Illumina MiSeq system
Medium throughput system
MiSeq Workflow

Analysis tools

DNA purification

DNA barcoding

Library
Tutorial on MiSeq workflow

MiSeq Sequencing Chemistry: ca. 20 min
http://support.illumina.com/training/courses/MiSeq_Sequencing_Chemistry/index.html?iframe
DNA purification

Example: EasyDNA from Invitrogen
The following figure describes the experimental process.

1. Add Solution A and Vortex
2. 65°C for 10 minutes
3. Add Solution B and Vortex
4. Add Chloroform and Vortex (Centrifuge)
5. Transfer aqueous phase to new tube
6. Add ethanol and precipitate DNA
Qubit DNA quantification

http://www.youtube.com/watch?v=6HtnVUHMX_8
Normal Illumina workflow
Video on Sample preparation

http://www.youtube.com/watch?v=fs1A_Ik7Smo
Simplified protocol
Nextera XT sample prep video

Manual:
Nextera XT tutorial

Nextera DNA Sample Preparation Kit

This course takes 20 minutes to complete and contains audio.

http://support.illumina.com/training/courses/Nextera_Sample_Preparation_Kits/index.html?iframe
Nextera XT library workflow

Nextera "Tagmentation" Technology

3. The Nextera transposome complex consists of a Transposase enzyme and transposon ends (DNA) containing adapter sequences.
Adapters added by PCR
Multiplexing DNA samples

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Multiplexing
18 E. coli
24 S. aureus
Multiplexing with Nextera XT

Dual Indexing Principle

The dual indexing strategy uses two 8 base indices, Index 1 (i7) adjacent to the P7 sequence, and Index 2 (i5) adjacent to the P5 sequence. Dual indexing is enabled by adding a unique Index 1 (i7) and Index 2 (i5) to each sample from 12 different Index 1 (i7) adapters (N701–N712) and 8 different Index 2 (i5) adapters (S501–S508) for the 96 sample Nextera XT Index Kit (FC-131-1002), and 6 different Index 1 (i7) adapters (N701–N706) and 4 different Index 2 (i5) adapters (N501–N504) for the 24 sample Nextera XT Index Kit (FC-131-1001). In the Index adapter name, the N refers to Nextera XT sample preparation, 7 or 5 refers to Index 1 (i7) or Index 2 (i5), respectively, and 01–12 refers to the Index number. A list of index sequences is provided for generating sample sheets to demultiplex the samples:

<table>
<thead>
<tr>
<th>Index 1 (i7)</th>
<th>Sequence</th>
<th>Index 2 (i5)</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>N701</td>
<td>TAAGGCGA</td>
<td>S501</td>
<td>TAGATCGC</td>
</tr>
<tr>
<td>N702</td>
<td>CGTACTAG</td>
<td>S502</td>
<td>CTCTCTAT</td>
</tr>
<tr>
<td>N703</td>
<td>AGGCAGAA</td>
<td>S503</td>
<td>TATCCTCT</td>
</tr>
<tr>
<td>N704</td>
<td>TCCTGAGC</td>
<td>S504</td>
<td>AGAGTAGA</td>
</tr>
<tr>
<td>N705</td>
<td>GGACTCCT</td>
<td>S505</td>
<td>GTAAGGAG</td>
</tr>
<tr>
<td>N706</td>
<td>TAGGCATG</td>
<td>S506</td>
<td>ACTGCATA</td>
</tr>
<tr>
<td>N707</td>
<td>CTCTCTAC</td>
<td>S507</td>
<td>AAGGAGTA</td>
</tr>
<tr>
<td>N708</td>
<td>CAGAGAGG</td>
<td>S508</td>
<td>CTAAGCCT</td>
</tr>
<tr>
<td>N709</td>
<td>GCTACGCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N710</td>
<td>CGAGGCTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N711</td>
<td>AAGAGGCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N712</td>
<td>GTAGAGGA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Library building
Library preparation movies

Nextera Best Practices Videos

- Dual Indexing
- Adhering Microseals
- Handling Magnetic Beads
- Library Normalization

Library Normalization
This process normalizes the quality of each library to ensure more equal library representation in your pooled sample.

https://support.illumina.com/sequencing/sequencing_kits/nextera_xt_dna_kit/training.ilmn
(Login might be required)
How many bacteria in a library?

- 16-18 genomes around 5-6 Mb
  - E. coli
  - Klebsiella
  - Salmonella

- 24 genomes around 3 Mb
  - Enterococcus
  - Staphylococcus
  - Campylobacter
The MiSeq principle

http://www.youtube.com/watch?v=l99aKKHcxC4
To the MiSeq

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