Oils
    • For droplet generation
    • For droplet reader
  • All the special plastics

• What you need:
  • Primers/probes, DNA
  • Tubes/tips etc. (available in PCR rooms)

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<thead>
<tr>
<th>Name</th>
<th>Image</th>
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<tbody>
<tr>
<td>QX200™ ddPCR™ EvaGreen Supermix</td>
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<tr>
<td>ddPCR™ Supermix for Probes (No UTP)</td>
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<tr>
<td>QX200™ Droplet Generation Oil for EvaGreen</td>
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<td>Droplet Generation Oil for Probes</td>
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<td>DG8™ Cartridges</td>
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<td>DG8™ Gaskets</td>
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<td>ddPCR™ 96-Well Plates</td>
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<td>Finntip Filter 100 µl tips (Thermo Fisher)</td>
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<td>PCR Plate Heat Seal, foil, pierceable</td>
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<tr>
<td>ddPCR™ Droplet Reader Oil</td>
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ddPCR core

**Personnel**

• Academic:
  • Annika Kohvakka, tel. 040 522 0696, email: annika.kohvakka@tuni.fi
  • (Heini Kallio, tel. 040 558 4354, email: heini.kallio@tuni.fi)

• Technical:
  • Ulla Kala, tel. 050 318 6300, email: ulla.kala@tuni.fi
  • Marjo Salminen, tel. 040 190 1507, email: marjo.salminen@tuni.fi

**Services**

• Reagents and consumables
• Hands-on training to use the instruments
• Reaction set-up and run by core staff