Eppendorf – In touch with life®
Streamlined processing of sample-to-sequencer workflows on a single instrument through automated liquid handling

Kanhav Khanna, Field Application Specialist
High Throughput Sequencing Symposium, May 2022
> Overview and Challenges of Microbiome Workflows
> Addressing the ‘pain points’
> Performance Evaluation
> Versatility of epMotion platforms
Agenda

> Overview and Challenges of Microbiome Workflows
> Addressing the ‘pain points’
> Performance Evaluation
> Versatility of epMotion platforms
Earth’s microbiome science

MICROBES have shaped our PLANET
…and even our built environments

Overview of Microbiome Research

Microbiome analysis and modifications

Modification/Intervention
“Use bugs as drugs”
Overview of Microbiome Research

Multi-omics approach
Overview of Microbiome Research

Multi-omics approach

Genomics

Who is there?
High-level community profiling

Marker-gene studies
(16S rDNA, ITS or 18S rDNA region)

How do they interact?
Functional Profiling

Shotgun metagenomics

How do they function?
Real-time functional profiling

Meta-transcriptomics

Proteomics

Understanding the biological system?
Protein expression

Meta-proteomics

Metabolomics

Understanding the biological system?
Metabolic productivity

Meta-metabolomics

NGS

Mass Spectrometry

Eppendorf SE | The 5th HTS Symposium | Streamlined processing of sample-to-sequencer workflows on a single instrument through automated liquid handling | Kanhav Khanna | May 19th, 2022
Overview of Microbiome Research

NGS workflow

16S rDNA sequencing
Bacterial diversity and abundance is surveyed by 16S rDNA amplicon sequencing

Meta-Transcriptomics
RNA sequencing to reveal community functional potential and activity

Signature profile of an environment or disease state – “Deep Dive”
High sequencing depth reveals total DNA/RNA content, relative abundance, metabolic pathways, gene content, novel genes, rare features

Principle of Diversity Assessment by NGS
Microbial fingerprinting

Sample Collection → Sample Prep → Amplification → Prepare Library → Sequence → Data Analysis

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Common sources of error in sample preparation

- Sample number is too low
- Sampling/preservation bias
- Contamination
- Extraction bias
- PCR-, Library preparation bias
- Carry-over and cross-contamination
Challenges of Microbiome Research

Complexity of sample processing

Sample Collection | Sample Prep | Amplification | Prepare Library | Sequence | Data Analysis

Library Preparation | Library QC | Sequencing

- Reaction setup
- Incubations
- PCR amplification
- Bead Cleanups

- Quantification
- qPCR
- Dilution & Normalization

- Library Pooling
Challenges of Microbiome Research

Complexity of sample processing

> Each process of the workflow contains multiple repetitive steps (e.g. Bead Cleanups)
> Potential failures amount to lost time and are costly
Challenges of Microbiome Research

Pipetting errors

Pipette
- Accuracy / Precision
- Pipetting System
- Calibration and maintenance

Operator
- Pipetting technique
- Pipetting angle
- Immersion depth

Pipette tip
- Reproducible fit
- Tip quality (material)

Environment
- Temperature
- Humidity

Liquid Type
- Viscosity
- Vapor Pressure

Major Sources of Pipetting Error in the Lab

Source: TTE Laboratories
## Pipetting errors

<table>
<thead>
<tr>
<th>Kit</th>
<th>Steps in manual</th>
<th>Total time for 8–24 samples</th>
<th>Cost per kit (96 samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S Metagenomics Library Prep</td>
<td>&gt; 59</td>
<td>&gt; 3 hrs</td>
<td>&gt; 3,000 USD</td>
</tr>
<tr>
<td>Nextera XT/Flex</td>
<td>&gt; 65</td>
<td>&gt; 4 hrs</td>
<td>&gt; 4000 USD</td>
</tr>
<tr>
<td>Stranded Total RNA</td>
<td>&gt; 150</td>
<td>&gt; 12 hrs</td>
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Overview and Challenges of Microbiome Workflows

Addressing the ‘pain points’

Performance Evaluation

Versatility of epMotion platforms
Addressing the ‘pain points’

Workflow standardization

Use of controls

> Negative Template Control (NTC)
  > Reagent and laboratory contaminants

> Positive Template Control (PTC)
  > Assay validity

> Internal Spike-In Control (IC)
  > Assay validity (level of detection and representation bias)
  > Quality check of sample preparation
  > Normalization of the data
Addressing the ‘pain points’

NGS library preparation

Evaluating targeted sequencing vs shotgun sequencing for diversity identification

> It is critical to be able to identify to the strain and sub-strain level
> 16S sequencing can be limited in some cases
> Shotgun sequencing allows to identify all the microbes
Addressing the ‘pain points’

Automated liquid handling

epMotion 5073

epMotion 5075
Addressing the ‘pain points’

Benefits of automation

> Peace of mind
  > Automate reaction setup and reaction cleanup
  > Dilution, Normalization, Pooling of samples
Addressing the ‘pain points’

Benefits of automation

- Peace of mind
- Handle difficult liquids
Addressing the ‘pain points’

Benefits of automation

> Peace of mind
> Handle difficult liquids
> Small liquid volumes
  > Accurately pipette sub-microliter volume
    > 1 µL CV < 0.6%
    > 200 nL (0.2 µL) CV < 1.8%
Addressing the ‘pain points’

**epMotion** reduced complexity

---

**Manual Illumina DNA Prep**
- Genomic DNA Extraction
  - Tagmentation
  - Cleanup Tagmented DNA
    - Indexing
    - Amplification
      - PCR Cleanup
      - Pooling
  - epMotion Method

**Automated Illumina DNA Prep**
- epMotion Method
  - Amplification
  - epMotion Method
Addressing the ‘pain points’

epMotion saves time

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*epMotion* saves time

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<th>Hands-On Time 96 samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S Metagenomics Library Prep</td>
<td>&gt; 59</td>
<td>&gt; 3 hrs</td>
<td>&lt; 0.5 hrs</td>
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<tr>
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> Addressing the ‘pain points’
> Performance Evaluation
> Versatility of epMotion platforms
Performance Evaluation

DNA extraction from soil

- Qiagen (MoBio) PowerSoil-htp and PowerMag Soil kits
  - Different soil types were used; DNA evaluated using Agarose gel electrophoresis and PCR

<table>
<thead>
<tr>
<th>Soil Site</th>
<th>Soil Type</th>
<th>Average DNA Yield (ng/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBS LTER T1R1</td>
<td>Standard row crop agriculture historically tilled</td>
<td>6.95 ± 0.2</td>
</tr>
<tr>
<td>KBS LTER T8R1</td>
<td>Managed grassland never tilled</td>
<td>20.3 ± 5.4</td>
</tr>
<tr>
<td>KBS LTER DFR1</td>
<td>Deciduous forest</td>
<td>18.0 ± 4.6</td>
</tr>
<tr>
<td>GLBRC Extensive Site PR06</td>
<td>Prairie</td>
<td>11.6 ± 1.1</td>
</tr>
<tr>
<td>GLBRC Extensive Site SW10</td>
<td>Switchgrass</td>
<td>17.6 ± 1.9</td>
</tr>
<tr>
<td>GLBRC Extensive Site C26</td>
<td>Corn</td>
<td>9.10 ± 1.6</td>
</tr>
</tbody>
</table>

Source: Eppendorf Application Notes 233 & 278
DNA extraction from microbial cultures

- Qiagen (MoBio) PowerFood and PowerMag Microbial kits
- Pure culture of E. faecalis or Listeria monocytogenes grown in ground beef or chocolate

Table: DNA yields from *E. faecalis* culture using the PowerMag Microbial kit

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (ng/μL)</th>
<th>$A_{260/280}$</th>
<th>Yield (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>512.90</td>
<td>1.86</td>
<td>51.3</td>
</tr>
<tr>
<td>2</td>
<td>517.25</td>
<td>1.86</td>
<td>51.7</td>
</tr>
<tr>
<td>3</td>
<td>502.84</td>
<td>1.86</td>
<td>50.3</td>
</tr>
<tr>
<td>4</td>
<td>513.44</td>
<td>1.85</td>
<td>51.3</td>
</tr>
<tr>
<td>5</td>
<td>506.53</td>
<td>1.86</td>
<td>50.7</td>
</tr>
<tr>
<td>6</td>
<td>529.14</td>
<td>1.86</td>
<td>52.9</td>
</tr>
<tr>
<td>7</td>
<td>543.52</td>
<td>1.84</td>
<td>54.4</td>
</tr>
<tr>
<td>8</td>
<td>528.71</td>
<td>1.83</td>
<td>52.9</td>
</tr>
</tbody>
</table>

Table: Average yields of DNA isolated from *Listeria monocytogenes*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Isolation Method</th>
<th>Average Conc. (ng/μL)</th>
<th>Average $A_{260/280}$</th>
<th>Average Yield (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>Silica spin filter</td>
<td>81.00</td>
<td>1.83</td>
<td>8.1</td>
</tr>
<tr>
<td>Beef</td>
<td>SwiftMag</td>
<td>126.86</td>
<td>2.02</td>
<td>12.9</td>
</tr>
<tr>
<td>Chocolate</td>
<td>Silica spin filter</td>
<td>70.18</td>
<td>1.86</td>
<td>7.0</td>
</tr>
<tr>
<td>Chocolate</td>
<td>SwiftMag</td>
<td>177.74</td>
<td>1.85</td>
<td>17.8</td>
</tr>
</tbody>
</table>

Source: Eppendorf Application Notes 277
Performance Evaluation

WGS library preparation

> Illumina’s DNA Prep kit (formally Nextera FLEX)
> 8 libraries prepared from 200 – 300 ng DNA from Coriell Institute female reference cell line NA12878

<table>
<thead>
<tr>
<th></th>
<th>Manual Preparation</th>
<th>Automated epMotion® 5075t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (Qubit), ng/μL</td>
<td>13.2</td>
<td>13.6</td>
</tr>
<tr>
<td>Yield CV (%)</td>
<td>5.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Index CV (%)</td>
<td>10.8</td>
<td>11</td>
</tr>
<tr>
<td>Median insert size range (bp)</td>
<td>352</td>
<td>339</td>
</tr>
<tr>
<td>Mean diversity</td>
<td>&gt; 2.3e⁹</td>
<td>&gt;3.0e⁹</td>
</tr>
<tr>
<td>Autosomal mean coverage (Fold)</td>
<td>30 - 32x</td>
<td>30 - 32x</td>
</tr>
<tr>
<td>Autosomal Callability (%)</td>
<td>95.0</td>
<td>95.5</td>
</tr>
</tbody>
</table>

Source: Eppendorf White Paper 13
16S metagenomics library preparation

- Illumina’s 16S Metagenomics Sequencing Library Preparation
- E.coli MG1655 with 12.5 ng genomics DNA

Source: Eppendorf Application Note 420
Performance Evaluation

16S metagenomics library preparation

> Illumina’s 16S Metagenomics Sequencing Library Preparation
  > Mock community of 20 Strain Staggered Genomics Material

Number of reads passing filter vs the % reads classified to genus

PCoA of the normalized relative abundance of all samples

Source: Eppendorf Application Note 420
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> Performance Evaluation
> Versatility of epMotion platforms
Versatility of epMotion platforms

epMotion family

epMotion 96  epMotion 5070  epMotion 5073  epMotion 5075

COMPLEXITY OF APPLICATION

THROUGHPUT

SIZE
Versatility of epMotion platforms

epMotion features

- High pipetting accuracy and precision (200 nL - 1000 uL)
- On-deck thermomixing of beads (up to 2,000 rpm)
- Walk away setup with on-deck heating and cooling for enzymatic reactions
Versatility of epMotion platforms

epMotion features

Flexible sample throughput with smart and automatic tool exchange

Flexible and Intuitive programming for easy adjustment of method steps
Versatility of epMotion platforms

epMotion features
Versatility of epMotion platforms

dMotion flexibility
Versatility of epMotion platforms

epMotion collaborations

- Revolution cfDNA
- MagAttract PowerMicrobiome
- MagAttract PowerSoil
- Maxwell HT Viral TNA kit
- NucleoMag kits
  - Blood
  - Pathogen
  - Plant
  - Tissue
  - Virus
- NucleoSpin Kits
  - Blood
  - PCR Cleanup
  - Plasmid Tissue
- PureLink 96 RNA Purification kit
- Wizard SV
  - gDNA Purification
  - Total RNA Isolation
  - and more...

Officially Qualified

- Olink Explore
- 16S rRNA
- AmpliSeq for Illumina
- Nextera XT DNA
- Illumina DNA Prep
- Illumina DNA Prep for Enrichment
- Illumina DNA PCR Free
- Illumina Stranded Total RNA
- Illumina Stranded mRNA
- Illumina RNA Prep with Enrichment
- TruSeq DNA PCR–Free
- TruSeq DNA Nano
- TruSeq RNA Fusion
- TruSeq RNA Exome
- TruSeq Stranded mRNA
- TruSeq Stranded Total RNA
- KAPA HyperPlus Kit
- NEBNext Ultra II Directional RNA
- and more...
Versatility of epMotion platforms

epMotion support
Helpful resources

> eppendorf.com/Own-Your-Solution

> eppendorf.com/NGS-Made-Easy

> Contact: bioapps@eppendorf.com
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