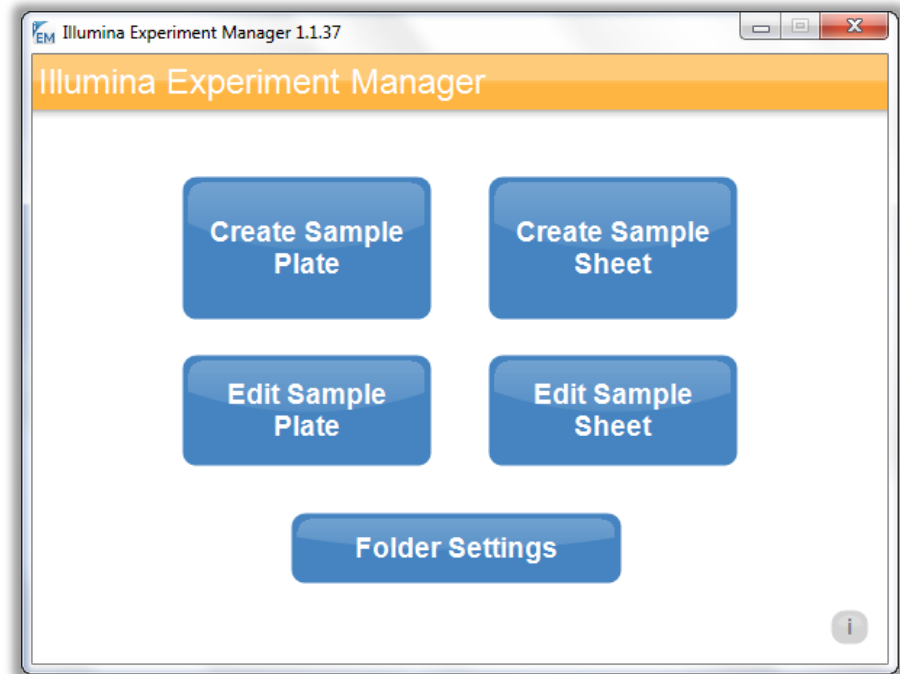# Illumina Experiment Manager

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Course Objectives

By the end of this course, you will be able to:

▶ Identify the purpose of Illumina Experiment Manager (IEM)

▶ Describe the IEM workflow

▶ Create and edit a sample plate

▶ Create and edit a sample sheet

IEM Overview

▶ IEM is an application to create and edit sample plates and sample sheets

▶ Sample plates store information regarding:
- Assay type to be performed
- Plate name
- Sample indices

▶ The sample sheet is a .csv file that stores information needed to set up, perform, and analyze a sequencing run

Use IEM to create a sample sheet **before starting sample preparation**.

Getting started

Download Software
- Log-in to MyIllumina
- From the Sequencing Support Page, select **Downloads** and locate **Illumina Experiment Manager**

Install Software
- To begin installation, open the Setup.exe file
- After the installation wizard opens, selected **Next** to accept all default settings
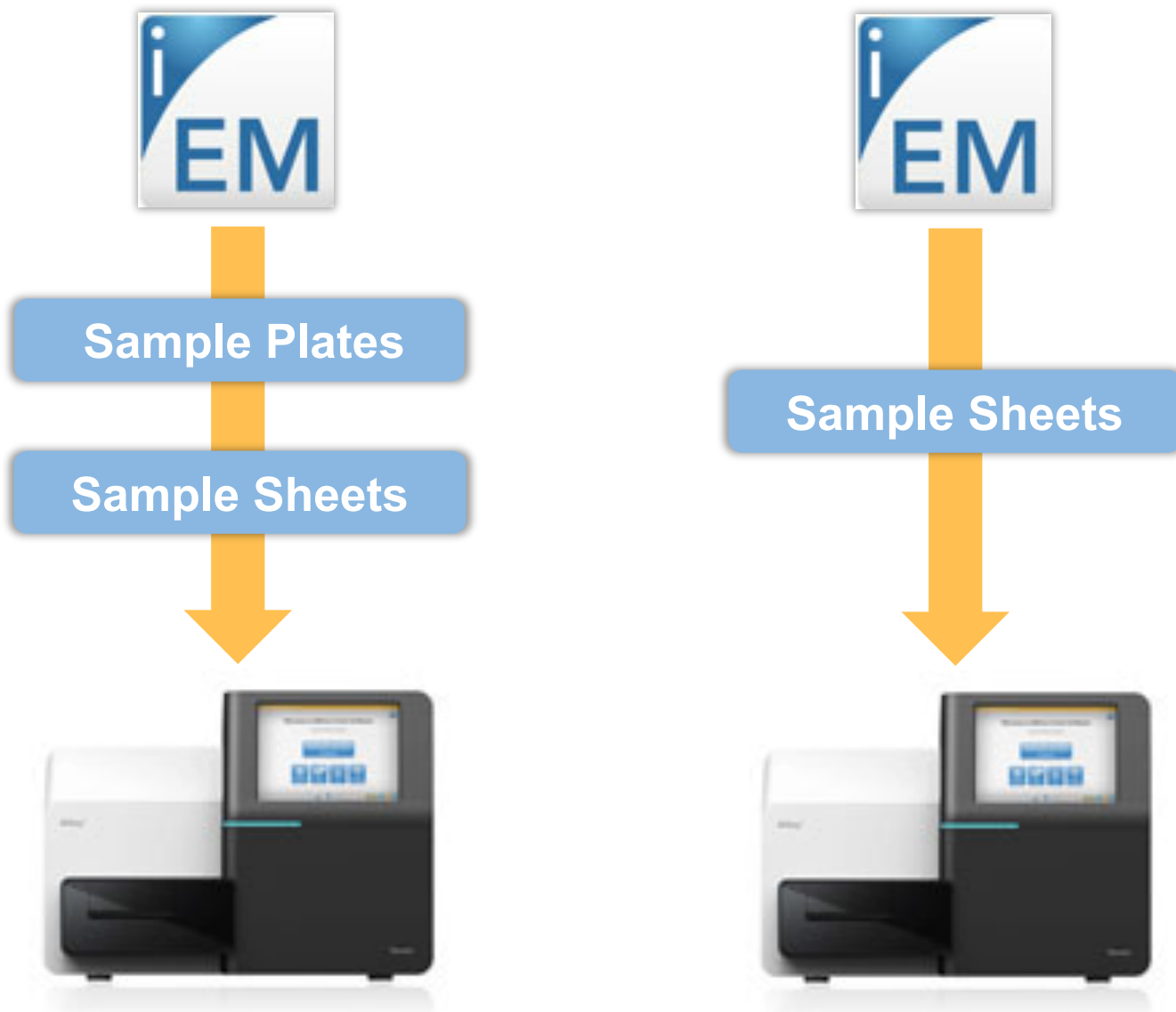
Start Software
- To start the software:
- Click on the Illumina Experiment Manager icon on your desktop

To ensure the files are accessible from the sequencing instrument in your lab, save them in a shared network folder or on a USB drive

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Two Workflow options

Sample Plates

Sample Sheets

Sample Sheets

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Data Analysis Overview

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Illumina Data Analysis Workflow

1 **Primary Analysis**

2 **Secondary Analysis**
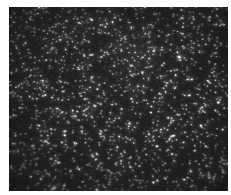
MiSeq Reporter   CASAVA

3 **Data Visualization**

BaseSpace™

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Primary and Secondary Analysis Overview

Analysis Type	Software	Outputs
Sequencing	**ICS/RTA**	**Images/TIFF files**
Primary Analysis	**ICS/RTA**	**Intensities** **Base Calling**
Secondary Analysis	BaseSpace™ MiSeq Reporter CASAVA	Alignments, Variant Detection and Counting

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Data Analysis File Formats and Terminology

| File Formats / Terminology	Definition
FASTQ	A text-based format for storing both a nucleotide sequence and its corresponding quality scores • Useful if you prefer to use third-party analysis tools to analyze the data
SAM	Sequence Alignment/Map
BAM	Binary Compressed SAM
VCF	Variant Call Format • A standard that lists and annotates almost the entire collection of human variants

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Sample Plate Considerations

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Sample Plate Assay Selection

Assay Type	File Extension Example	Index Reads/Cycles
TruSeq Custom Amplicon	.amp28.plt	Only 2/8 possible
Nextera	.nex0.plt	0, 1/8 and 2/8 possible
TruSeq DNA/RNA	.tru16.plt	0, and 1/6 possible
Small RNA	.smr16.plt	0, and 1/6 possible

Click "Apply Default Index Layout" to auto-populate the indices for all index reads, you can edit them later on
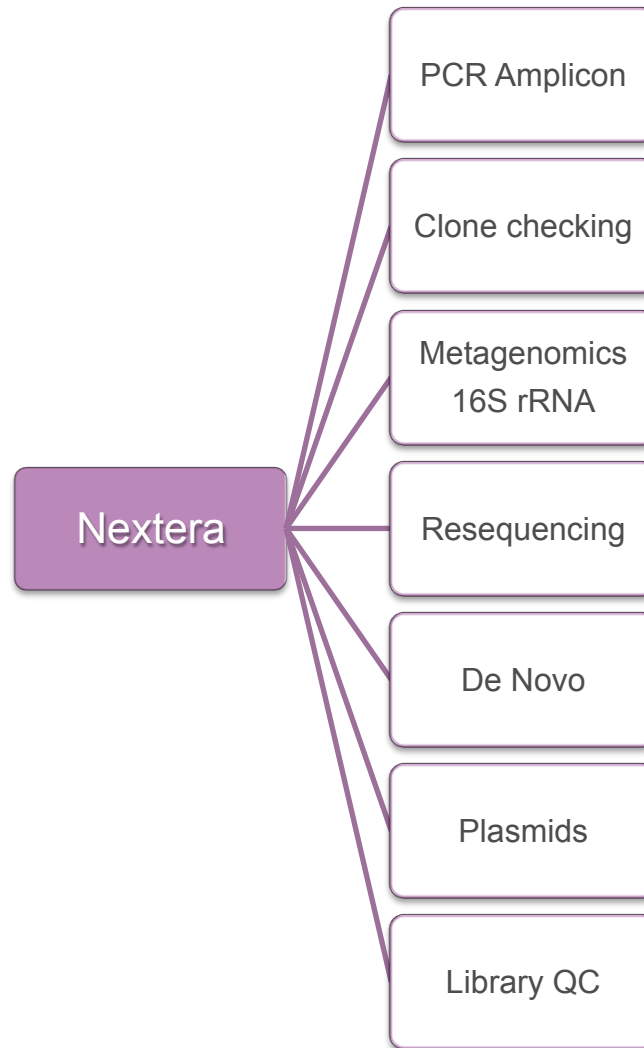
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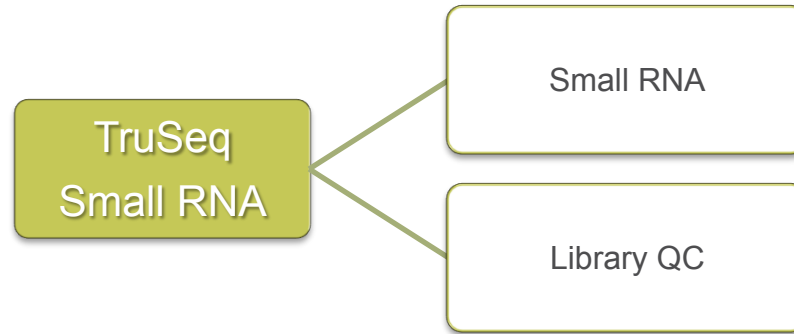
Sample Plate Assay Selection:
TruSeq DNA/RNA
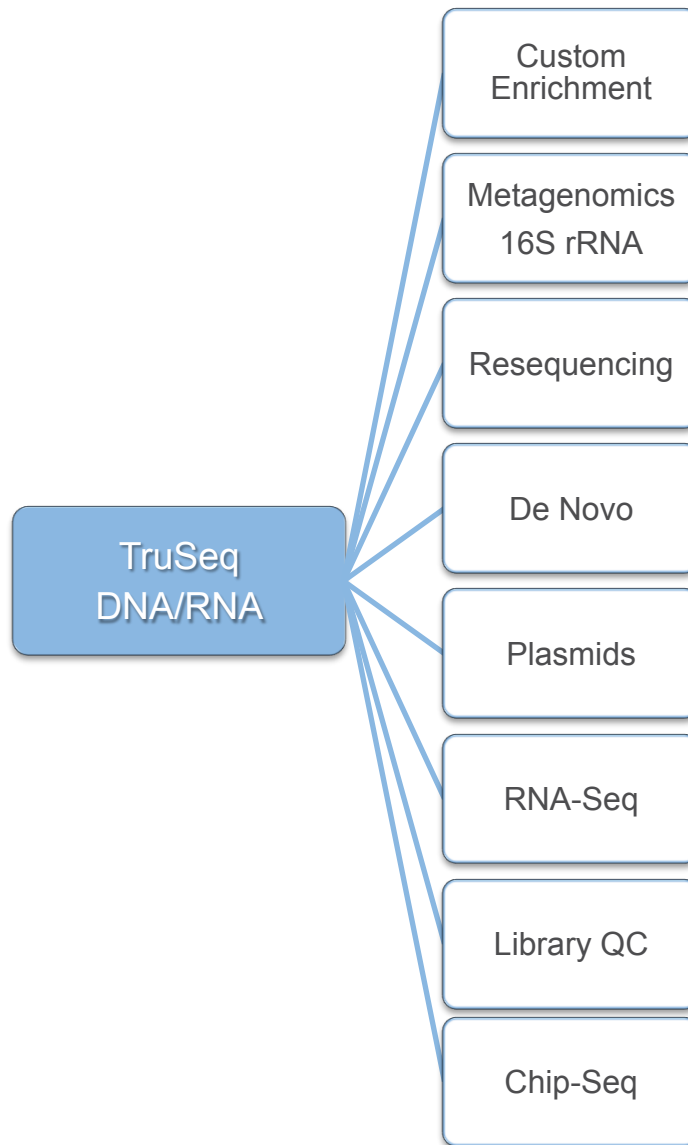
Sample Plate Assay Selection:
Nextera

Sample Plate Assay Selection:
TruSeq small RNA

```
              ┌──────────────┐
              │  Small RNA   │
              └──────────────┘
  ┌──────────┐
  │  TruSeq  │
  │ Small RNA│
  └──────────┘
              ┌──────────────┐
              │  Library QC  │
              └──────────────┘
```

Sample Sheet Considerations

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Creating a Sample Sheet:

Sample Sheet Application and Workflow Overview

Application

Workflow

Targeted Resequencing
- Custom Amplicon
- Custom Enrichment
- PCR Amplicon
- Metagenomics 16S rRNA
- Clone Checking

Small Genome Sequencing
- De novo
- Resequencing
- Plasmids

RNA Sequencing
- Small RNA
- RNA-Seq
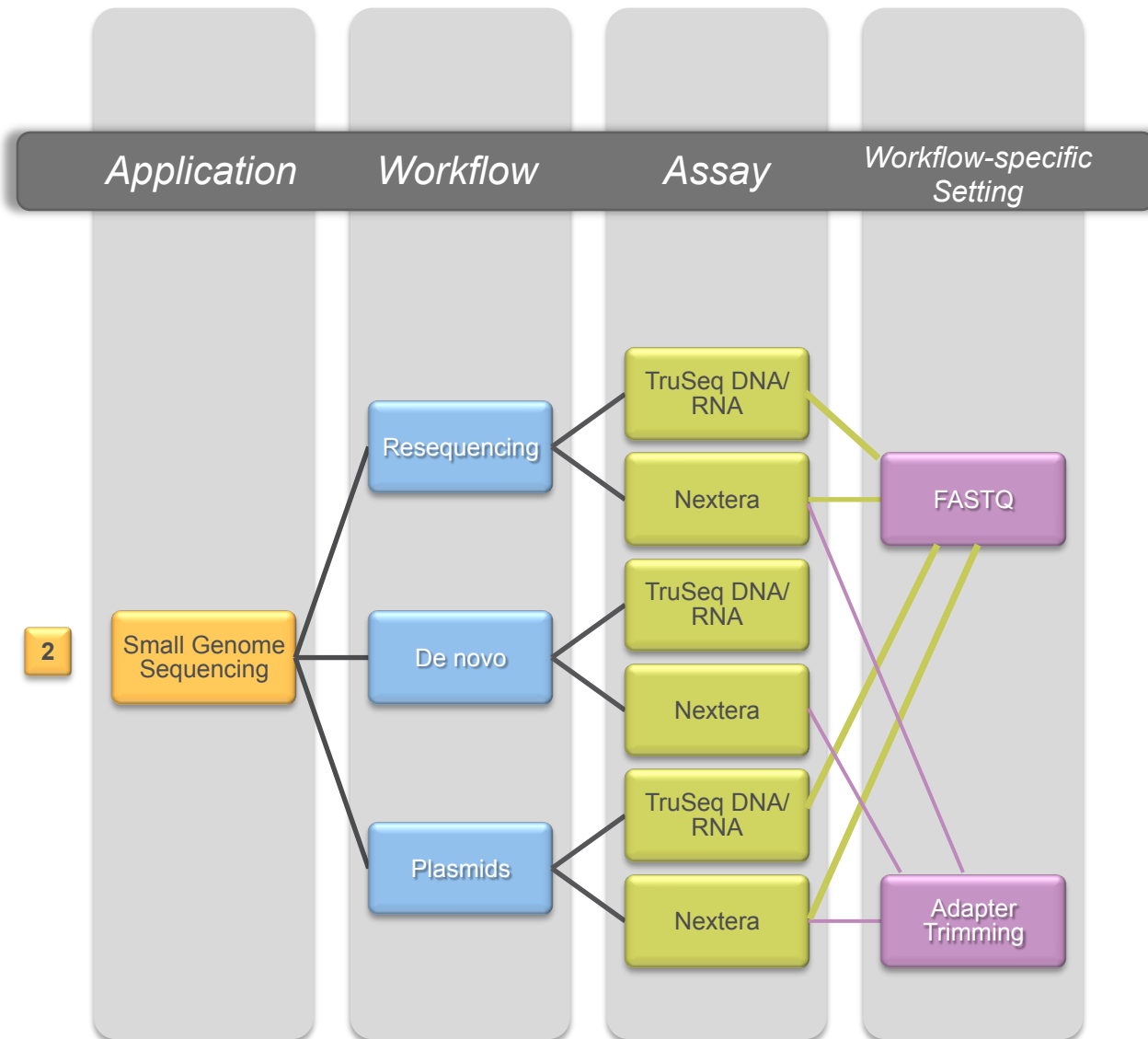
Other
- Library QC
- ChIP-Seq

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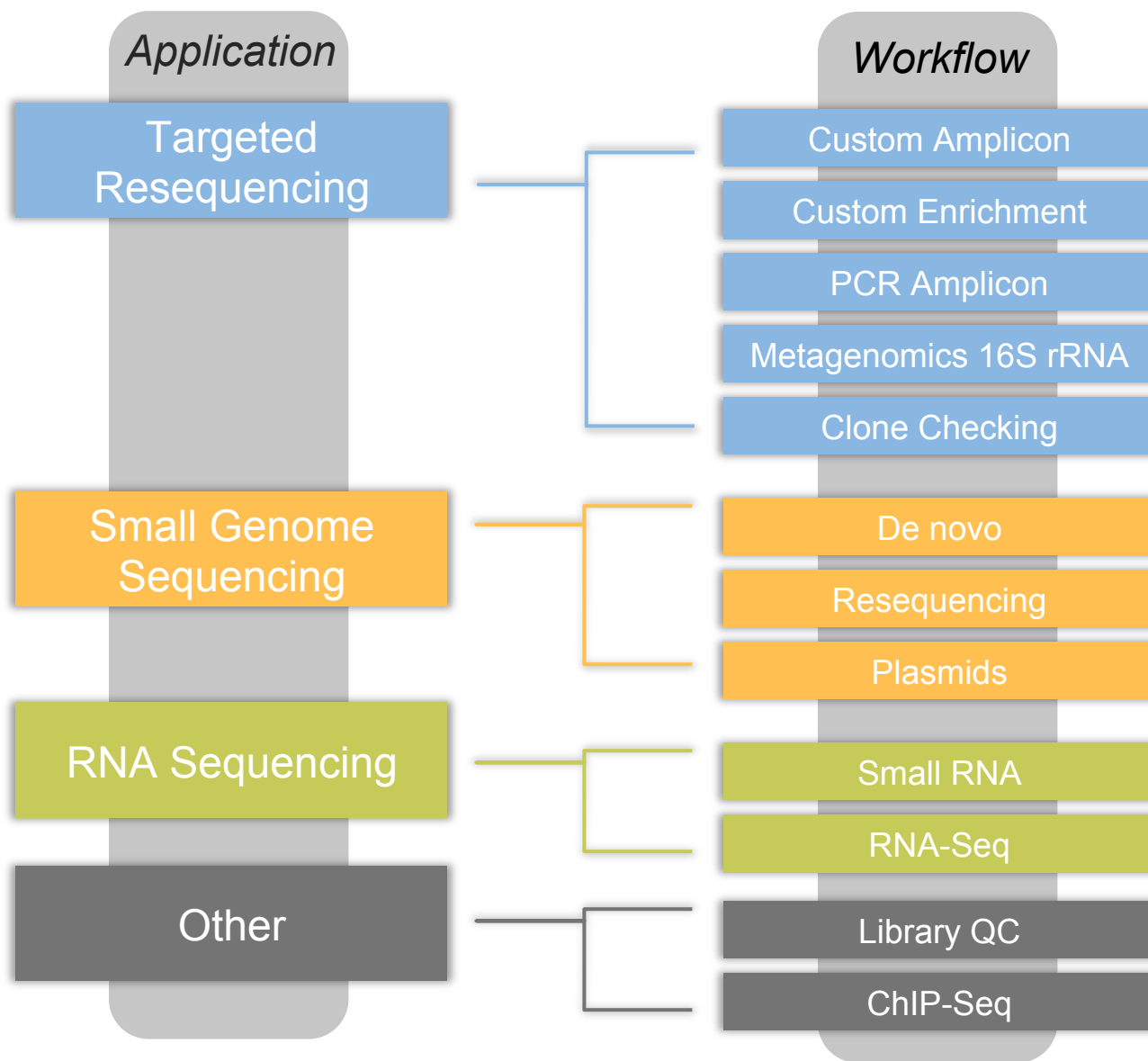
Workflow Selection Path
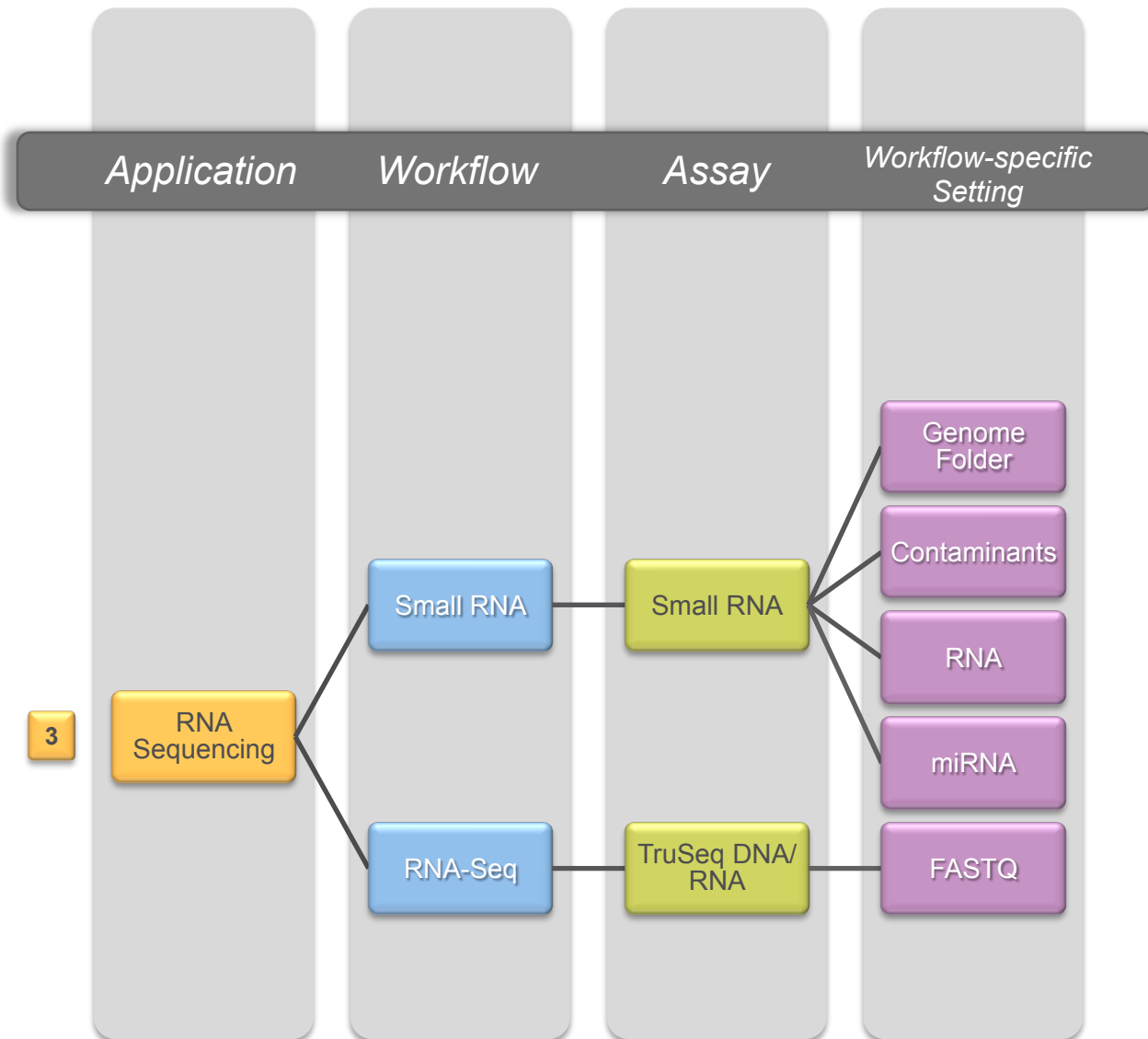
Targeted Resequencing
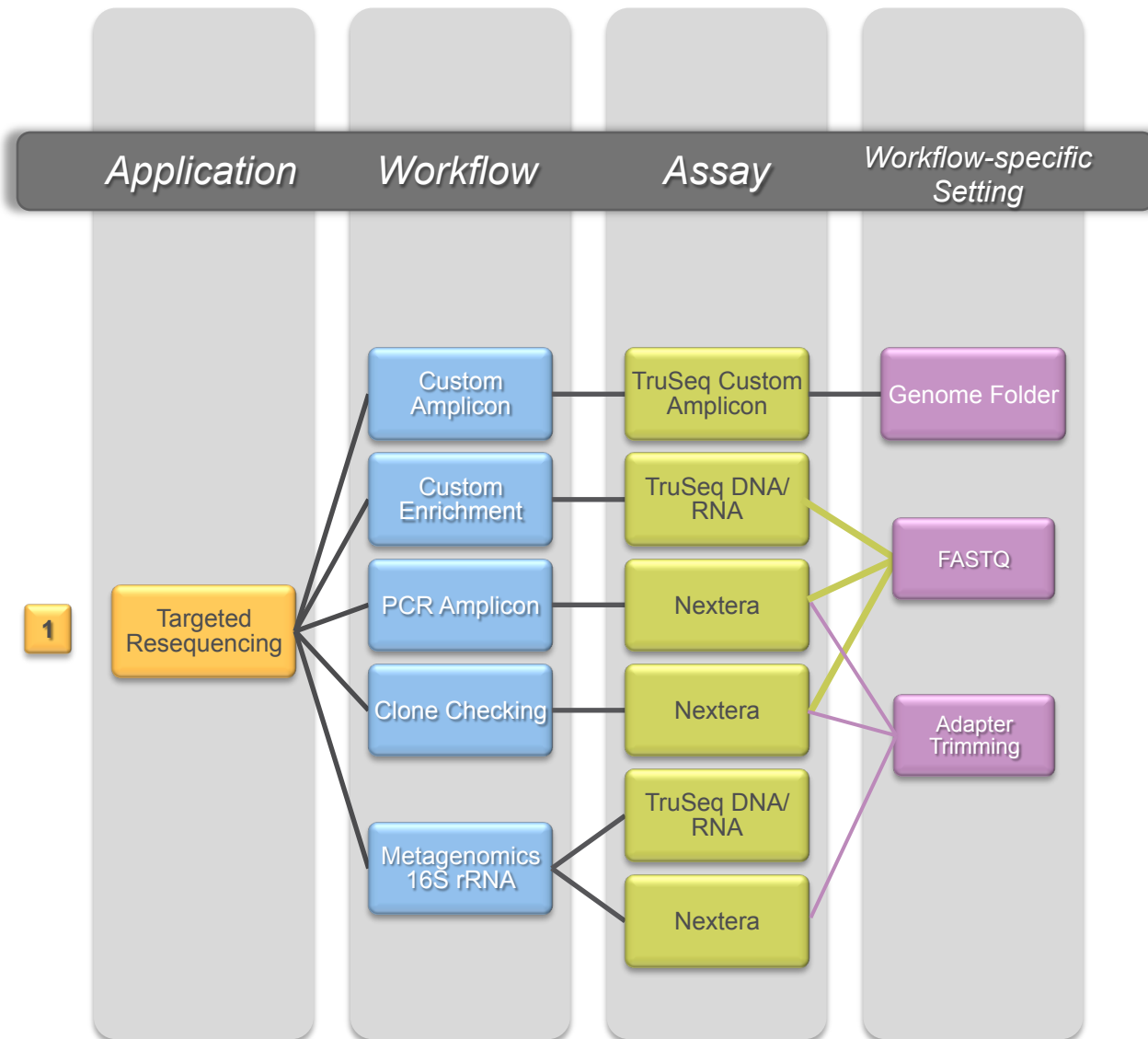
Workflow Selection Path
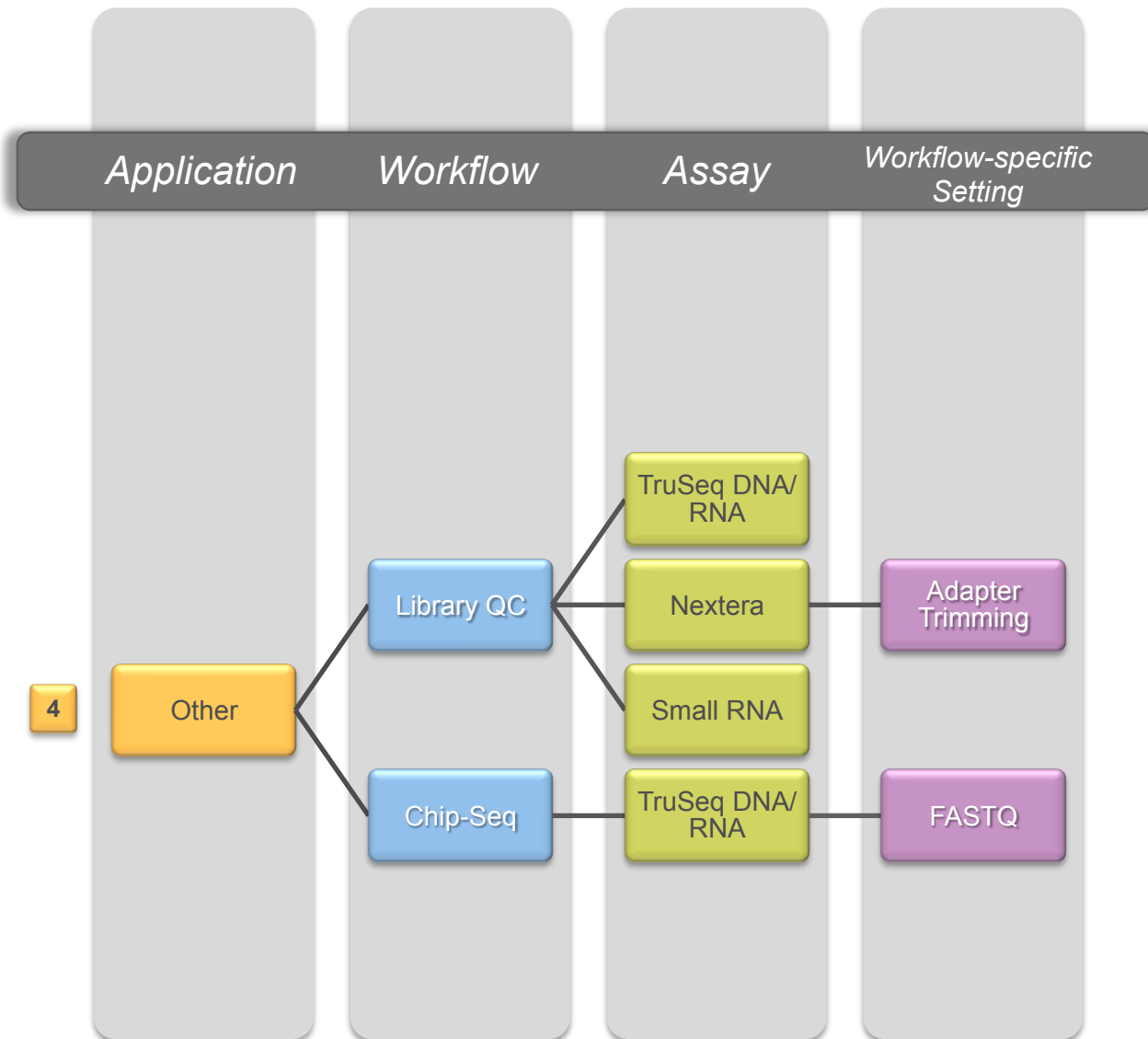## *Small Genome Sequencing*

Workflow Selection Path
RNA Sequencing
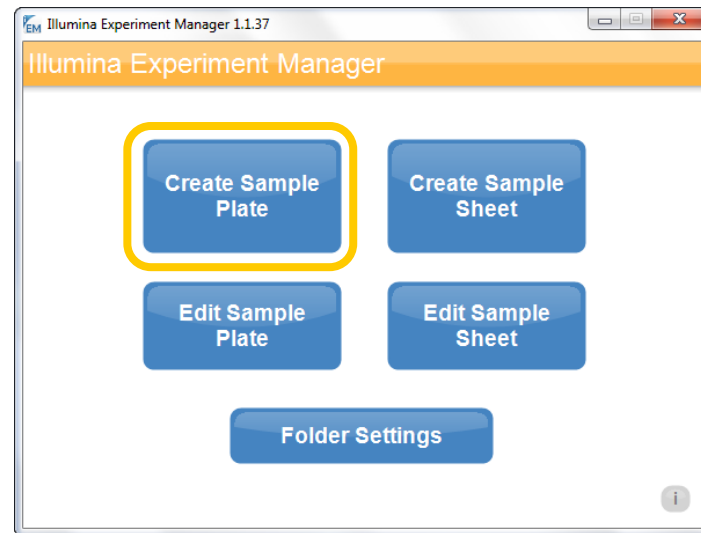
Workflow Selection Path
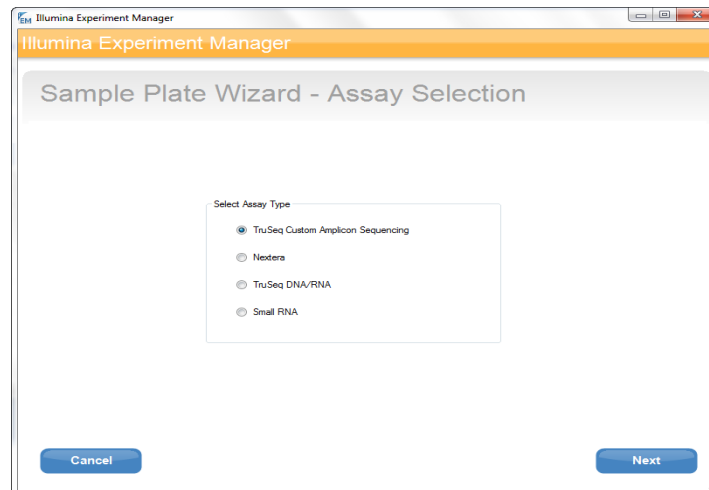
Other

Questions?

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IEM Training

▸ PPT given by the FAS

▸ Live demonstration of IEM

▸ Exercises for the customer

Live demonstration of IEM; items to show:

▸ Create a sample plate

▸ Select assay

Create a sample plate

▶ Discuss general plate
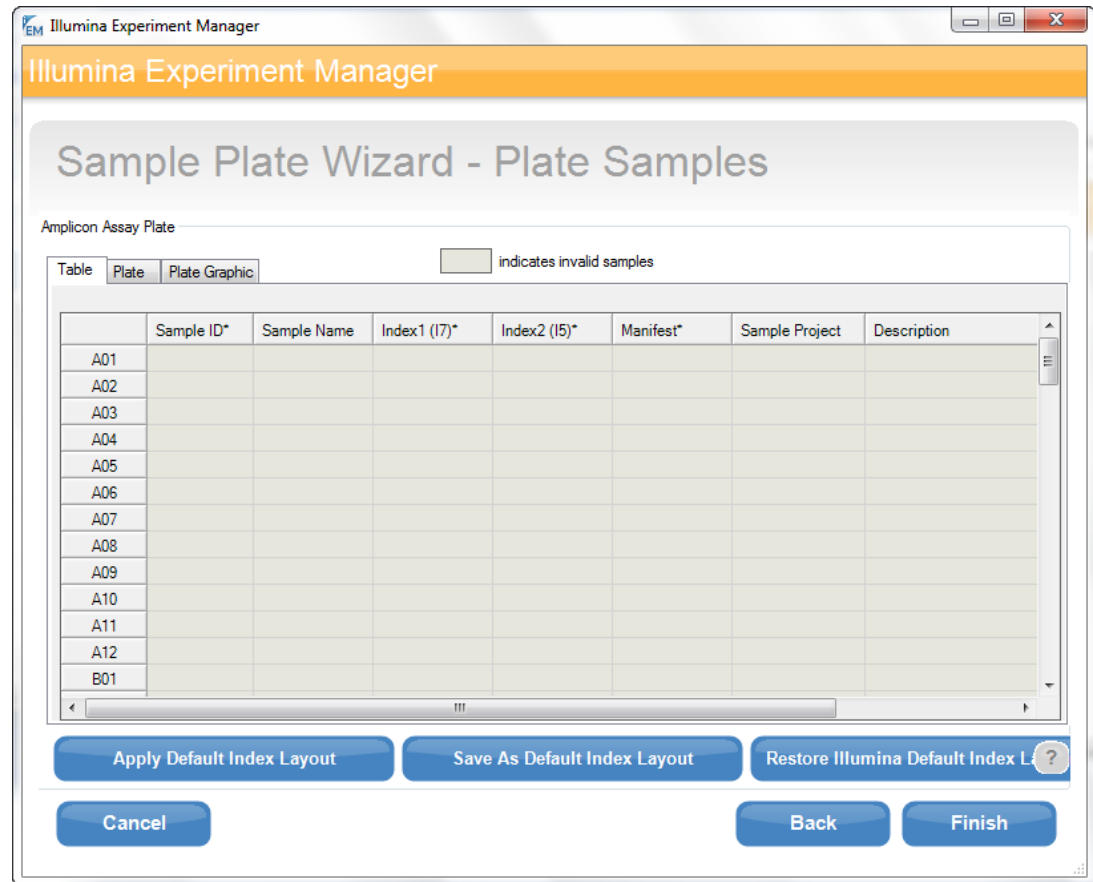   parameters :
   – Plate name
   – Index reads
   – Index cycles

Create a sample plate

▸ Discuss the table tab and the plate tab on the plate sample screen

▸ Demonstrate default index layout buttons

Create a sample plate

▶ Show the plate graphic including copy to clipboard and print buttons
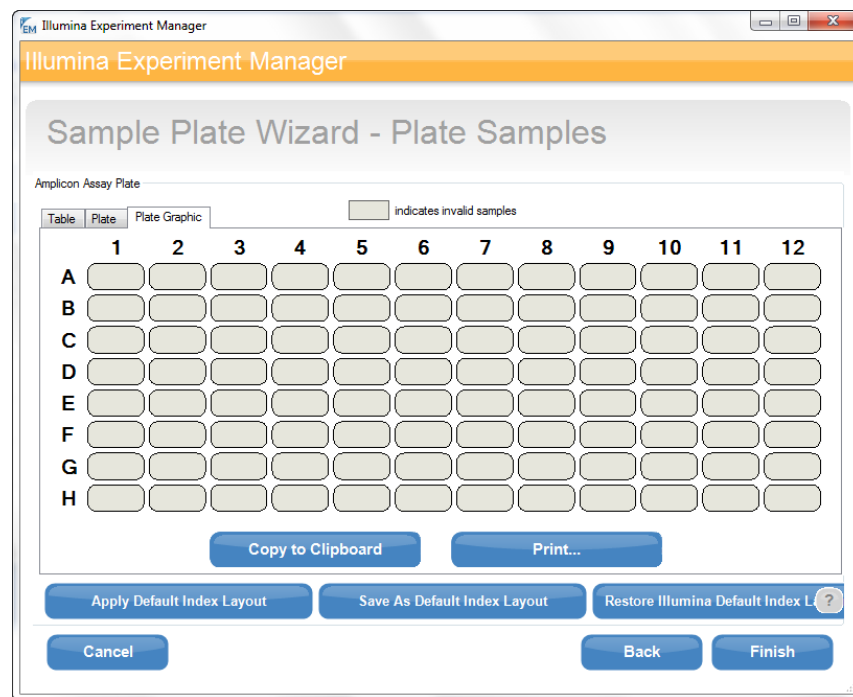
Create a sample plate

▸ Finish, discuss save as and file extension

▸ Edit a sample plate (open the plate you just created and edit this plate)

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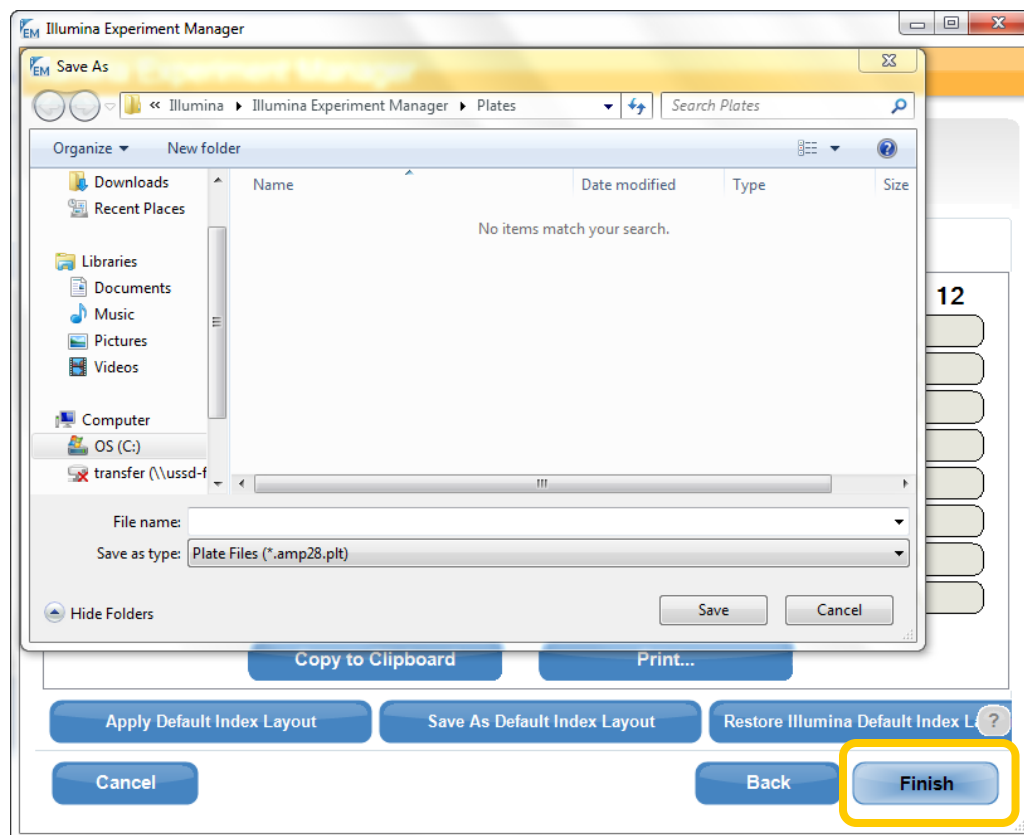
Creating a Sample Sheet

▶ Create Sample sheet
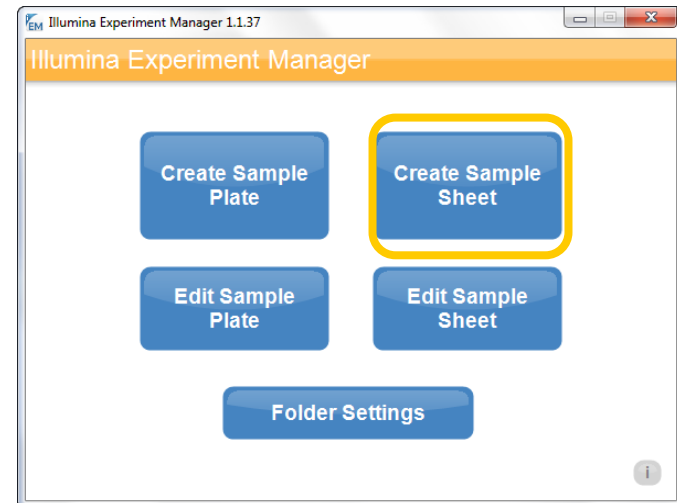
▶ Select instrument

Creating a Sample Sheet

▶ Select application and workflow
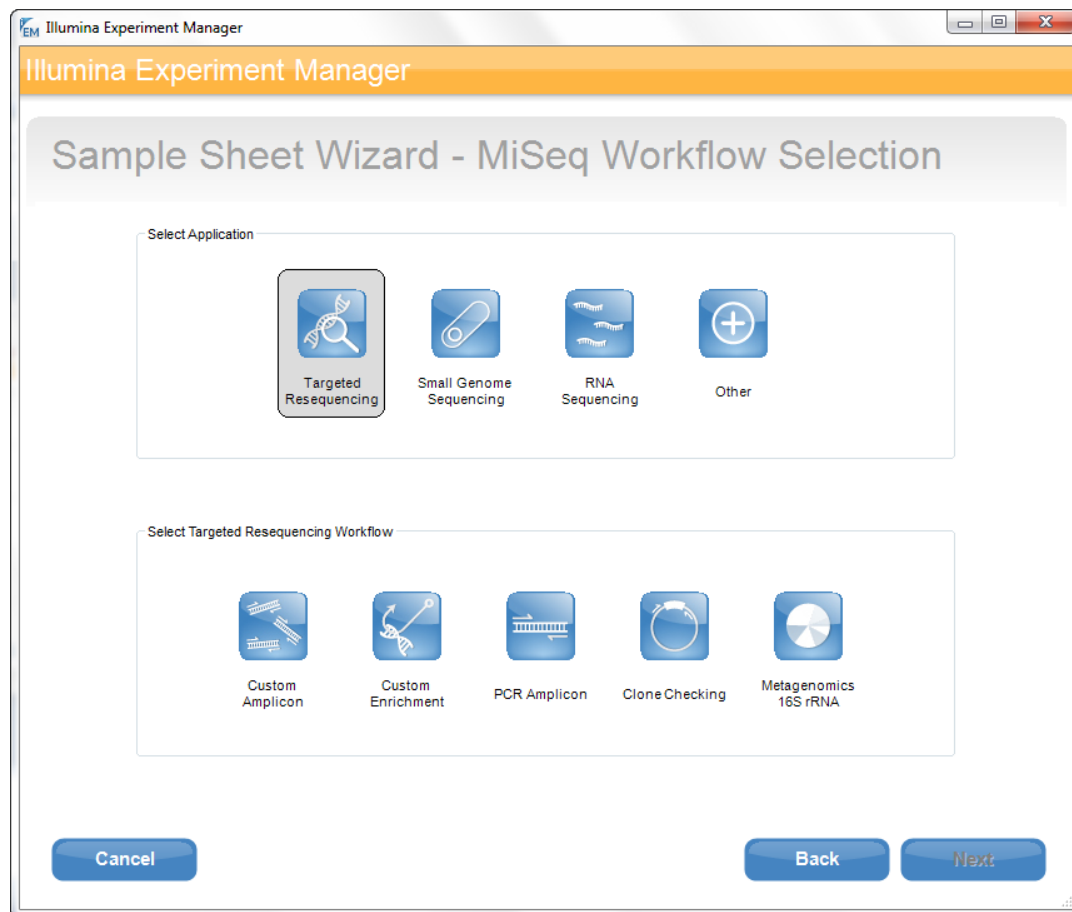
Creating a Sample Sheet

▸ Discuss run settings and specific settings box on the workflow parameter screen

- Make sure you point out the assay drop down menu

Creating a Sample Sheet

▶ Navigate to sample plate you created before, add selected samples.

▶ Demonstrate alternative option without sample plate, adding blank rows to the sheet

▶ Finish and save

Exercises

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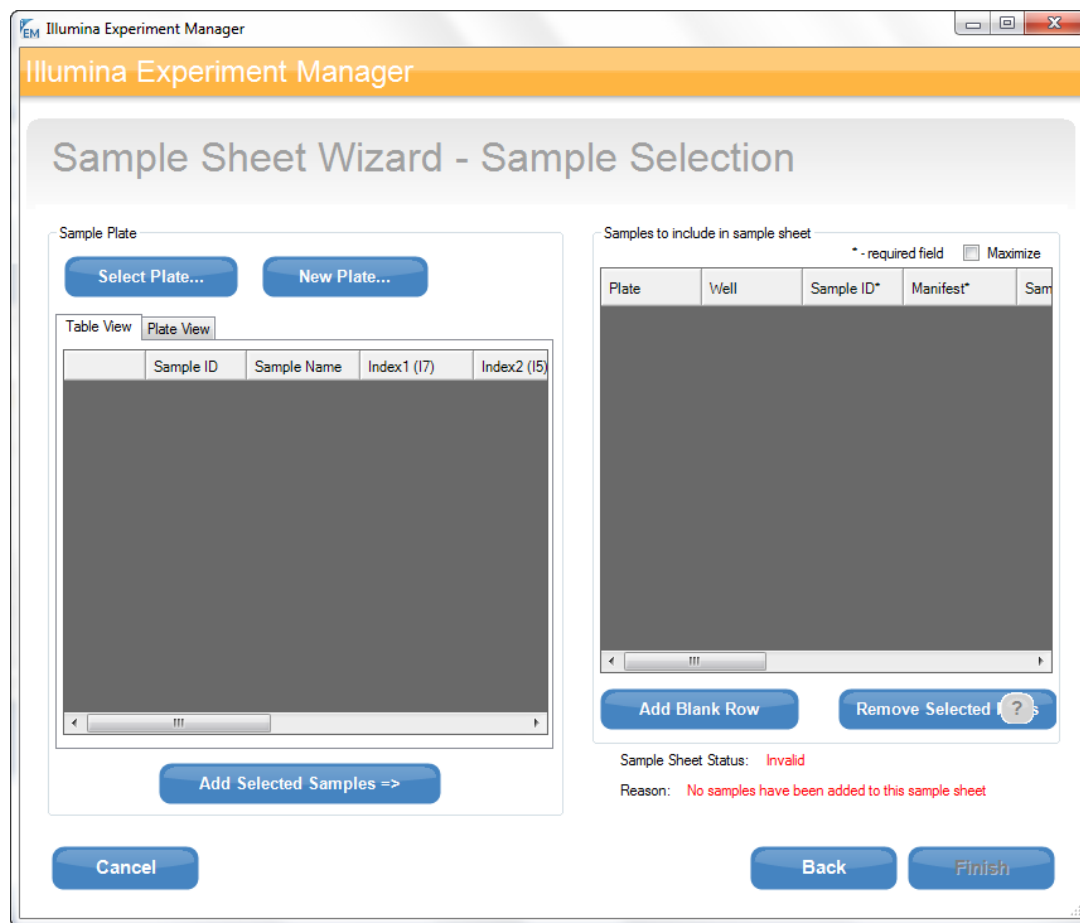
Exercises: Create a Sample Plate

1. Create a sample plate for a TruSeq DNA assay one index read (96 Samples, apply default index layout). Save as test 1

2. Create a sample plate for a Nextera assay dual index reads (48 Samples, choose your own indices). Save as test 2
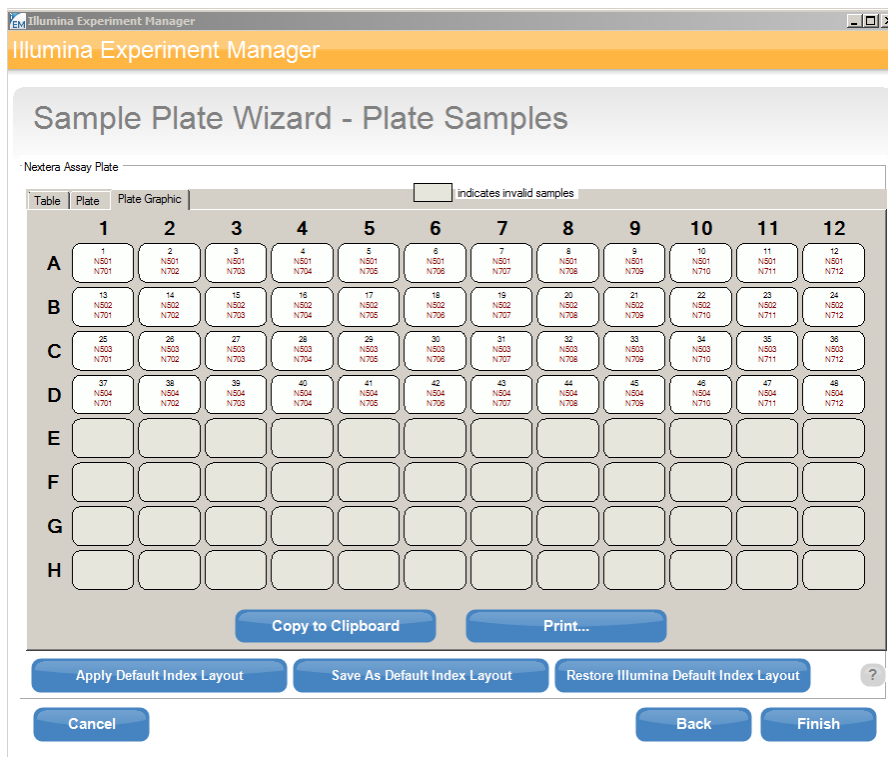
Exercises: Edit a Sample Plate

1. Edit the Sample Plate that you have saved as test 1 and change the indices for the first 12 samples

Exercises: Create a Sample Sheet

1. Sample Sheet Wizard Fun
   - Open the Sample Sheet Wizard Screen
   - Ask the students where to find the following workflows:
     - Chip-Seq
     - De-novo
     - Metagenomics
     - Small RNA
     - Resequencing
     - Custom Amplicon
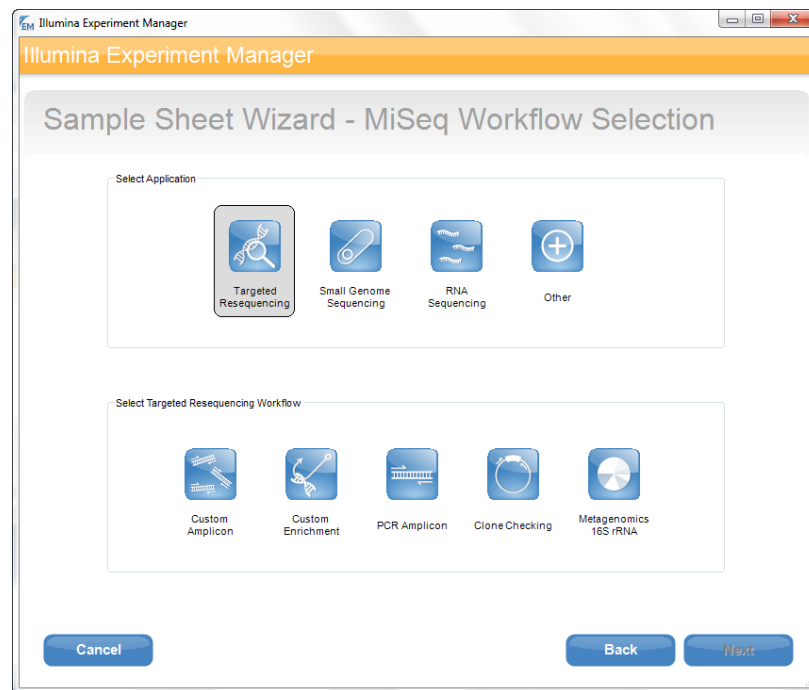
Exercises: Create a Sample Sheet

▶ Resequencing Experiment (Nextera assay, dual index reads, 2x151 cycles, adapter trimming, use sample plate test 2)

[Header]										
IEMFileVersion	3									
Investigator Name	SuperStudent									
Project Name	Training									
Experiment Name	2									
Date	########									
Workflow	Resequencing									
Assay	Nextera									
Description										
Chemistry	Amplicon									
[Reads]										
151										
151										
[Settings]										
OnlyGenerateFASTQ	0									
Adapter	CTGTCTCTTATACACATCT									
[Data]										
Sample_ID	Sample_N	Sample_P	Sample_W	Sample_P	index	I7_Index_	index2	I5_Index_	Descriptic	GenomeFolder
1		Test 2	A01		TAAGGCG	N701	TAGATCG(	N501		human
2		Test 2	A02		CGTACTA(	N702	TAGATCG(	N501		human
3		Test 2	A03		AGGCAGA	N703	TAGATCG(	N501		human
4		Test 2	A04		TCCTGAG(	N704	TAGATCG(	N501		human
5		Test 2	A05		GGACTCC	N705	TAGATCG(	N501		human
6		Test 2	A06		TAGGCAT(	N706	TAGATCG(	N501		human
7		Test 2	A07		CTCTCTAC	N707	TAGATCG(	N501		human
8		Test 2	A08		CAGAGAG	N708	TAGATCG(	N501		human
9		Test 2	A09		GCTACGC	N709	TAGATCG(	N501		human
10		Test 2	A10		CGAGGCT(	N710	TAGATCG(	N501		human
11		Test 2	A11		AAGAGGC	N711	TAGATCG(	N501		human
12		Test 2	A12		GTAGAGG	N712	TAGATCG(	N501		human

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Edit a Sample Sheet, use custom primers

▸ Locate the [Settings] section, after the [Reads] section and before the [Data} section

▸ Add a line for each of your custom primers according to the following example;

[Settings]

CustomRead1PrimerMix	C1
CustomIndexPrimerMix	C2
CustomRead2PrimerMix	C3

▸ If you are using illumina primers for read 1 and 2 and a custom primer for the index read edit your [Settings] section as follows:

[Settings]

CustomIndexPrimerMix	C2

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Example of custom primers sample sheet

	A	B	C	D	E	F	G	H
1	[Header]							
2	IEMFileVersion	3						
3	Investigator Name	Dr_YourName						
4	Project Name	Custom_Primers						
5	Experiment Name	Custom_Primers						
6	Date	1/16/2012						
7	Workflow	Resequencing						
8	Assay	TruSeq DNA/RNA						
9	Description	Test						
10	Chemistry	Default						
11	[Reads]							
12	151							
13	151							
14	[Settings]							
15	CustomRead1PrimerMix	C1						
16	CustomIndexPrimerMix	C2						
17	CustomRead2PrimerMix	C3						
18	[Data]							
19	Sample_ID	Sample_Name	Sample_Plate	Sample_Well	Sample_Prc	index	I7_Index_	Descriptic
20	Sample_1		Custom_Primers	A01		ATCACG	A001	
21	Sample_2		Custom_Primers	A02		TTAGGC	A003	
22	Sample_3		Custom_Primers	A03		GGCTAC	A011	

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