



Technology Transition Workshop

Laboratory Exercise Overview

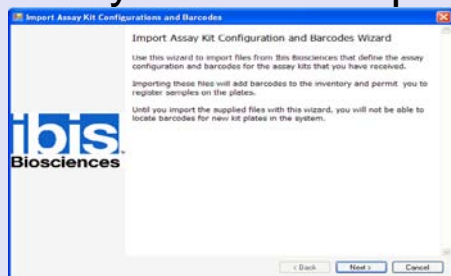
Kristin S. Lowery, Ph.D.

Ibis Biosciences, Inc.

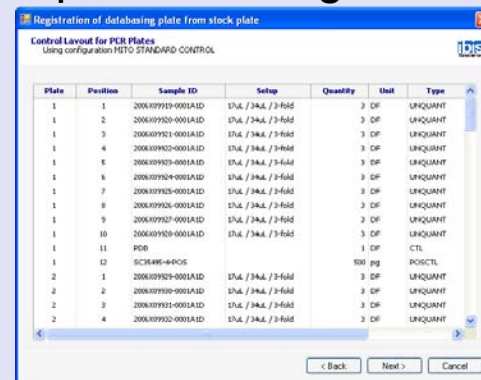
Technology Transition Workshop

Overview of Exercise

Assay Barcode Import



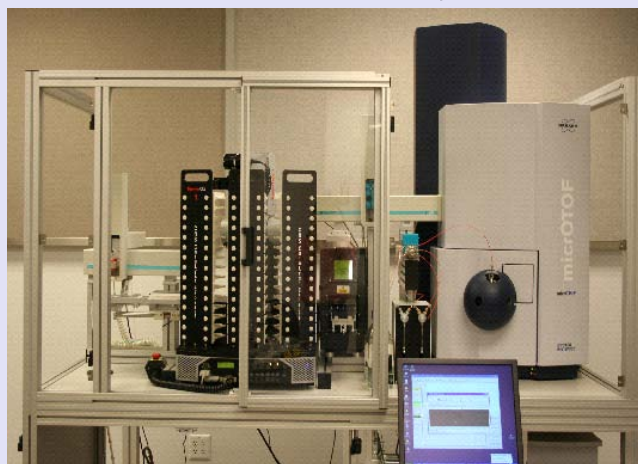
Experiment Registration



Thermocycling



Ibis T5000™



Robotic Setup





Goal of Exercise

- Import wizard example
- Practice using registration wizards
- Practice PCR setup using JANUS®
- Practice setting up and running Ibis T5000™



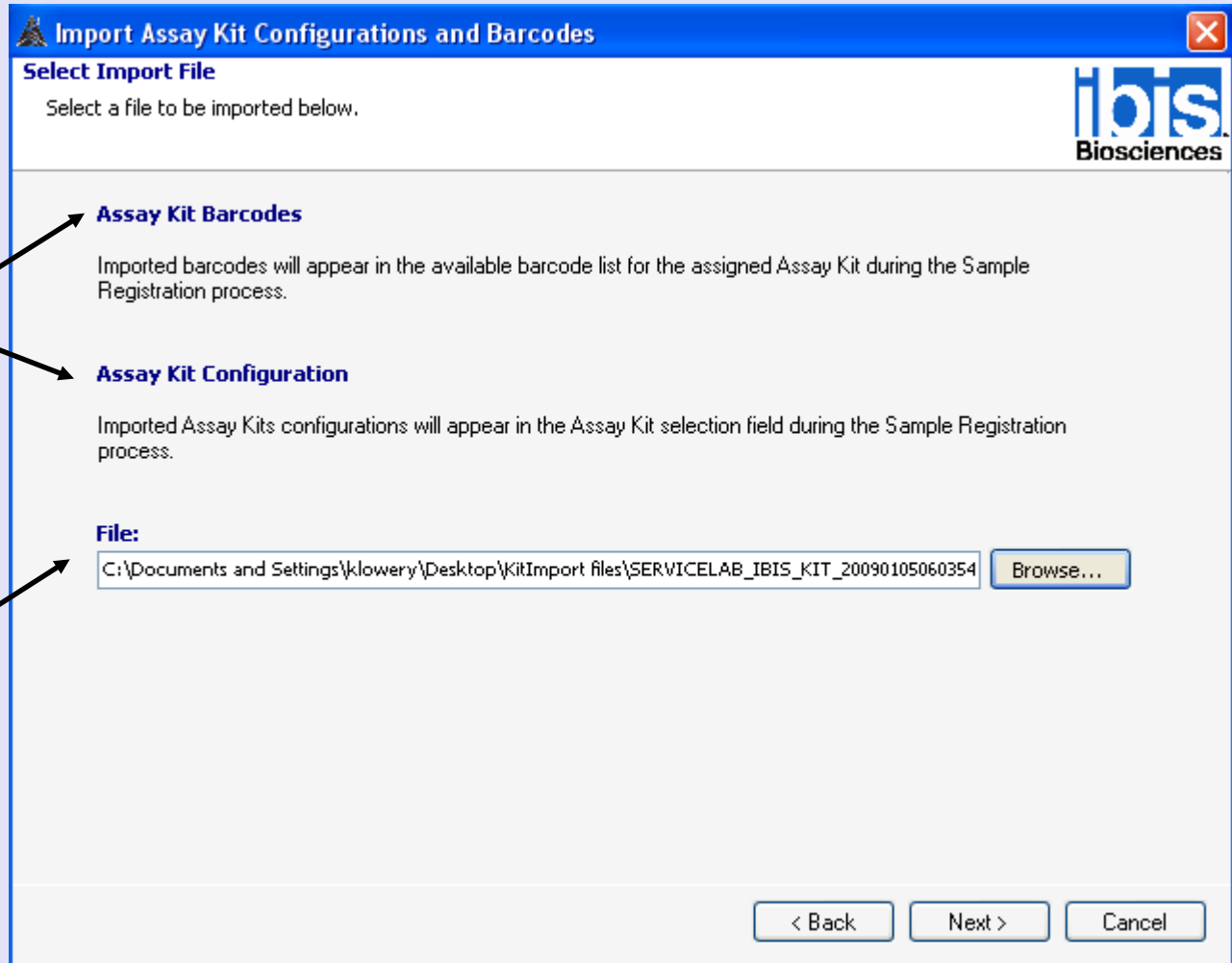
Goal of Exercise

- Two types of plates will be made
 - Blinded sample plate
 - 10 samples, 1 negative control, and 1 positive control
 - Goal – Correctly identify sample by searching database
 - Known sample plate
 - 10 samples, 1 negative control, and 1 positive control
 - Goal – Compare your profile to previously registered profile for the sample

Import Wizard Example

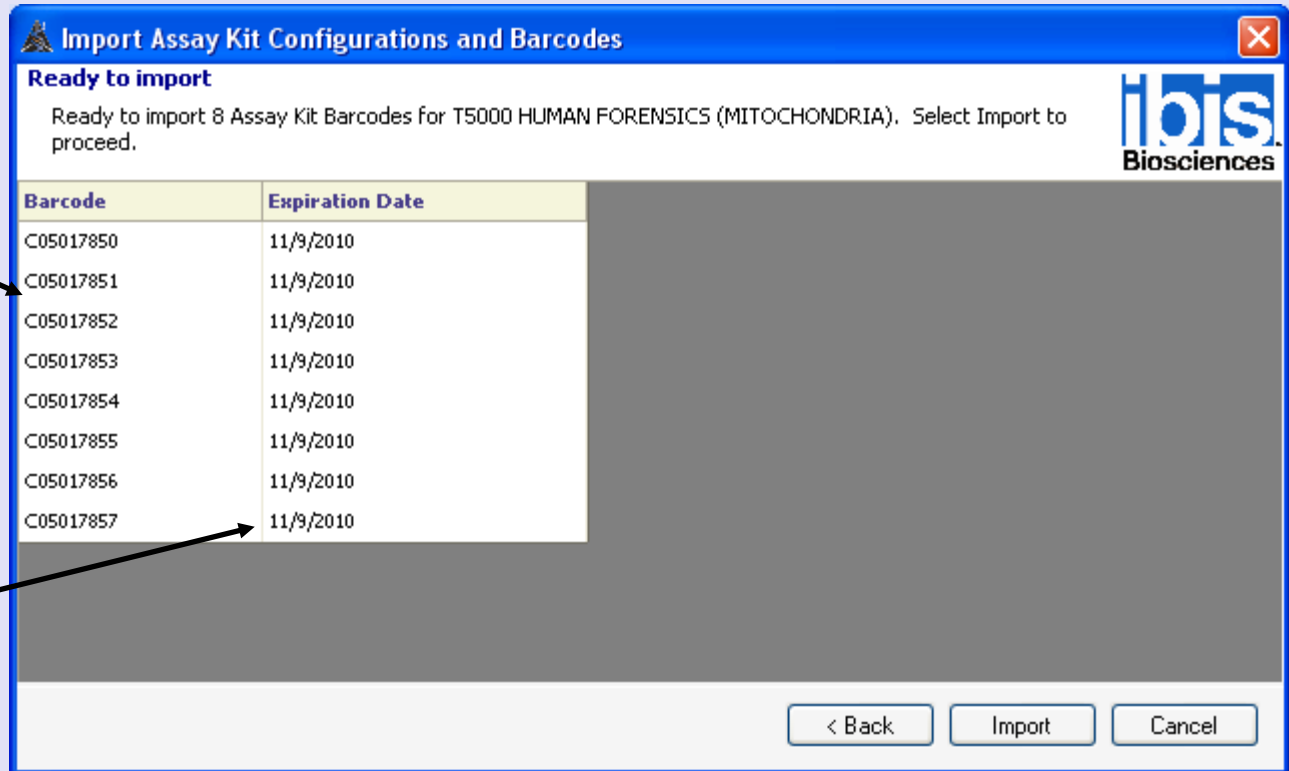
Use to import
barcodes or
assay plans

Type of
import
selected
based on file



Import Wizard Example

Barcodes to
be imported



Expiration
date

- Click **Import** button
- “Importing has completed” message will display at bottom
- Click **Finish** button to close wizard



Assay Registration

- Register 10 samples and controls to assay plate
- Data analysis requires a positive control and a negative control be registered to a plate
- Show example with Casework wizard
 - Setup for 10 samples from tubes

Casework Wizard

Register a New Experiment

Assay Kit Properties
Assay Kit, Project and Experiment fields below are required.

Select an Assay Kit
CASEWORK FOR PCR BUFFER II TEST

Select a Project
FORENSIC-829

Select an Experiment
Casework registration for training

Inventory
59

Enter a Comment

< Back Next > Cancel

Indicates number of barcodes available

➤ If “0” barcodes in inventory, import necessary assay barcodes before continuing

- Select an Assay Kit from the drop down box
- Select a Project and type in an Experiment title
- Click **Next** button

Register a New Experiment

Select Sample Type
Choose a sample type from the options below.

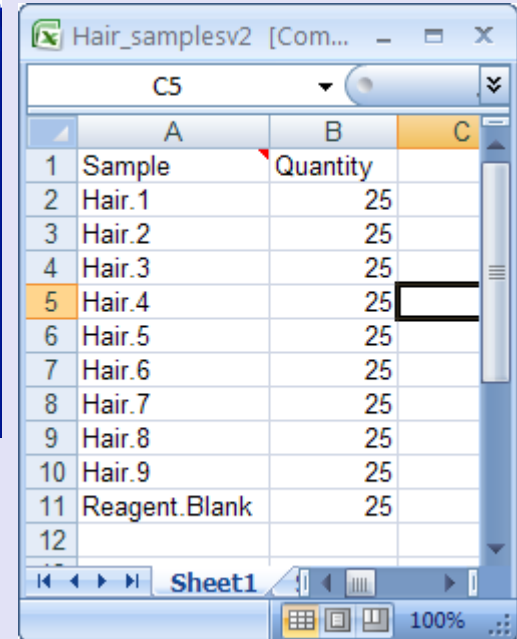
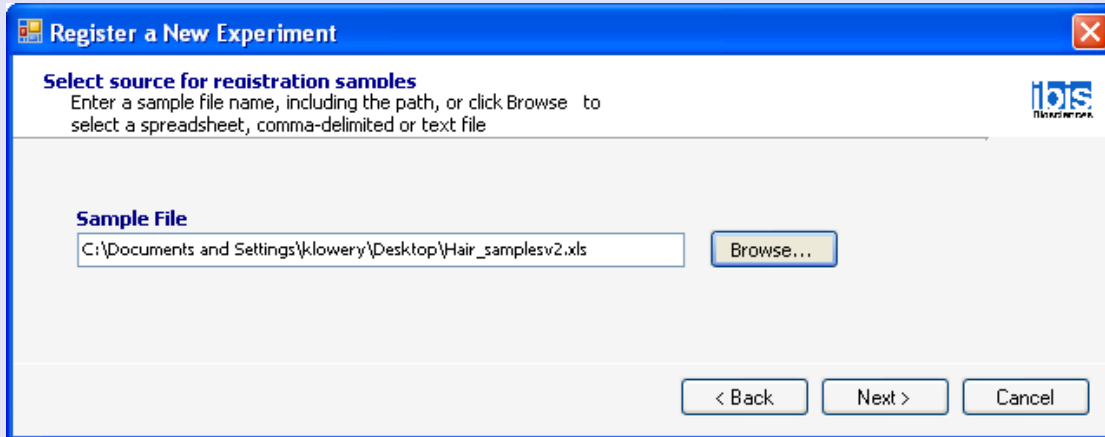
Unquantified
Unquantified samples have not been characterized for the amount of genomic material present. No quantity is expected on input.

Quantified (Mass)
Samples quantified by mass indicate the amount of genomic material in nanograms. The amount is specified in the Quantity field on input and the value supplied is the mass in a single PCR well for the assay plate.

Quantified (Copies)
Samples quantified by copies specify the number of genomes or organisms present in the sample.. The amount is specified in the Quantity field on input and the number represents the copies present in a single PCR well for the assay plate.

< Back Next > Cancel

- Select Sample Type
 - Unquantified (enter in DF for PCR plate)
 - Quantified (enter in picograms or copies for PCR Plate)
- Click **Next** button



	A	B	C
1	Sample	Quantity	
2	Hair.1	25	
3	Hair.2	25	
4	Hair.3	25	
5	Hair.4	25	
6	Hair.5	25	
7	Hair.6	25	
8	Hair.7	25	
9	Hair.8	25	
10	Hair.9	25	
11	Reagent.Blank	25	
12			

- Select file with sample information and click **Next** button
- Sample file is validated
- Click **Next** button

Casework Wizard

Select
control
layout

Automatically
interleaves controls
with samples based
on control layout

Register a New Experiment

Control Layout
Choose a control layout below. The default layout is samples with no controls interleaved.

Control Layout
MITO STANDARD CONTROL

Plate	Position	Sample ID	Quantity	Unit	Type
1	1	HAIR.1	25	DF	Unquantified
1	2	HAIR.2	25	DF	Unquantified
1	3	HAIR.3	25	DF	Unquantified
1	4	HAIR.4	25	DF	Unquantified
1	5	HAIR.5	25	DF	Unquantified
1	6	HAIR.6	25	DF	Unquantified
1	7	HAIR.7	25	DF	Unquantified
1	8	HAIR.8	25	DF	Unquantified
1	9	HAIR.9	25	DF	Unquantified
1	10	REAGENT.BLANK	25	DF	Unquantified
1	11	PDB	1	DF	CTL
1	12	SC35495-4-POS	500	pg	POSTL

< Back Next > Cancel

- Select Control Layout – predefined in a previous step
- Click **Next** button

Number of
barcodes
required

Register a New Experiment

Selecting PCR Plate Bar Codes
For assay kit CASEWORK FOR PCR BUFFER II TEST

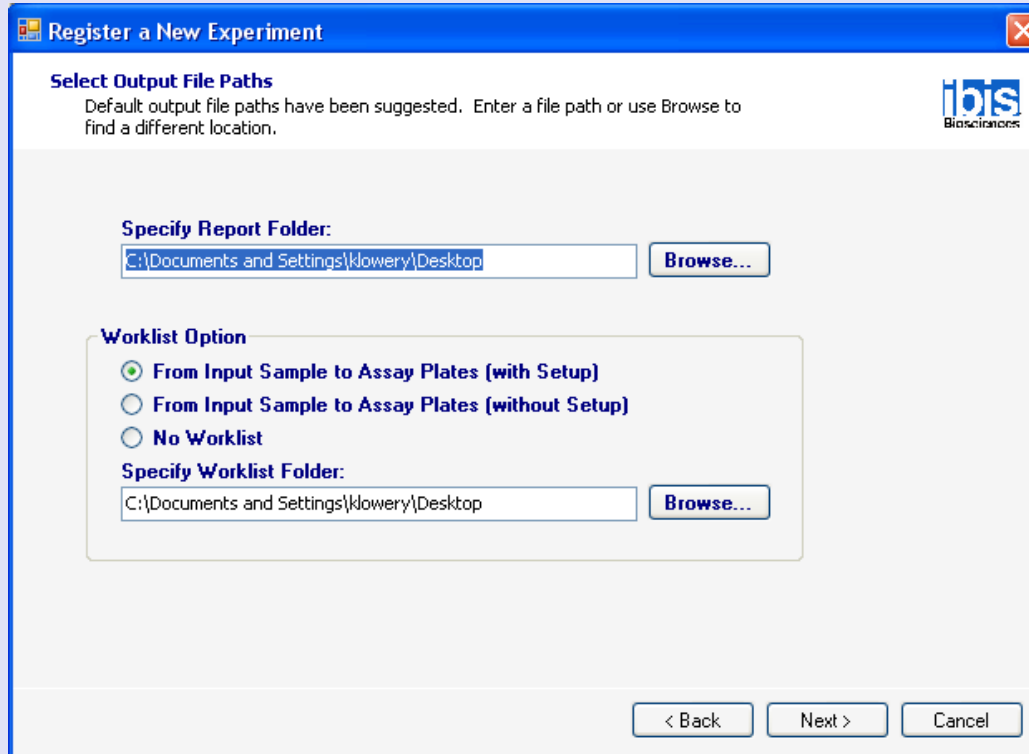
MITOTILING - PCR BUFFER II TEST → **1 PCR bar code required**

Available	
BarCode	
C00016820	
C00016821	
C00016822	
C00016823	
C00016824	
C00016825	
C00016826	
C00016827	
C00016828	
C00016829	
C00016830	
.....	

Assigned	
Plate	BarCode
1	C00016819

< Back Next > Cancel

- Highlight barcodes and click **Left Arrow** button
- Click **Next** button



Register a New Experiment

Select Output File Paths
Default output file paths have been suggested. Enter a file path or use Browse to find a different location.

Specify Report Folder:
C:\Documents and Settings\klowery\Desktop **Browse...**

Worklist Option

From Input Sample to Assay Plates (with Setup)
 From Input Sample to Assay Plates (without Setup)
 No Worklist

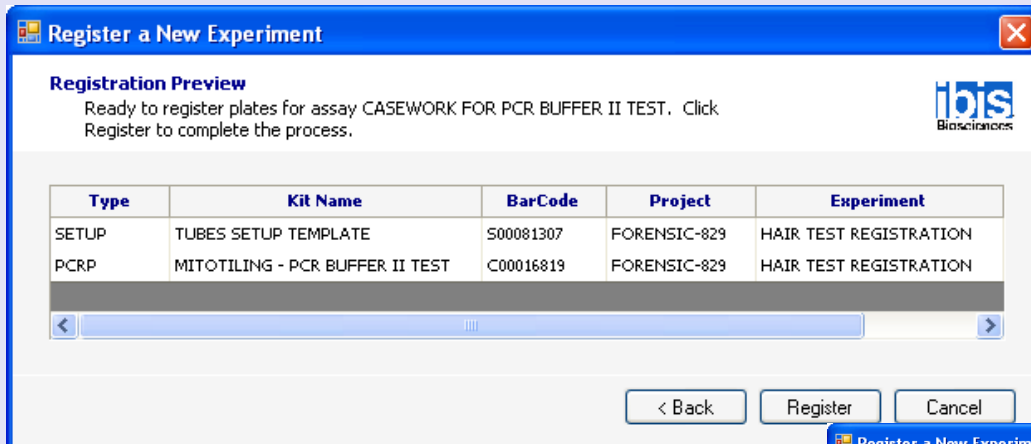
Specify Worklist Folder:
C:\Documents and Settings\klowery\Desktop **Browse...**

< Back Next > Cancel

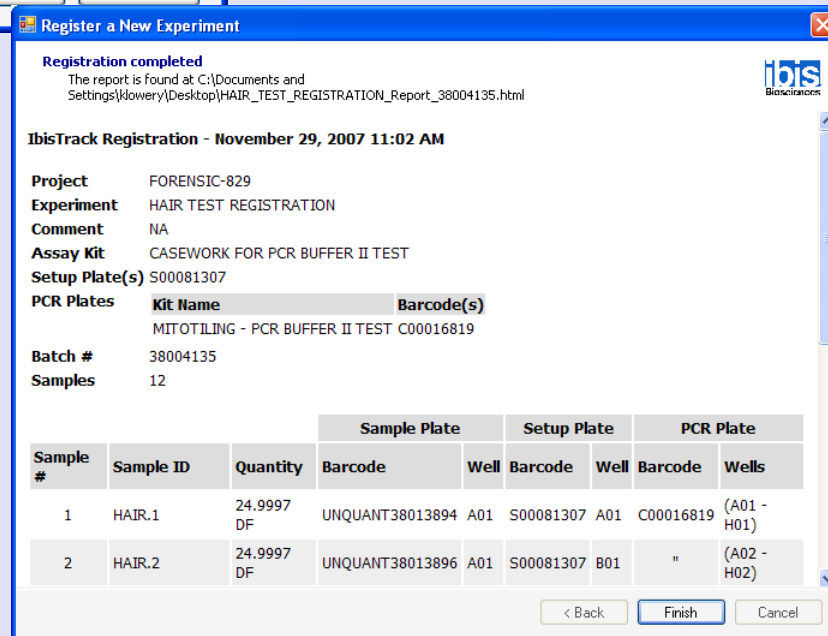
- Select a folder for the Report and Worklist
- Select Worklist option
 - If using the JANUS[®], select the first option
- Click **Next** button

NIJ National Institute of Justice *Technology Transition Workshop*

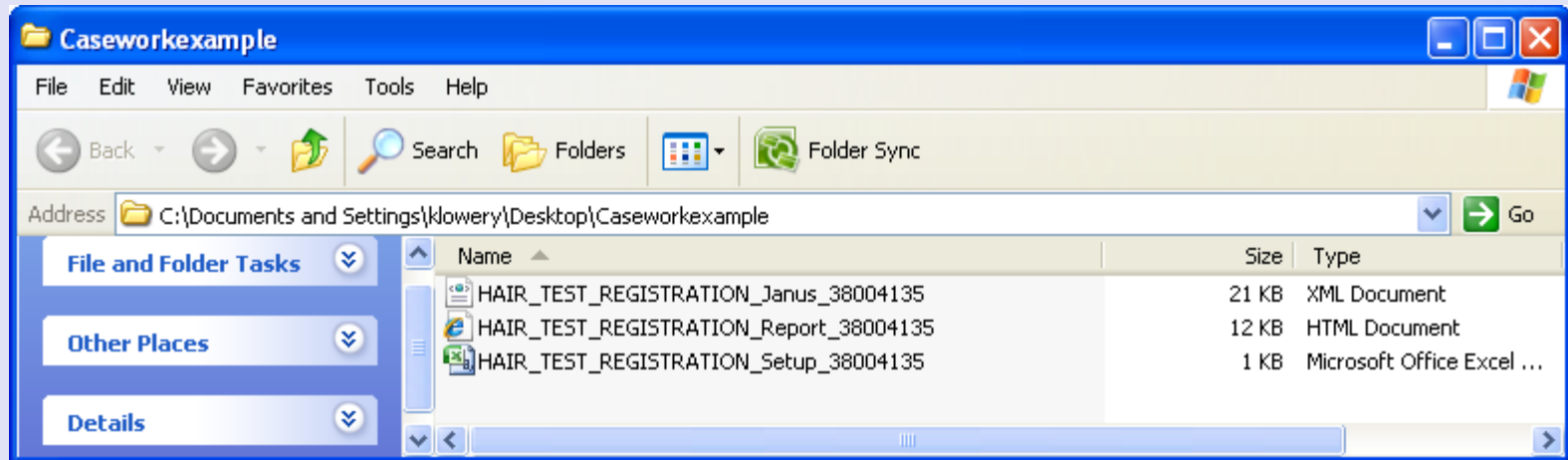
Casework Wizard



- Click **Register** button
- Report generated with sample layout
- Go to file location to print out report and worklists



Casework Wizard



- The Janus file (.xml) needs to be copied to the JANUS[®] computer
- The Report file (.html) details sample layout as well as Setup and PCR barcodes required
- The Setup file (.xls) shows how to place samples in the tube rack for use with the JANUS[®]

Contamination Control

- Gowning procedure
 - Bouffant, disposable lab coat, booties, face mask, and gloves
- Decontamination of PCR hood, pipettes, and consumables
 - Spray work surface with bleach and let set for ~ 5-10 minutes before wipe down
 - Wipe down work surfaces with DNA-Erase™
 - Wipe down pipettes with DNA-Erase™
 - Place new consumables in hood – tips, tubes, reservoirs, etc.
 - Turn on UV light for 15 minutes



PCR Setup

- 5 μL of sample is added per well
 - A minimum of 50 μL is needed for each sample
 - 40 μL required for sample + 10 μL for waste
 - Use a new tip each time
- Sample dilution is performed in PCR hood
 - For quantitated sample, dilute to 500 pg/5 μL with PDB or DNA-free water
 - For non-quantitated sample, dilute to set volume
 - For example, 17 μL stock + 34 μL of PDB



PCR Setup

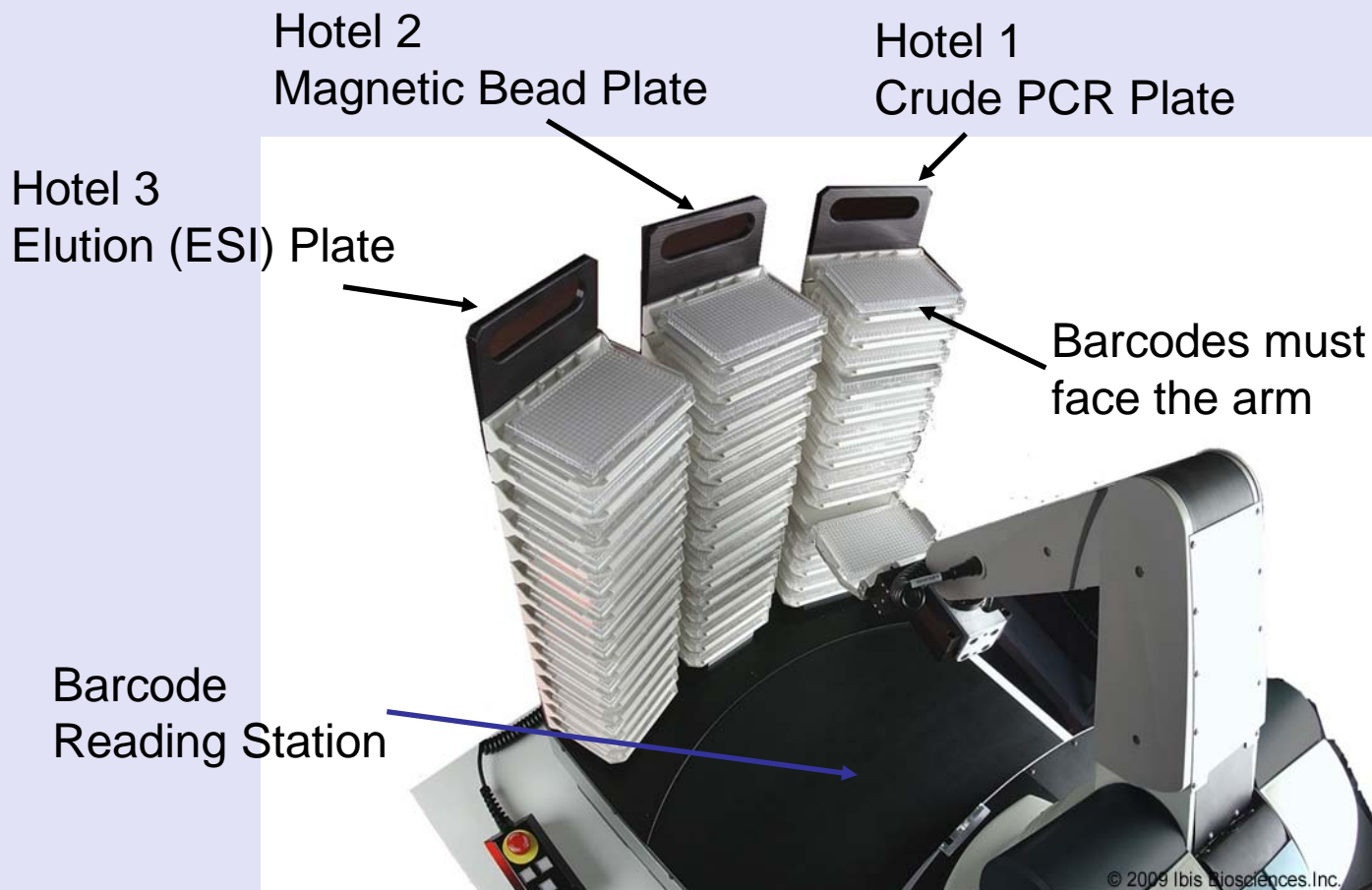
- After all samples have been added, seal the plate with a Super Pierce heat seal
- Vortex plate for 30-60 sec.
- Centrifuge the plate for ~15 sec at 2500 rpm
- Plate is ready to be thermocycled



Thermocycling

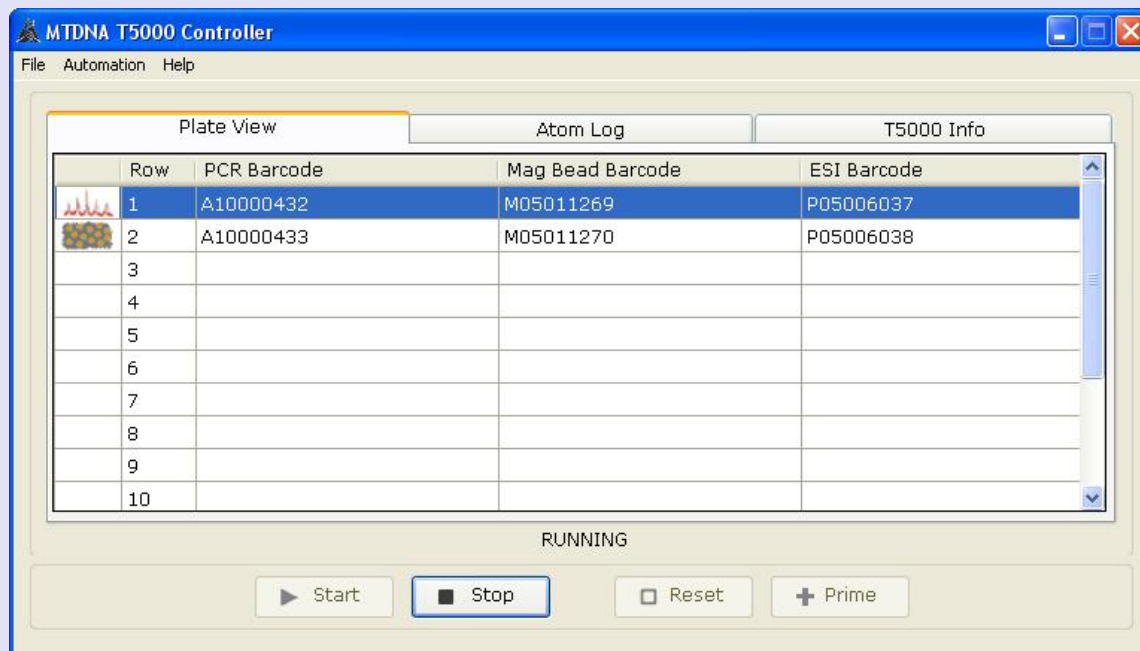
- Eppendorf® thermal cyclers are required
 - Assay validated on these thermocyclers
 - Assay plates fit block
- Thermocycler program
 - 3:16:15 total time
 - 36 cycles
 - Includes a heat kill step to minimize enzyme activity
- After thermocycling, centrifuge the plate for ~15 sec at ~ 800 rpm
- Plate may be frozen until put on Ibis T5000™

Ibis T5000™ system



Ibis T5000™ system

- Fill reagents and empty waste as necessary
- Start T5000 controller software
- Prime system





Ibis T5000™ system

- Select the 'Start' button
- Enter the number of plates to be run
- Allow System QC to be performed
 - This will spray Clean-up Reagent 3 into the mass spectrometer to ensure that signal is being acquired



Schedule for Tuesday

- Divide into 2 groups
- Morning
 - Group 1 – Lab exercise
 - Group 2 – Data analysis practice
- Afternoon
 - Group 1 – Data analysis practice
 - Group 2 – Lab exercise
- Setup Ibis T5000™ as group



Schedule for Tuesday

- Register 20 samples in pairs
 - Use premade sample list

- PCR plate setup
 - Setup blinded or known samples with JANUS[®] in pairs

- PCR hood and supplies will already be decontaminated



Technology Transition Workshop

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