



Illumina Experiment Manager

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Course Objectives

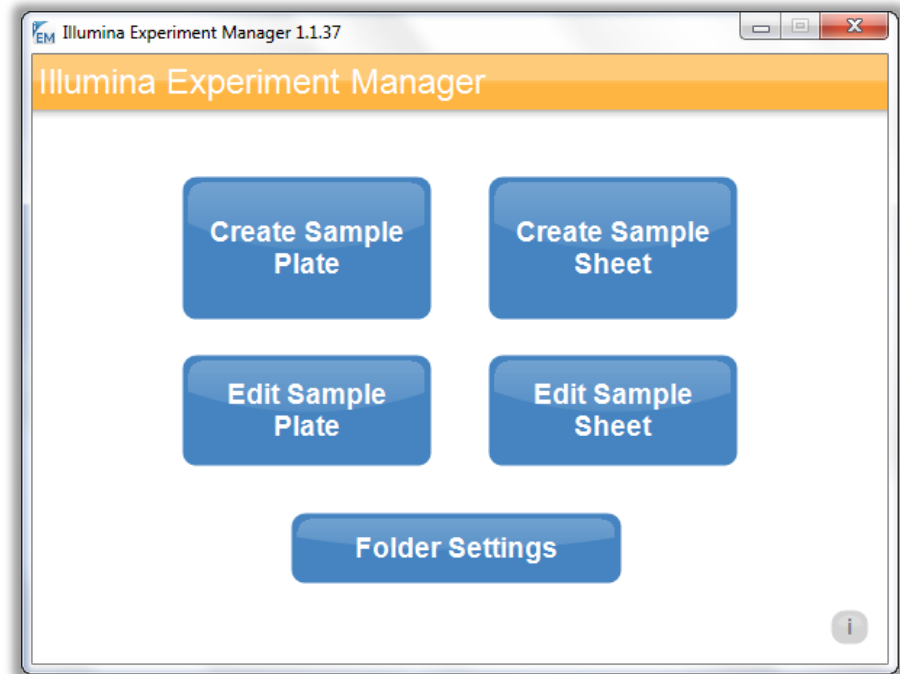
By the end of this course, you will be able to:

- ▶ 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

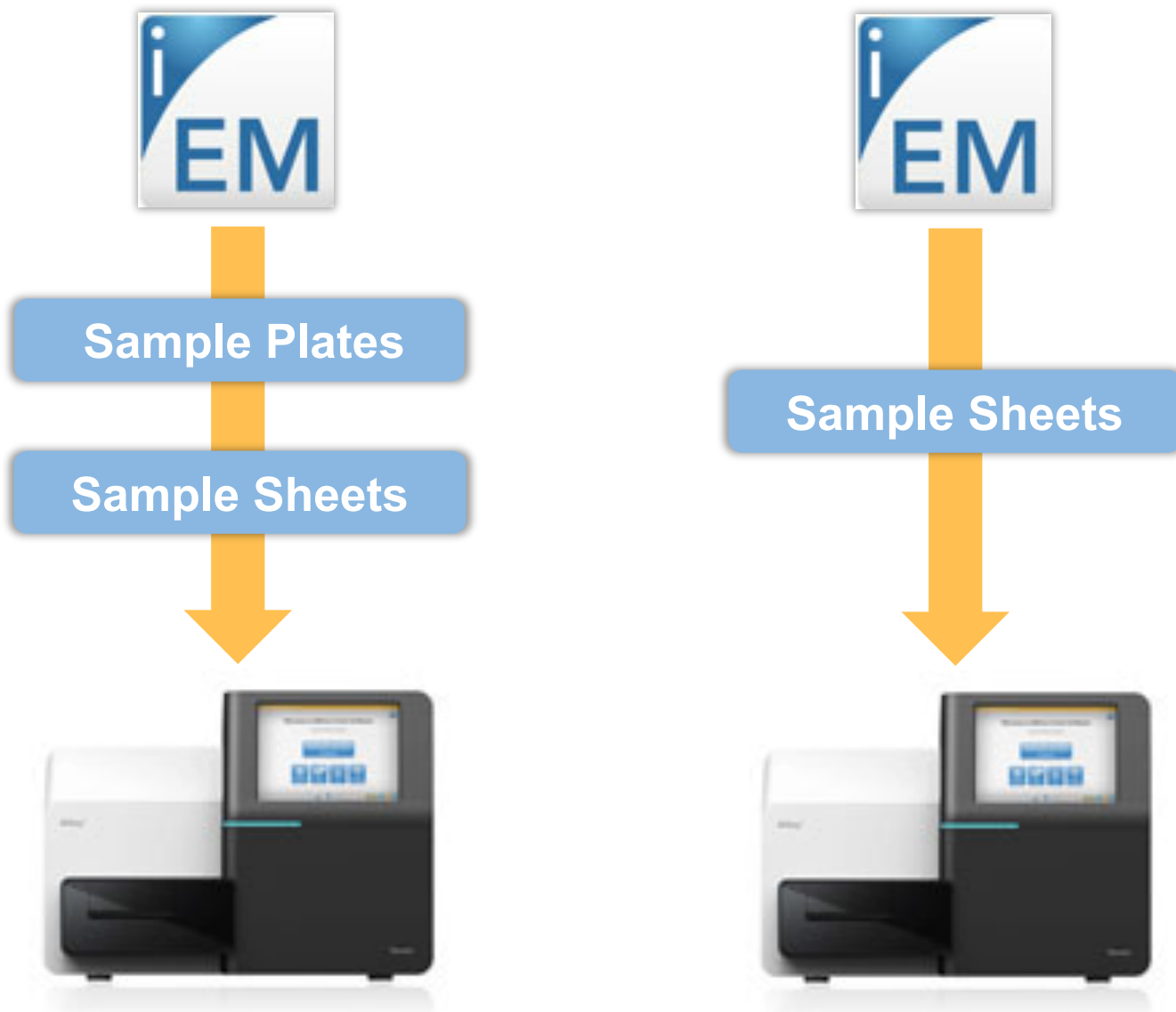
Start Software

- 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

Two Workflow options





Data Analysis Overview

Illumina Data Analysis Workflow

1

Primary Analysis



2

Secondary Analysis



MiSeq Reporter




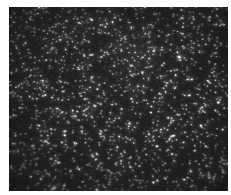




CASAVA

3

Data Visualization



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

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 <ul style="list-style-type: none">• 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 <ul style="list-style-type: none">• A standard that lists and annotates almost the entire collection of human variants



Sample Plate Considerations

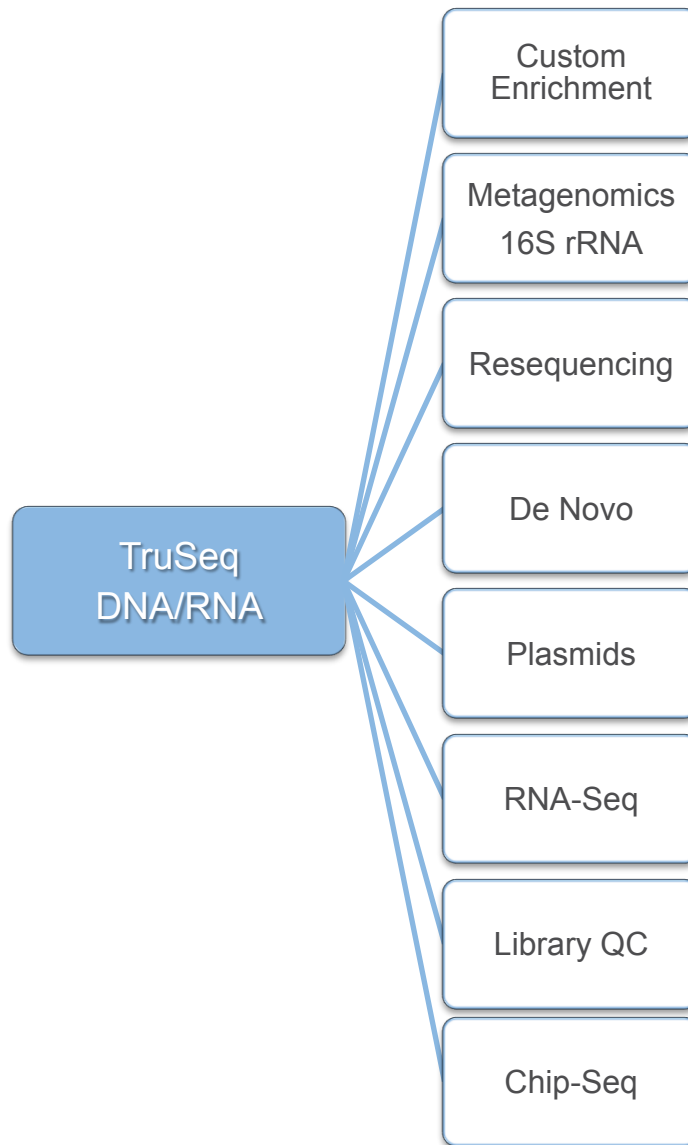
Sample Plate Assay Selection

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

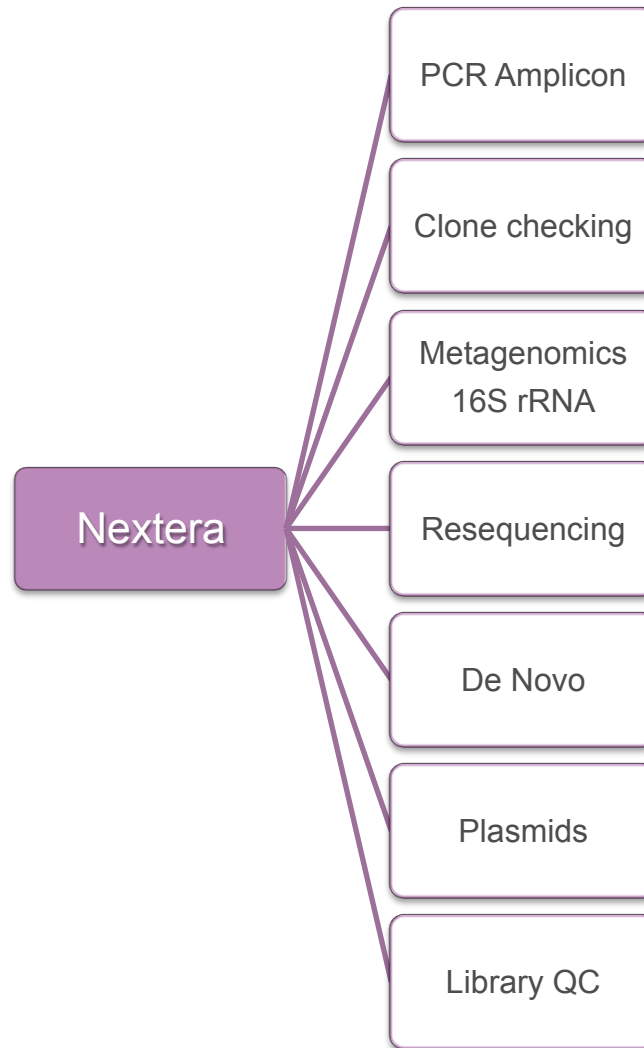
Sample Plate Assay Selection:

TruSeq DNA/RNA



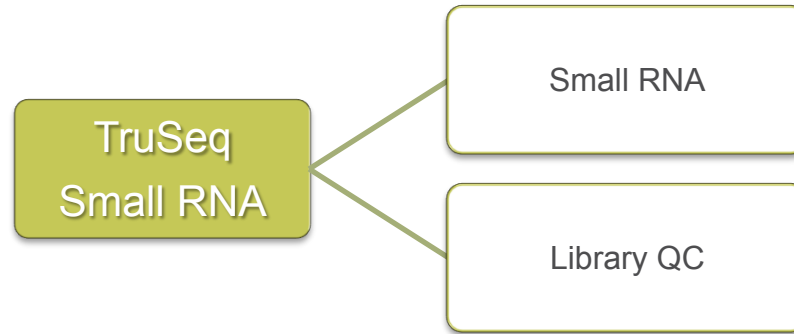
Sample Plate Assay Selection:

Nextera



Sample Plate Assay Selection:

TruSeq small RNA





Sample Sheet Considerations

Creating a Sample Sheet:

Illumina Experiment Manager

Sample Sheet Wizard - Instrument Selection

MiSeq

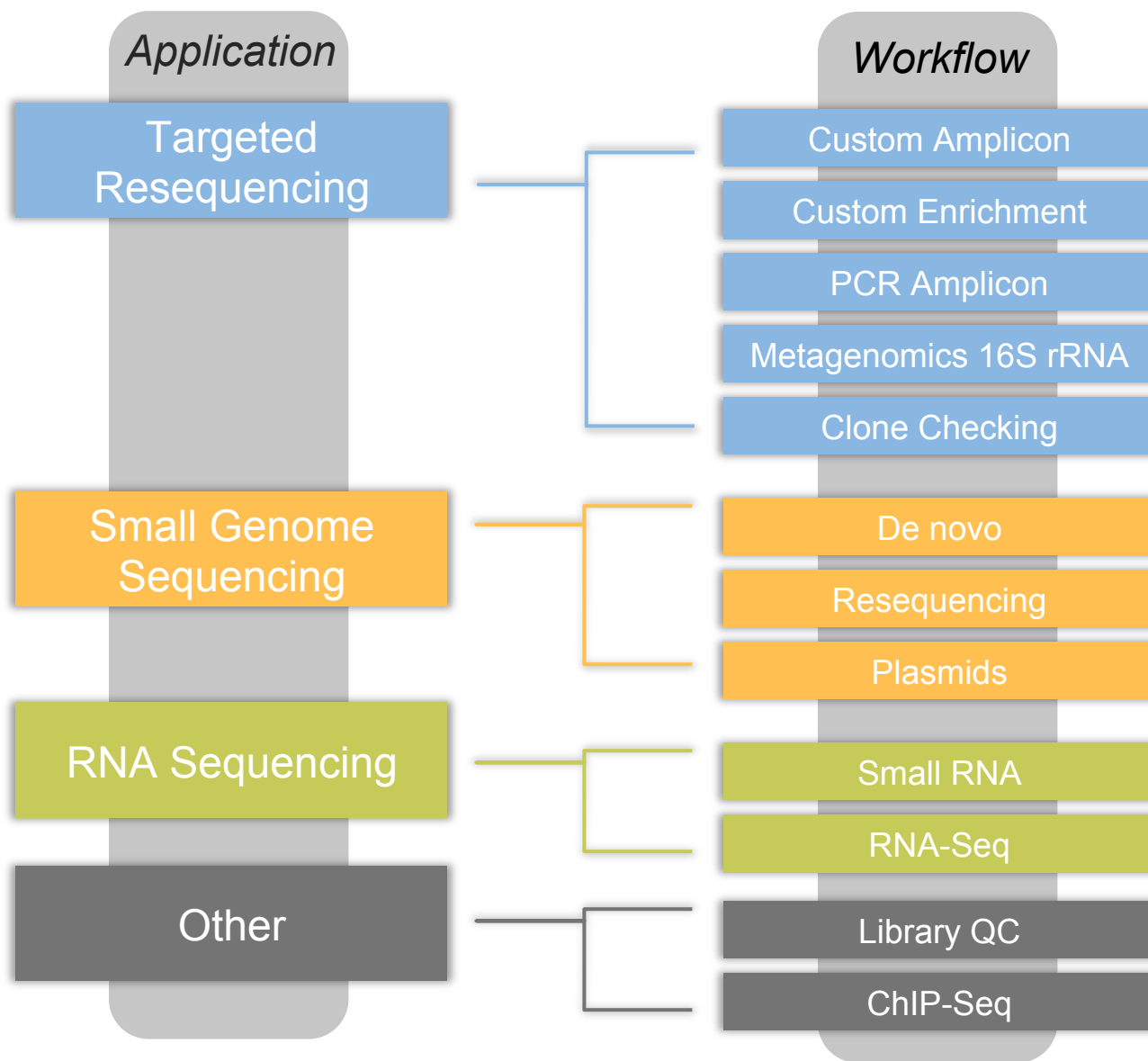
1

HiSeq 2000/1000
HiScanSQ
GA

Data analysis:
Will take place on server

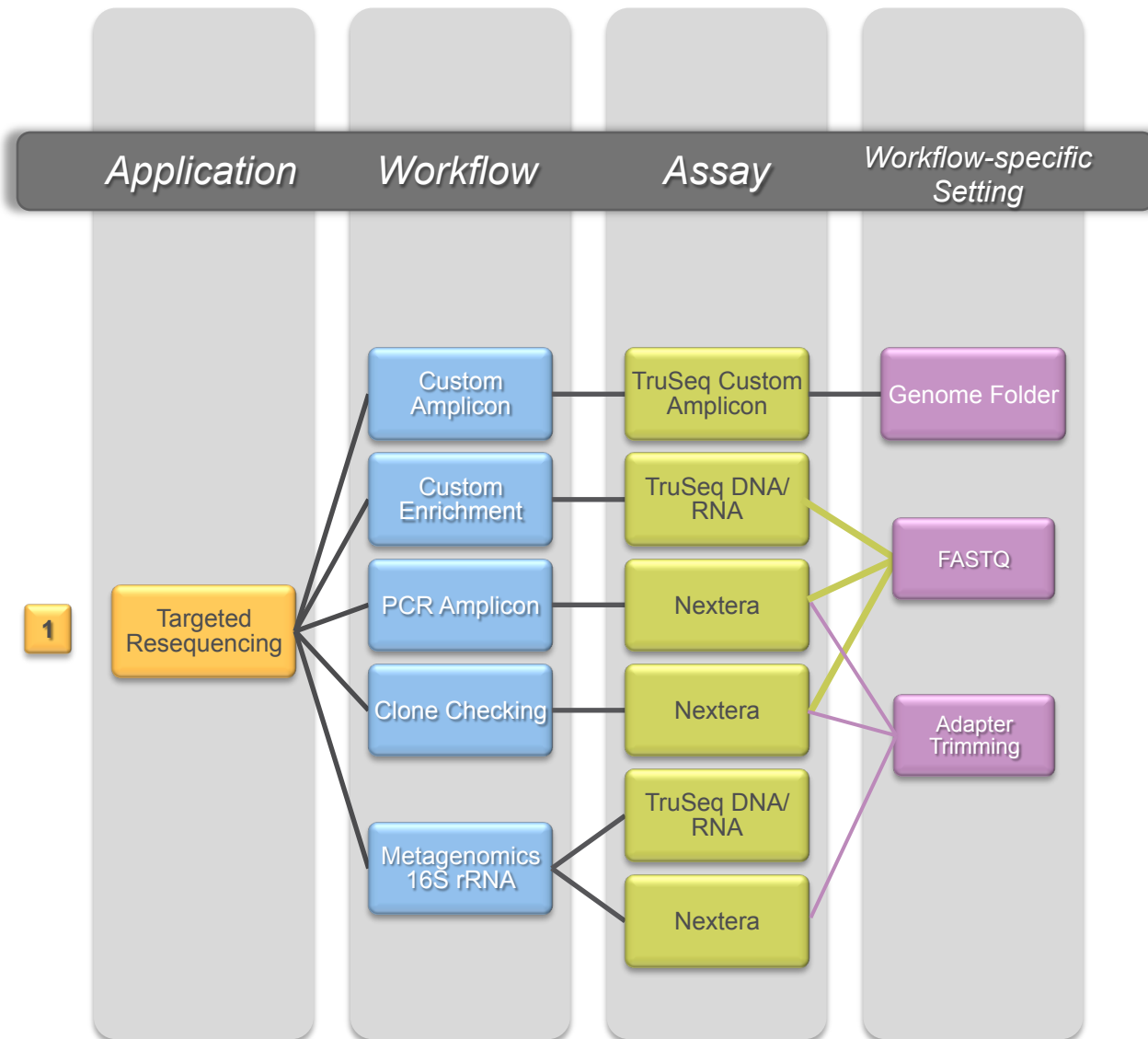
Cancel Next

Sample Sheet Application and Workflow Overview



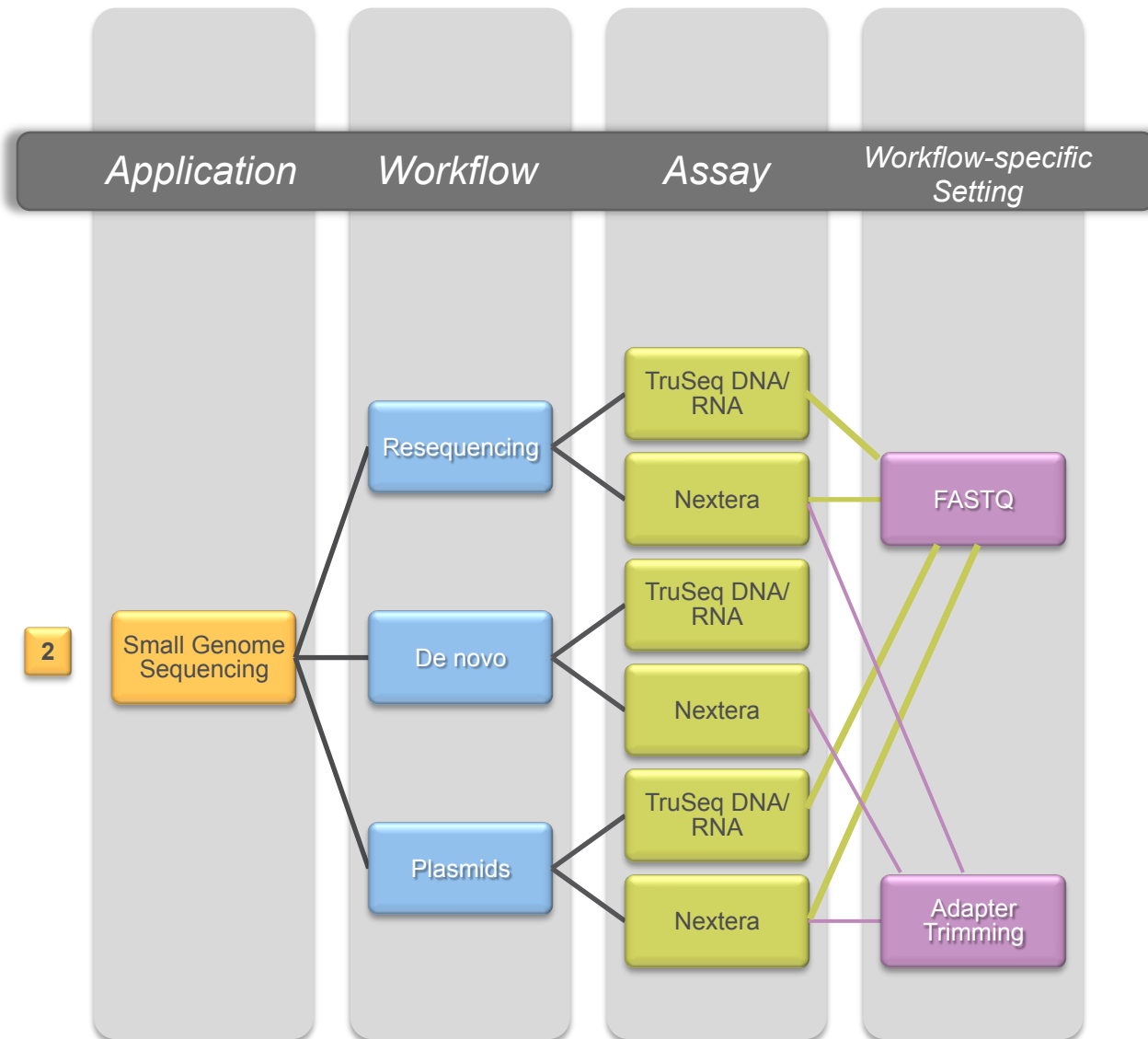
Workflow Selection Path

Targeted Resequencing



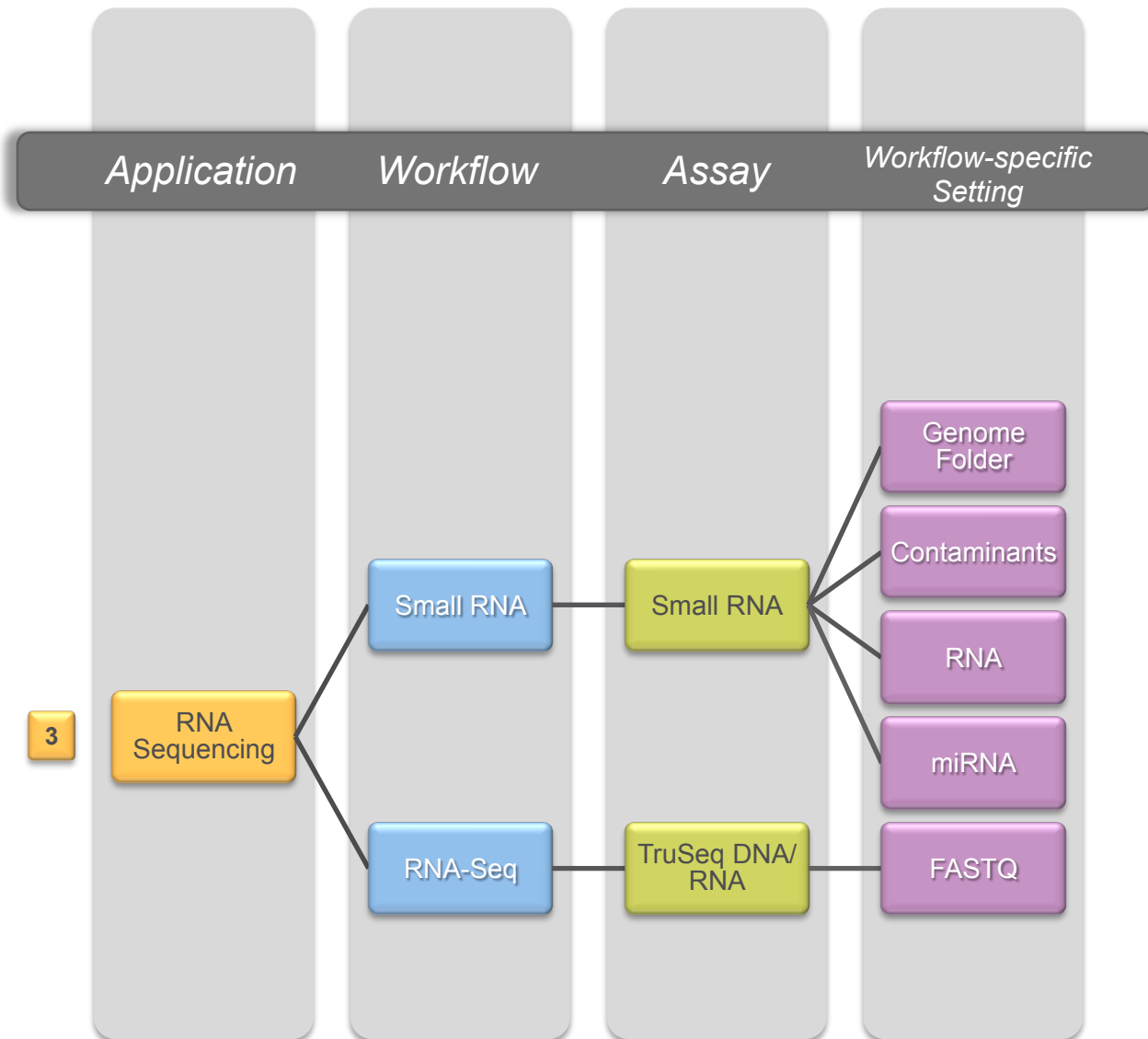
Workflow Selection Path

Small Genome Sequencing



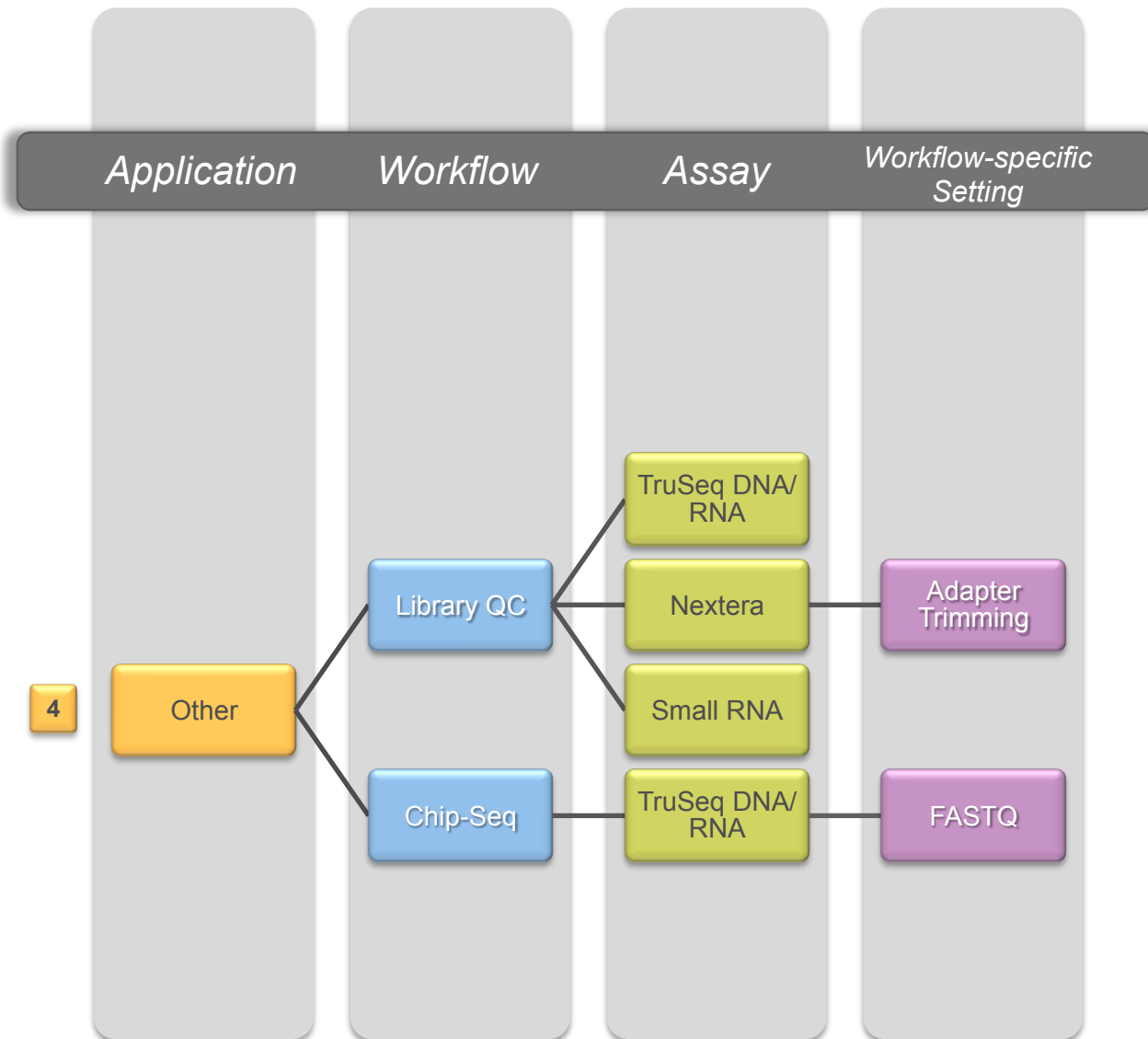
Workflow Selection Path

RNA Sequencing



Workflow Selection Path

Other



4



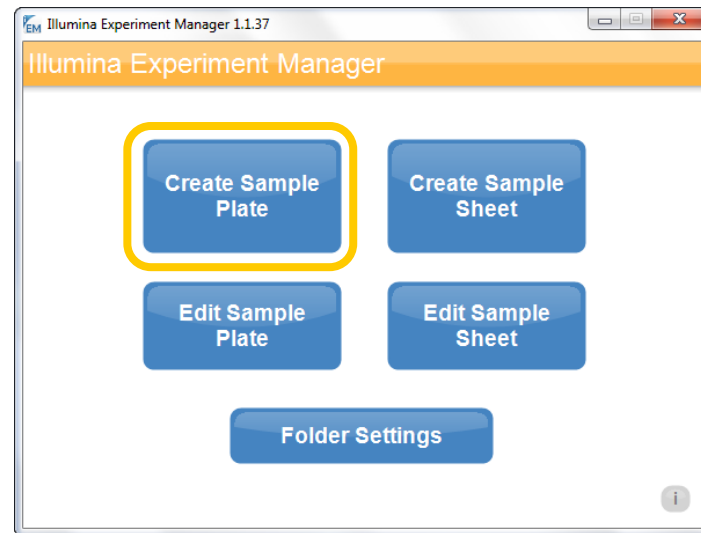
Questions?

IEM Training

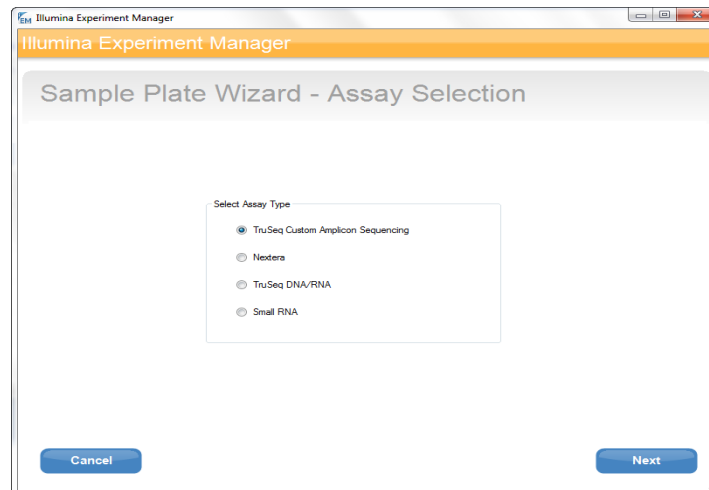
- ▶ PPT given by the FAS
- ▶ Live demonstration of IEM
- ▶ Exercises for the customer

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

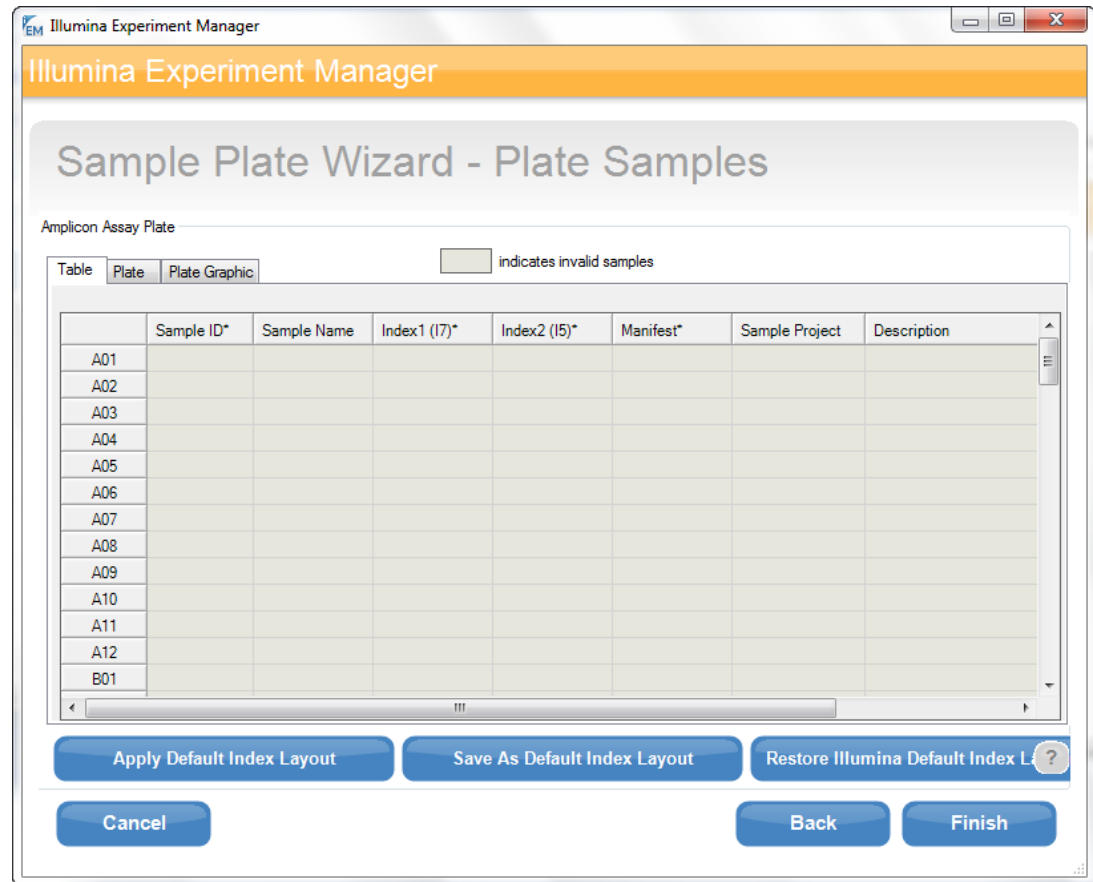
The screenshot shows the 'Illumina Experiment Manager' window with the 'Sample Plate Wizard - Assay Parameters' dialog box open. The dialog box contains the following fields and options:

- Unique Plate Name ***: A text input field.
- Index Reads**: Radio buttons for 0, 1, and 2. The '2' option is selected.
- Index Cycles**: Radio buttons for N/A, 6, and 8. The '8' option is selected.
- * - required field**: A note indicating that the Unique Plate Name field is mandatory.

At the bottom of the dialog box, there are two buttons: 'Cancel' on the left and 'Next' on the right.

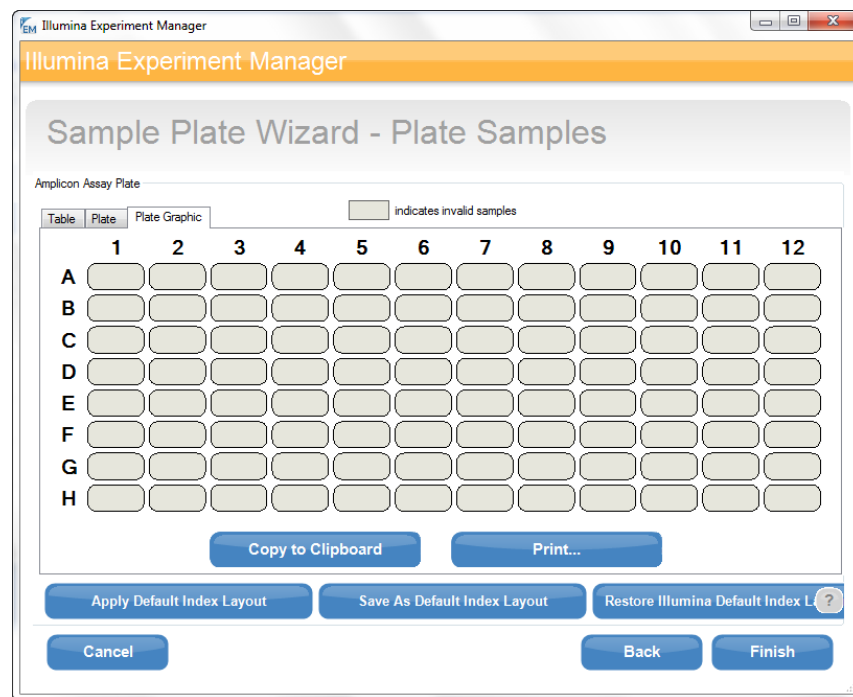
Create a sample plate

- ▶ Discuss the table tab and the plate tab on the plate sample screen
- ▶ Demonstrate default index layout buttons



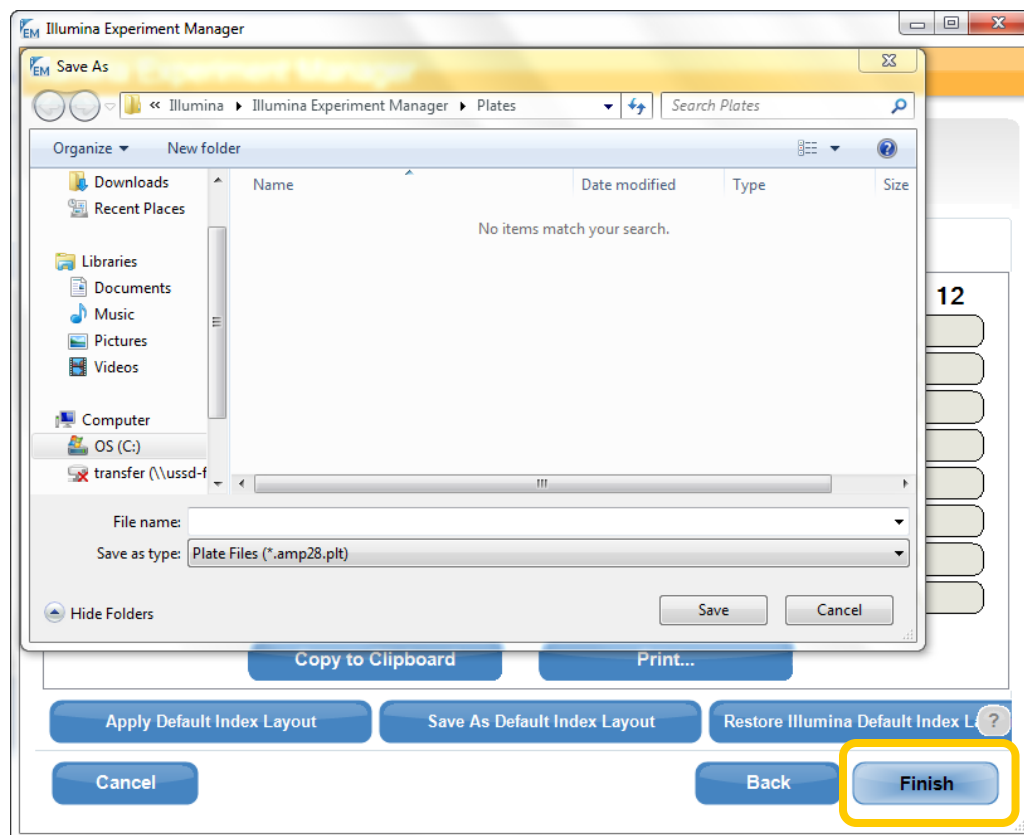
Create a sample plate

- ▶ Show the plate graphic including copy to clipboard and print buttons



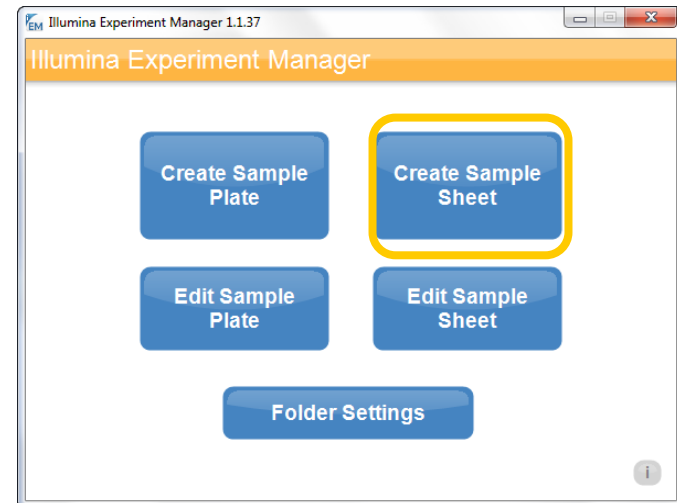
Create a sample plate

- ▶ Finish, discuss save as and file extension
- ▶ Edit a sample plate (open the plate you just created and edit this plate)



Creating a Sample Sheet

- ▶ Create Sample sheet

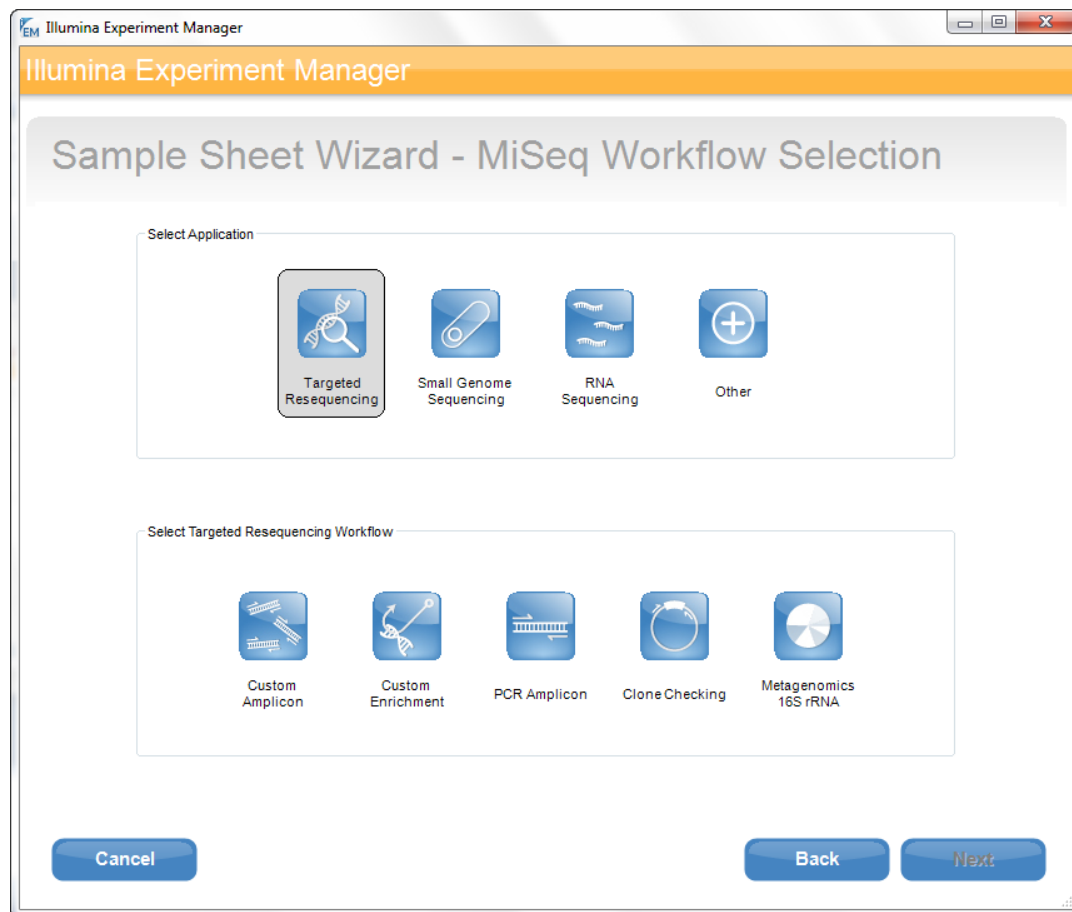


- ▶ Select instrument



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

The screenshot shows the 'Illumina Experiment Manager' window with the 'Sample Sheet Wizard - Workflow Parameters' dialog box open. The dialog is divided into two main sections: 'Istom Amplicon Run Settings' and 'Amplicon Workflow-Specific Settings'.

Istom Amplicon Run Settings:

- Reagent Cartridge Barcode*:
- Assay: **TruSeq Custom Amplicon Sequencing** (dropdown menu)
- Index Reads: 0 1 2
- Index Cycles: N/A 6 8
- Project Name:
- Experiment Name:
- Investigator Name:
- Description:
- Date: 12/12/2011 (calendar icon)
- Read Type:
- Cycles Read 1: 151 (spinner)
- Cycles Read 2: 151 (spinner)
- * - required field

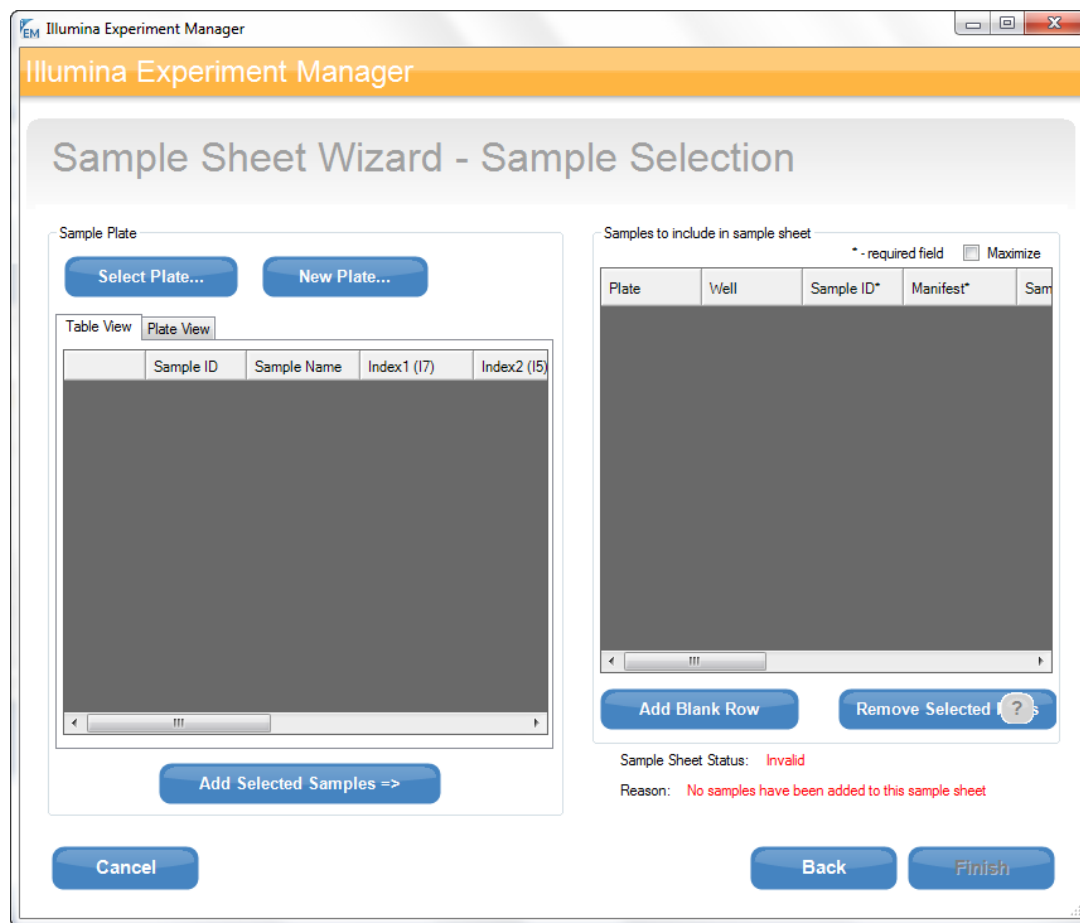
Amplicon Workflow-Specific Settings:

- Genome Folder:

At the bottom of the dialog are three buttons: 'Cancel', 'Back', and 'Next'. A help icon (?) is located in the bottom right corner of the 'Amplicon Workflow-Specific Settings' section.

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

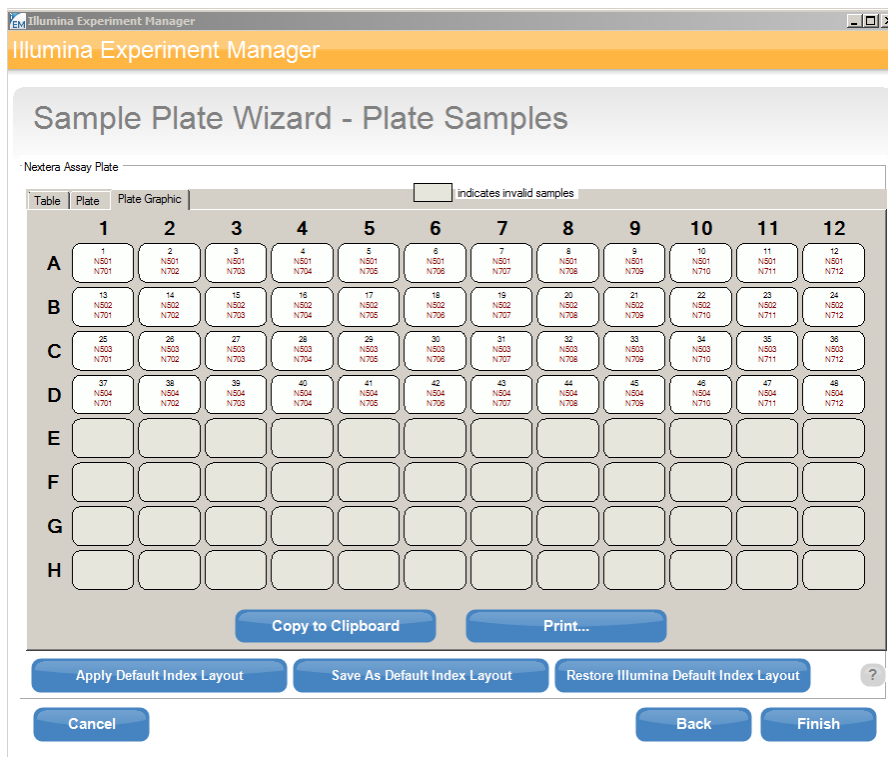




Exercises

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

Illumina Experiment Manager

Sample Plate Wizard - Plate Samples

TruSeq DNA/RNA Assay Plate

Table | Plate | Plate Graphic indicates invalid samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 A013	2 A014	3 A015	4 A016	5 A018	6 A019	7 A020	8 A021	9 A022	10 A023	11 A025	12 A027
B	13 A001	14 A002	15 A003	16 A004	17 A005	18 A006	19 A007	20 A008	21 A009	22 A010	23 A011	24 A012
C	25 A001	26 A002	27 A003	28 A004	29 A005	30 A006	31 A007	32 A008	33 A009	34 A010	35 A011	36 A012
D	37 A001	38 A002	39 A003	40 A004	41 A005	42 A006	43 A007	44 A008	45 A009	46 A010	47 A011	48 A012
E	49 A001	50 A002	51 A003	52 A004	53 A005	54 A006	55 A007	56 A008	57 A009	58 A010	59 A011	60 A012
F	61 A001	62 A002	63 A003	64 A004	65 A005	66 A006	67 A007	68 A008	69 A009	70 A010	71 A011	72 A012
G	73 A001	74 A002	75 A003	76 A004	77 A005	78 A006	79 A007	80 A008	81 A009	82 A010	83 A011	84 A012
H	85 A001	86 A002	87 A003	88 A004	89 A005	90 A006	91 A007	92 A008	93 A009	94 A010	95 A011	96 A012

Copy to Clipboard Print...

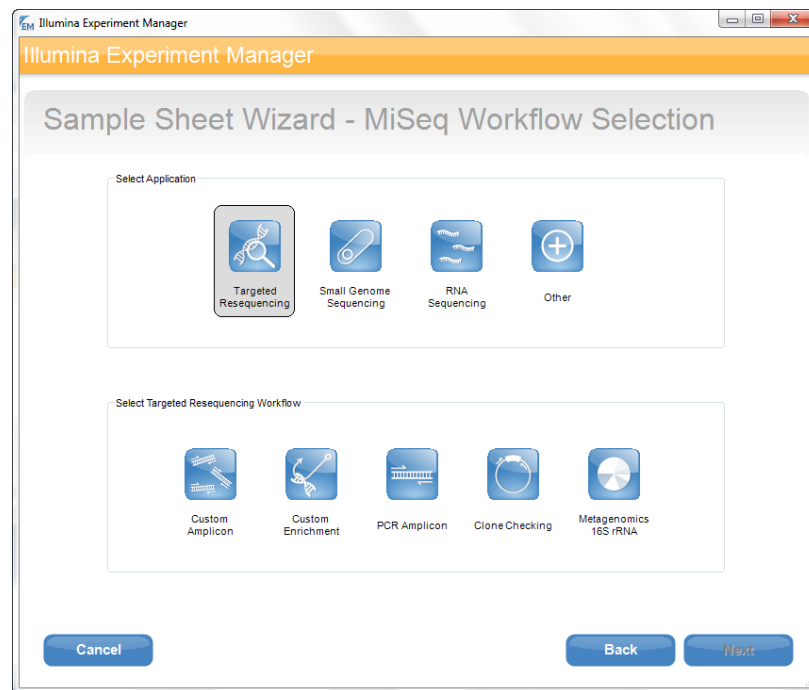
Apply Default Index Layout Save As Default Index Layout Restore Illumina Default Index Layout ?

Cancel Back Finish

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										
Investigator Name	SuperStudent									
Project Name	Training									
Experiment Name	2									
Date	#####									
Workflow	Resequencing									
Assay	Nextera									
Description										
Chemistry	Amplicon									
[Reads]										
	151									
	151									
[Settings]										
OnlyGenerateFASTQ	0									
Adapter	CTGTCTCTTATACATCT									
[Data]										
Sample_ID	Sample_N	Sample_P	Sample_V	Sample_P_index	I7_Index_index2	I5_Index	Descriptive	Genome	Folder	
	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	

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
----------------------	----

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_Prcindex	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	