Next Generation Sequencing: Pushing the Boundaries of Clinical Microbiology Research

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EMEA Channel Partners
Our Mission

To improve human health by unlocking the power of the genome
The Illumina Sequencing Portfolio

*iSeq™ 100*: NGS capabilities tailored to your lab

**Personal Scale**
- iSeq™ 100

**High Throughput**
- Large WGS | WES | T-OME | Many Samples
- NextSeq™ 500
- HiSeq™ Series
- MiSeq™

**Low Throughput**
- Targeted Sequencing | WGS (Microbes)
- MiniSeq™

**Production Scale**
- NovaSeq™
Traditional Genomic Technologies

The Challenge
How do I cost-effectively expand my research?

qPCR
First instrument released in 1996

- Low complexity assays
- Lack of discovery power
- Single assay reactions
- Cumbersome workflow

Sanger/CE Sequencing
First instrument released in 1987

- Low complexity assays
- Low sensitivity and detection power
- Lack of discovery power
- Missed calls
Cost of Traditional Genomics Technologies

Gene Expression on qPCR & Targeted Resequencing
Sanger/CE

Sanger/CE and qPCR Price per sample

Costs increase linearly with # of targets

$100/sample at 20 amplicons/targets per sample

Time, cost per sample, and sample input quantity all increase linearly with number of targets:

limiting discovery power

Assuming an average cost of $5/reaction/sample based on customer conversations (qPCR and Sanger)
Cost of Traditional Genomics Technologies

Gene Expression or Sequencing Project across 24 samples

Number Of 96 Well Plates Required

<table>
<thead>
<tr>
<th>qPCR</th>
<th>Targets</th>
<th>Sanger/CE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>14</td>
</tr>
<tr>
<td>18</td>
<td>48</td>
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<tr>
<td>36</td>
<td>200</td>
<td>113</td>
</tr>
<tr>
<td>150</td>
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</tbody>
</table>

Time, cost per sample, and sample input quantity all increase linearly with number of targets: **limiting discovery power**

Assumes single-plex reactions run in triplicates for qPCR and duplicates, f.wd. and r.ev, for Sanger/CE
Increase Your Discovery Power
*Rapid, high throughput workflow*

Assumes single-plex qPCR reactions
How Much Data Can You Get for $150?

*iSeq™ 100 Targeted Resequencing*

Assumptions:
Sanger sequencing calculations took into account 96 reactions of 1kb target amplicons. In this hypothetical case, 3 samples were batched on the Sanger sequencing run. $5 per reaction for Sanger/CE was assumed. Standard price and throughput calculations were used for *iSeq™ 100*.

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iSeq™ 100 Benefits

- **Lowest cost option** to acquire and use **proven SBS NGS**
- **Simplest NGS interface** and **workflow** from Illumina
- Single panel, **multi-target testing capability**
- **Plug & play** for **immediate use**
- Ideal for **small batch, quick turn-around** time testing

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Sample to Answer Workflow Solutions

AMPLICON SEQUENCING
TSS mRNA SEQUENCING
MICROBIAL WHOLE GENOME
TRANSCRIPTOMICS
SHOTGUN METAGENOMICS

Sample Prep
Sequence
Answer

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Challenges with DNA library Prep Tools
Whole-Genome Library Prep

<table>
<thead>
<tr>
<th>Challenges</th>
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</thead>
<tbody>
<tr>
<td><strong>Enzymatic Fragmentation</strong></td>
</tr>
<tr>
<td>Limited Performance</td>
</tr>
<tr>
<td><strong>Mechanical Shearing</strong></td>
</tr>
<tr>
<td>Slow</td>
</tr>
<tr>
<td><strong>No Direct Input</strong></td>
</tr>
<tr>
<td><strong>No Library Quant and Normalization</strong></td>
</tr>
</tbody>
</table>

Result: High performing, fast genomic library directly from genomic DNA, Blood, Saliva, or Microbial colonies

Solution
Nextera™ DNA Flex Library Prep Kit
Introducing Nextera™ DNA Flex Library Prep

One DNA prep, multiple solutions

Versatile
Broad DNA input range
Integrated protocol for blood, saliva, dried blood spot* & microbe colony* input

Robust and Reproducible
High uniformity of coverage
Consist insert size

Flexible Workflow
Integrated library normalization process
Minimal hands-on time

*Demonstrated protocol

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Nextera™ DNA Flex Library Prep
Workflow overview

A. Isolate and purify DNA
gDNA, blood, saliva, or microbe

B. Add DNA to bead-linked transposomes (BLT)
Transposome attached to magnetic beads

C. DNA is tagmented and remains bound to the bead

No additional tagmentation can occur after bead saturation
Allowing a large DNA input range (1–500ng)
Resulting in consistent insert size and normalized libraries

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Nextera™ DNA Flex Library Prep

Workflow overview

**PCR Amplification**

Index and sequencing adapter addition through PCR

**Sequence-Ready Library**

Normalized sequence-ready library

Library quantification, QC, and normalization not required

(100ng–500ng)

Allowing a large range of DNA input

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Save Time and Increase Efficiency

*With fewer steps*

**Standard mechanical**
- DNA extraction
- DNA quantification
- DNA shearing
- Adapter ligation and index tagging
- Normalization

**Standard enzyme-based**
- DNA extraction
- DNA quantification
- Tagmentation and index tagging
- Normalization

**Nextera™ DNA Flex**
- DNA extraction, tagmentation and index tagging, and normalization

**Nextera™ DNA Flex provides the entire* workflow solution**
- Blood/saliva to normalized library (~3.5 hours)
- Genomic DNA to normalized library (~3 hours)

*Prepared sample to normalized library*
Nextera DNA Flex allows sample preparation from microbial colonies

DNA Isolation Direct Colony Input (demonstrated protocol)

Qiagen
Powerbead tube, glass

SPB reagent, as needed for DNA isolation, is provided in sufficient quantity within the Nextera DNA Flex Kit

Sample Purification Beads (SPB)
Polyethylene Glycol (PEG)

Purified DNA
Ready to add to Tagmentation master mix

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Next Generation Sequencing (NGS) & Clinical Microbiology

High resolution genomic information enables a range of applications

Discovery
Path of transmission
Whole picture
Host Pathogen Interaction
Epidemiology and Whole-Genome Sequencing (WGS)
Real time application of whole genome sequencing for outbreak investigation – What is an achievable turnaround time?

Patrick McGann a,*, Jessica L. Bunin b, Erik Snesrud a, Seema Singh c, Rosslyn Maybank a, Ana C. Ong a, Yoon I. Kwak a, Scott Seronello c, Robert J. Clifford a, Mary Hinkle a, Stephen Yamada d, Jason Barnhill c, Emil Lesho a

The Case and challenge

- Outbreak of vancomycin resistant Enterococcus faecium (VRE) among 3 patients in the ICU, Hospital Acquired Infection (HAI)
- All three patients died within days of +ve blood culture
- Real time 2-day turnaround WGS “simulation” of an outbreak
Epidemiology and Whole-Genome Sequencing

Analysis for an Outbreak of VRE in a Hospital

Workflow

- **Sample prep**
  - Overnight culture, DNA extraction & purification

- **Prepare Library**
  - Nextera® XT Kit

- **Sequence**
  - MiSeq® System
    - 2x 75, 2x 150
  - 2 x 75 – 3hrs shorter run
  - 2 x 150 – 16x faster assembly

- **Analyze data & annotate variants**
  - Newbler v2.7 de novo assembly, reference mapping, and SNP detection SWs

<table>
<thead>
<tr>
<th>Sequeing run metrics</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>150 bp kit</td>
</tr>
<tr>
<td>Culture</td>
<td>12</td>
</tr>
<tr>
<td>DNA isolation</td>
<td>0.5</td>
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<tr>
<td>Library preparation</td>
<td>7</td>
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<tr>
<td>Run time</td>
<td>21</td>
</tr>
<tr>
<td>Assembly and QC</td>
<td>8</td>
</tr>
<tr>
<td>Analysis and Report generation</td>
<td>5.5</td>
</tr>
<tr>
<td>Total time</td>
<td>54</td>
</tr>
</tbody>
</table>

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Results

Single nucleotide difference identified between each patient, indicative of nosocomial infection

Rather than transmission from patient #1 → #2 → #3, two separate events from 1→2 and 1→3

Range of antibiotic resistance genes identified and characterized

Illumina WGS is a powerful way to identify and understand transmission of HAIs within a clinically-relevant timeframe
  • Feasible for small-scale labs

Diagnosis with Metagenomic Deep Sequencing
Patient history......

- 74 yo female presents to the hospital with altered mental state
  - Rewind= 4 weeks prior, admitted 3 times to hospitals for intermittent fever and altered mental status
    - Diagnosed- endophthalmitis
    - Treated- antibiotics, no growth in cultures, neg PCR for viral infections
      - Treatment for Toxoplasma gondii
    - MRI- areas of restricted diffusion, stroke work up
    - Cough, TB clinical scheduled
Progression....

- TB Clinical (2 days later)
- Altered mental state, transfer to ER
- Fever, moved arms/legs spontaneously, non-verbal and unable to follow commands
- Lapsed into a coma
- 3 weeks in ICU
- Antibiotics, antifungals...
kitchen sink
- MRI
- Brain biopsy
- Spinal tap, CSF

Wilson et al., ANN NEUROLOGY 2015; DOI: 10.1002/ana.24499
Workflow and Results

Sample prep
CSF, RNA extracted, converted to cDNA

Prepare Library
Nextera® XT Kit

Sequence
HiSeq 2500® System

Analyze data & annotate variants
SURPI publicly available on GitHub
<15 minutes
Total time ~2 days

- Total reads collected for this sample were 19,642,962
- After filtering, mapping to human, remained 33,093 (0.1%)

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Outlook and Conclusion

- Extensive traditional testing did NOT reveal the cause of her illness; metagenomic deep sequencing pointed to the culprit.
- Total patient care > $1,000,000
  - Sequencing costs ~$1,000
- Today; no known treatment BUT the team at UCSF is going through the FDA drug treatments; “promising” for the future
Microbial Diversity
A 46-year-old man presented with a mastoid abscess and lower-lobe pneumonia. Abscess debridement sample was collected.

**Results:**

- Gram-stain revealed rare branching gram + rods, indicative of *Norcardia* or *Actinomyces* bacteria.
- 16S Sanger sequencing for *Fusobacterium nucleatum* revealed gram - rods.

NGS performed on the MiSeq to give clarity to discrepancies in results. MISEQ, 2x250, 16S amplicon v1-2.
16S rRNA Sequencing

Analysis for an Infection with conflicting cause

Workflow

Sample prep
Extraction of RNA or DNA

Prepare Library
16S protocol
Nextera® XT Index Kit

Sequence
MiSeq® System

Analyze data
Cloud-BaseSpace®,
Local MiSeq Reporter
16S Metagenomics
Next Generation Sequencing can “characterize the composition of even very complex bacteria communities” in a culture-independent method.

The MiSeq was able to pull together the story where traditional methods were conflicting; “nondiagnostic or discrepant”.

Illumina WGS is a powerful way to identify and understand truth within a mixed sample in clinically-relevant timeframe.

Path of Transmission
These isolates included subtypes:

- H1N1 (n = 3)
- H1N2 (n = 32),
- H3N2 (n = 78), and multiple subtype codetections (n = 9)

Multiple gene segment exchange events among and within subtypes leading to new viruses.

Choi et al, Clin Infect Dis 2015; DOI: 10.1093/cid/civ618
Workflow and Results

Sample prep
IAV genome 8-segments RT-PCR

Prepare Library
TruSeq HT library prep

Sequence
MiSeq® System

Analyze data & annotate variants
Bowtie 2.0 (assembly), SAMtools (consensus), FLAN (FLuANotation by NCBI), MEGA5 (phylogenetic trees)

- These isolates included subtypes: H1N1 (n = 3), H1N2 (n = 32), H3N2 (n = 78), and multiple subtype codetections (n = 9)

- Multiple gene segment exchange events among and within subtypes leading to new viruses

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Live animal markets in Minnesota: A potential source for emergence of novel Influenza A viruses and interspecies transmission

Zoonotic transmission- 12yo boy showed symptoms 3 days after visiting the market.

- 7 segments 99-100% similarity to H3N2
- 8th segment 100% similarity to H1N2 isolated in the live animal market

Conclusion:

- MiSeq aided in identifying Multiple strains IAV and subtypes cocirculating, as well as new viral resortments and evidence of interspecies transmission.
- Epidemic concerns, if H3N2 transmitted more efficiently from human to human

Choi et al, Clin Infect Dis 2015; DOI: 10.1093/cid/civ618
“WGS exceeds any other typing method currently available….. particularly when trying to **elucidate** the mechanism of transmission”

“As an alternative to conventional assays, MDS represents an **unbiased and rapid diagnostic tool**…”

“In addition to implicating or ruling out specific agents of disease in patient specimens in a **culture-independent** fashion, it will become tractable to assess the overall **composition of a polymicrobial community**…..”
Thank You

Questions?