Laboratory Exercise Overview

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Ibis Biosciences, Inc.

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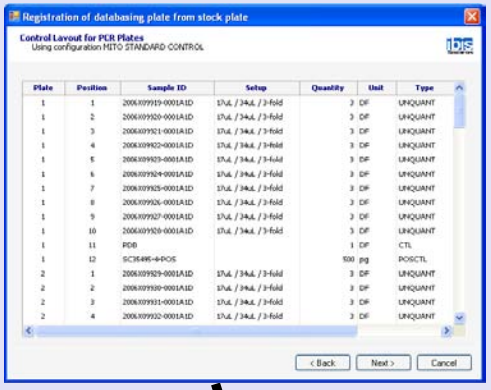
Overview of Exercise

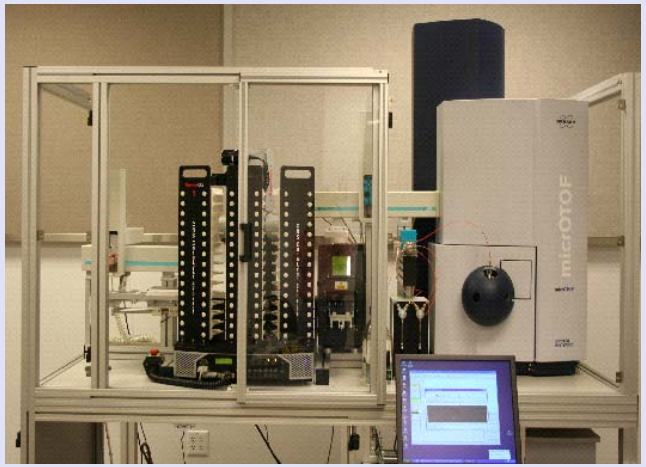
Assay Barcode Import

Experiment Registration



Thermocycling

Ibis T5000™



Robotic Setup

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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

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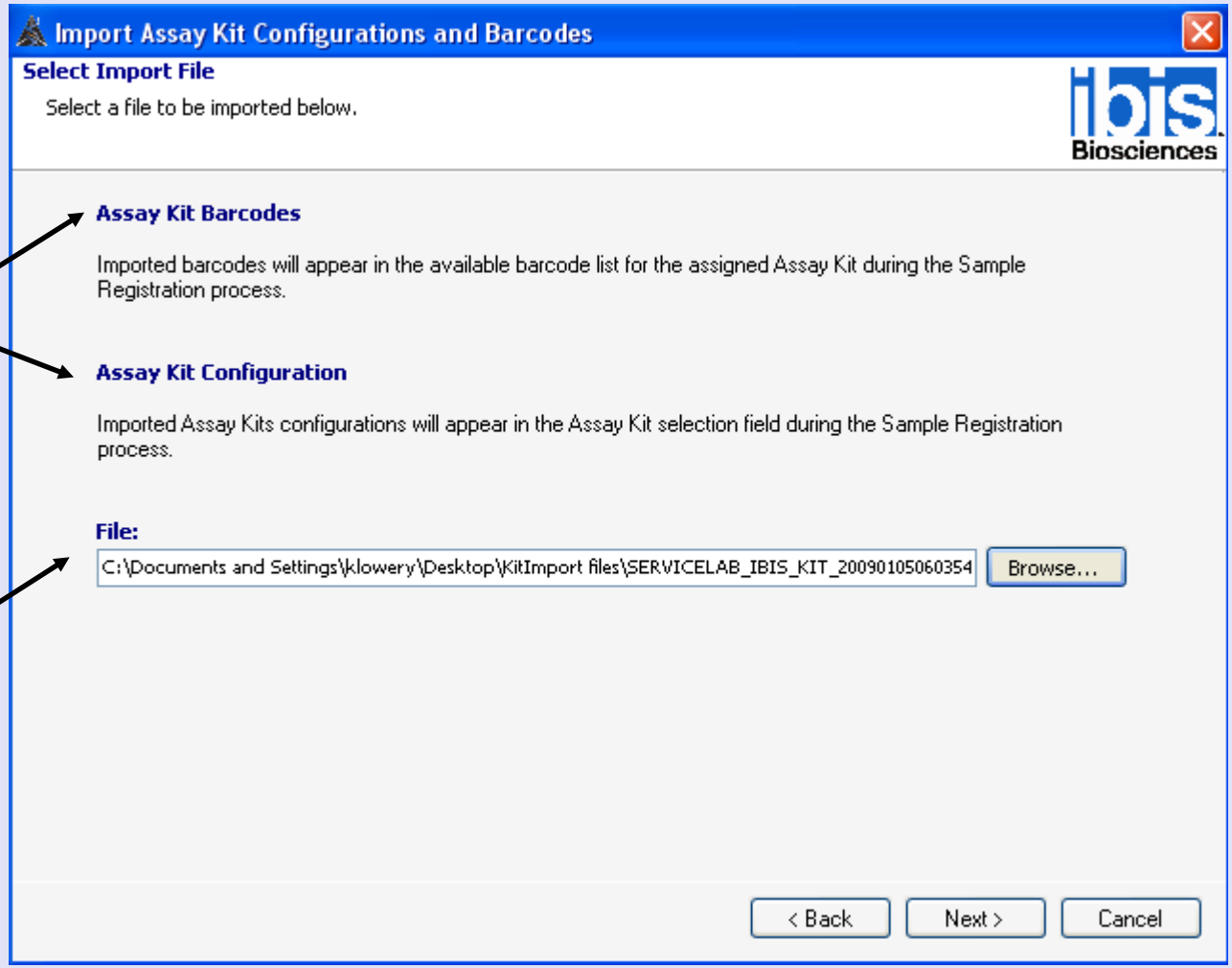
Import Wizard Example

Use to import barcodes or assay plans

Type of import selected based on file



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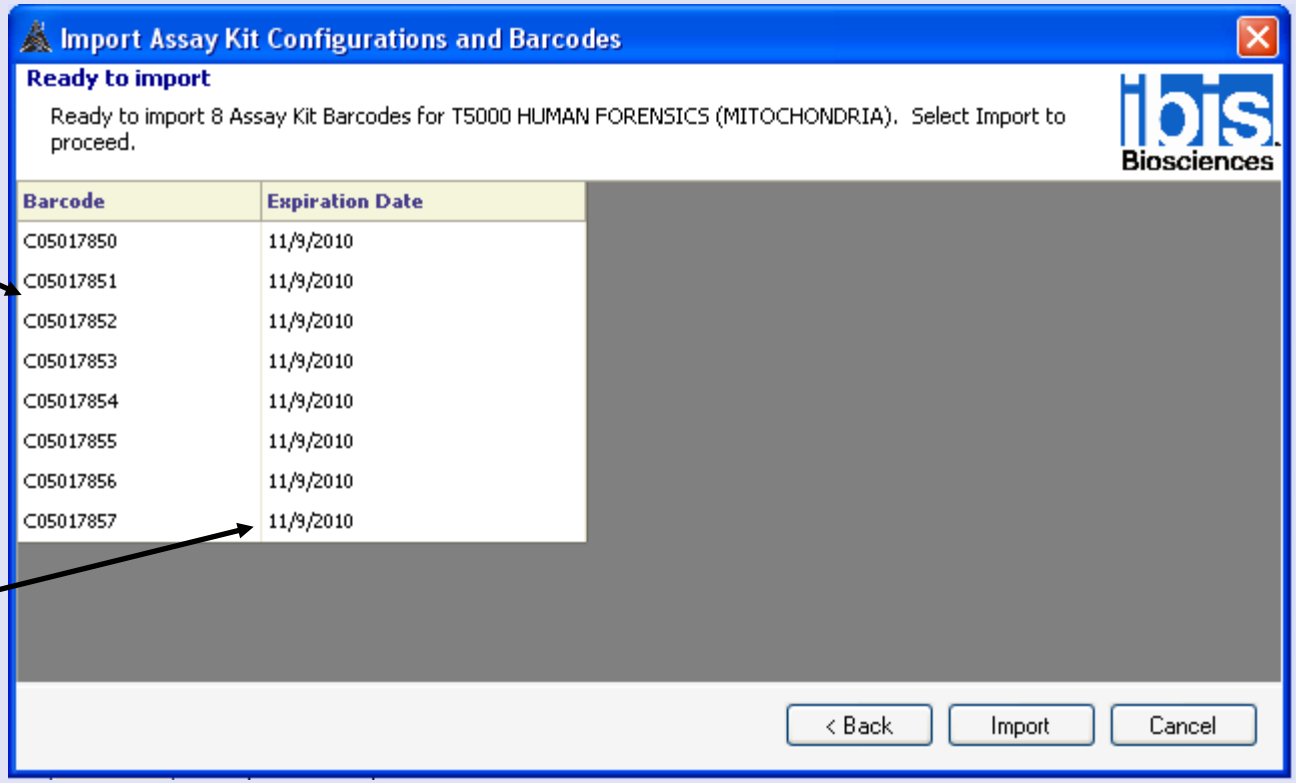
Import Wizard Example

Barcodes to be imported

Expiration date



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

IMAGE COURTESY OF KRISTIN S. LOWERY, PH.D.

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

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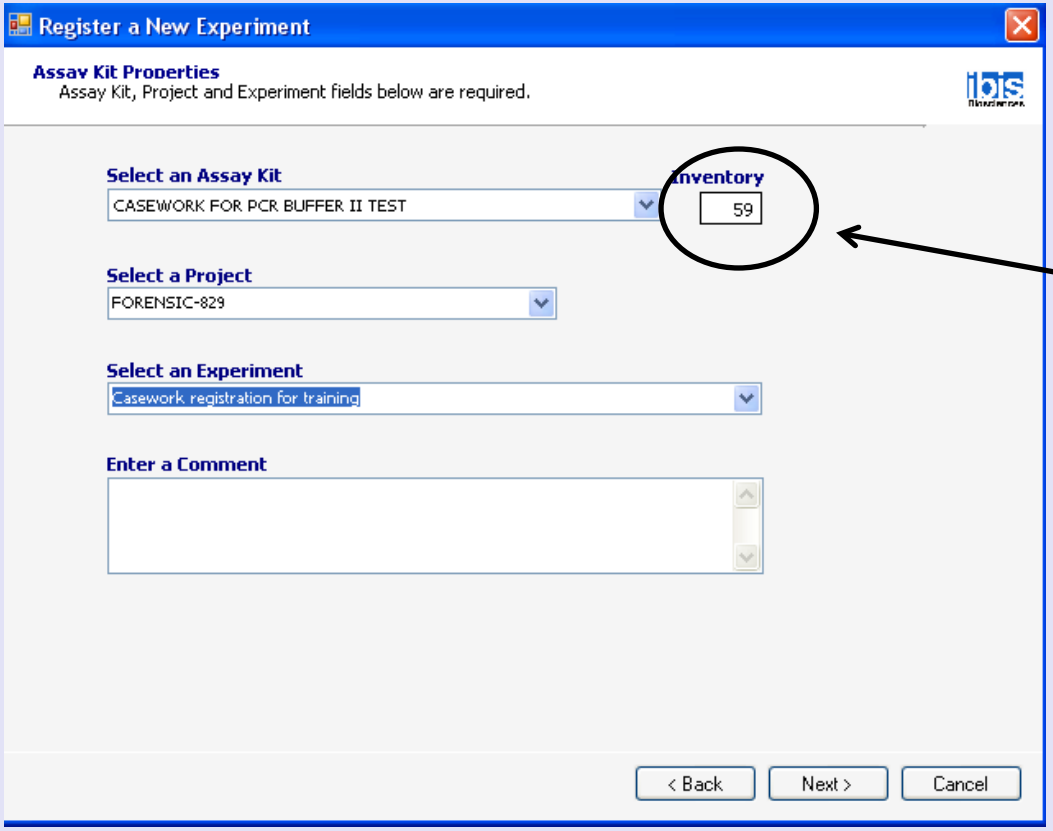
Casework Wizard



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

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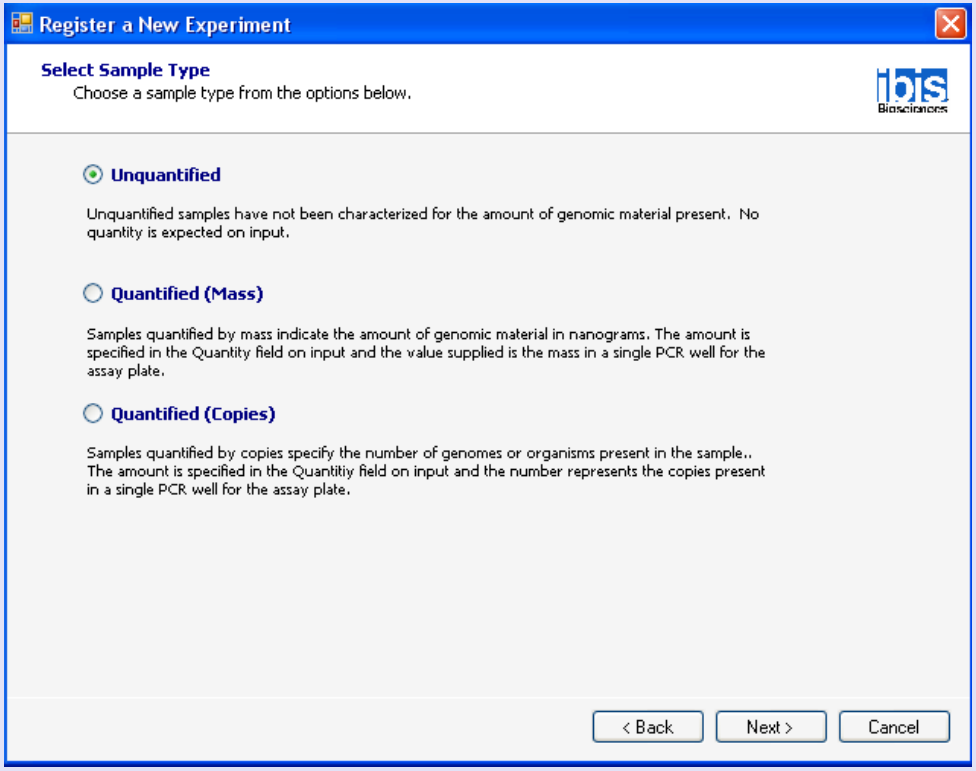
Casework Wizard



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

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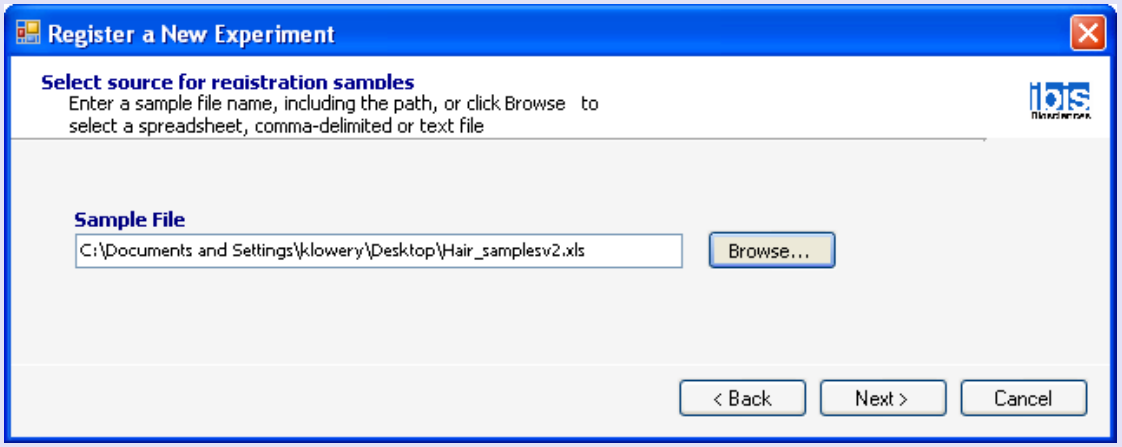
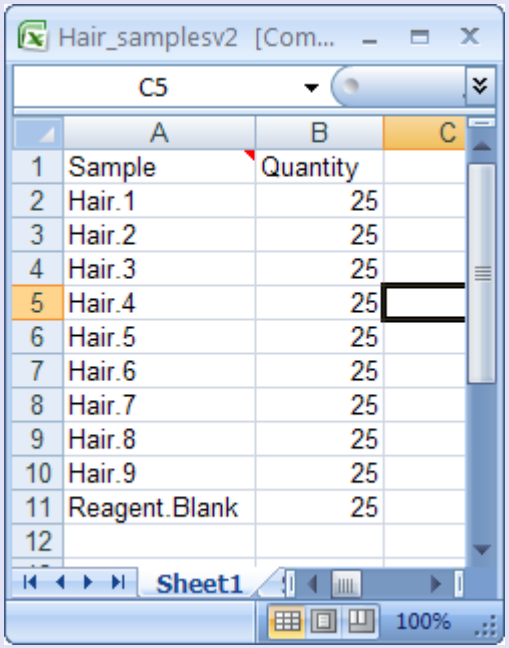
Casework Wizard





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

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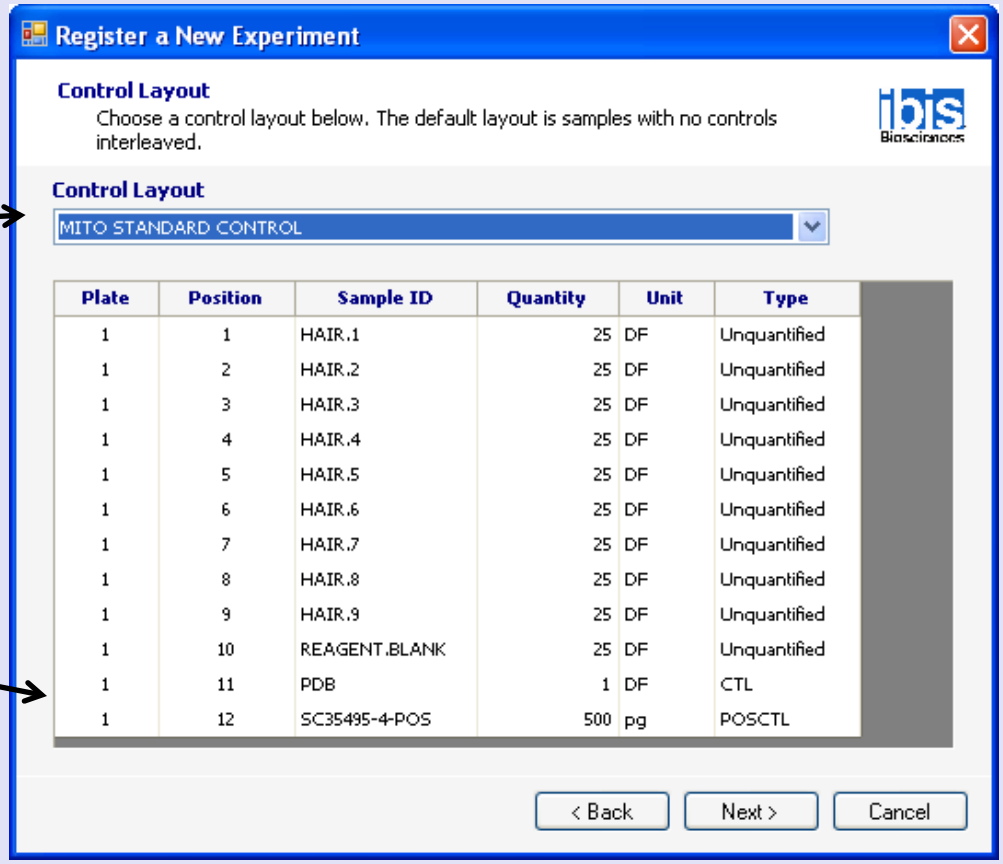
Casework Wizard

Select control layout

Automatically interleaves controls with samples based on control layout



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

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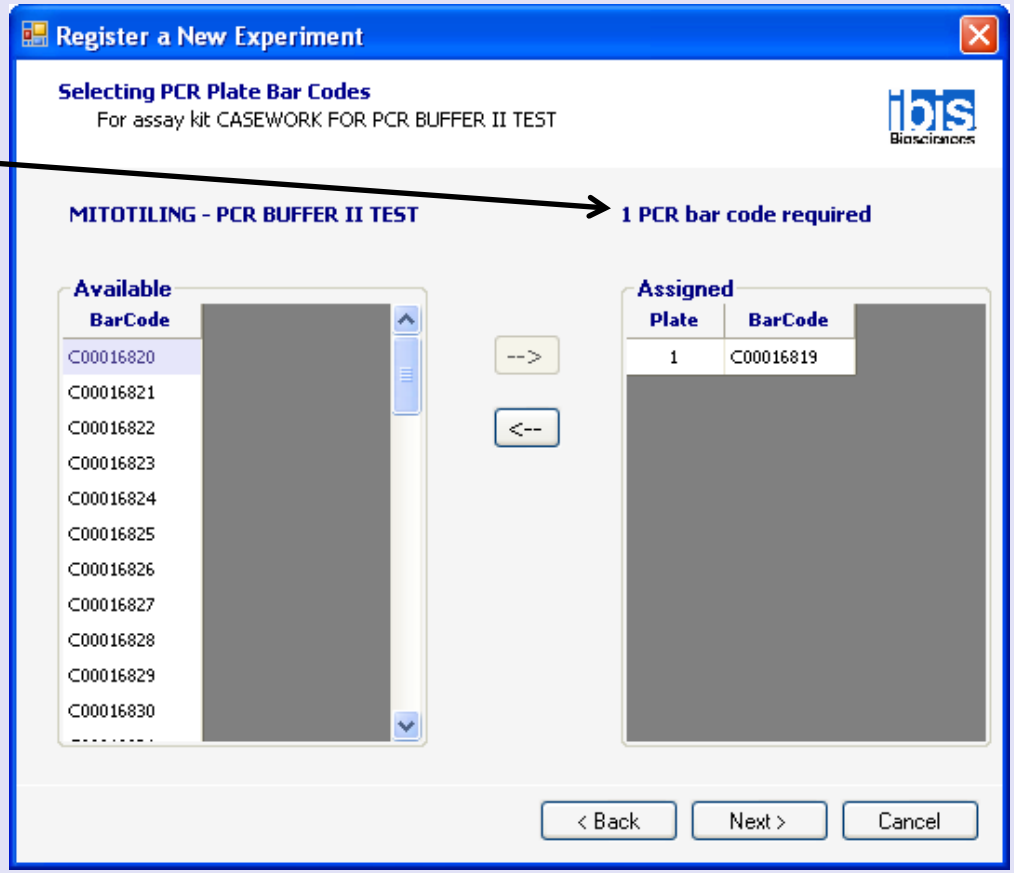
Casework Wizard

Number of barcodes required



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

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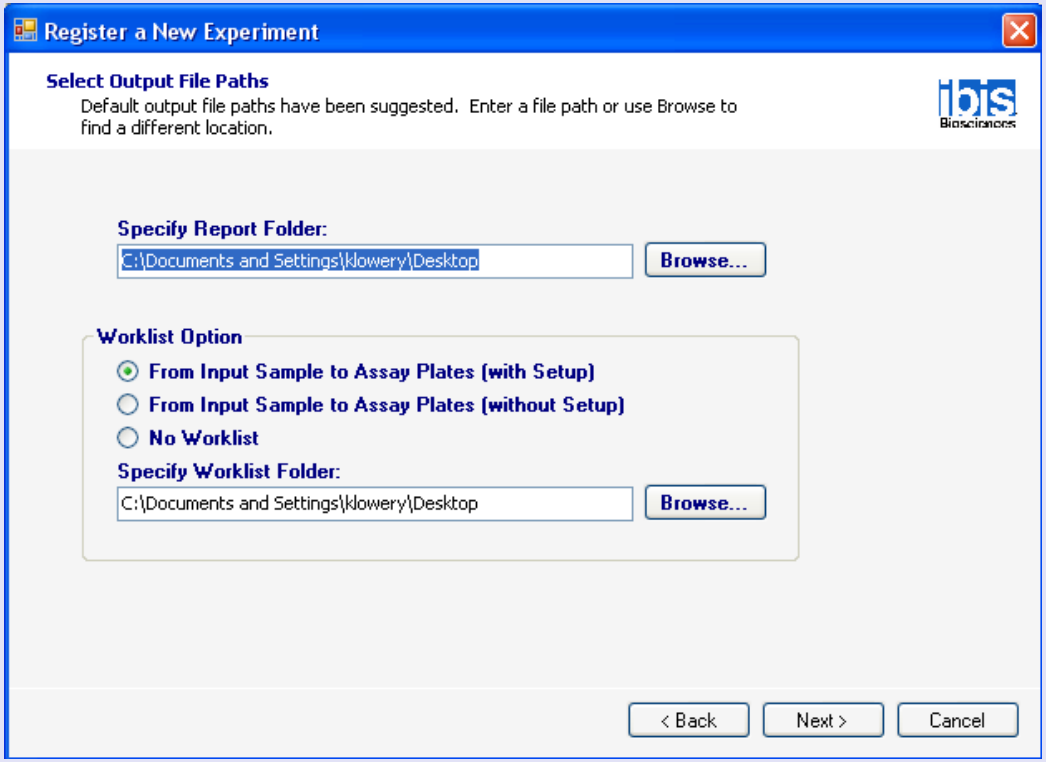
Casework Wizard



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

IMAGE COURTESY OF KRISTIN S. LOWERY, PH.D.

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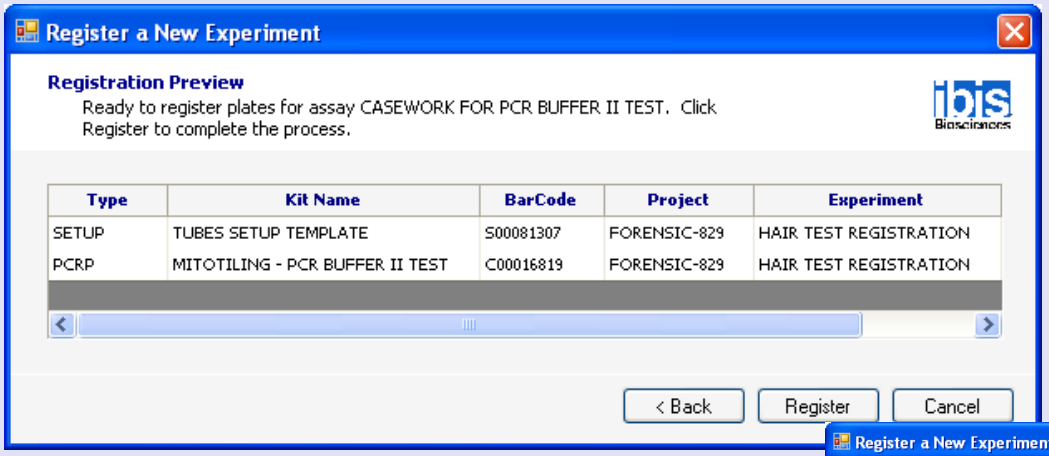
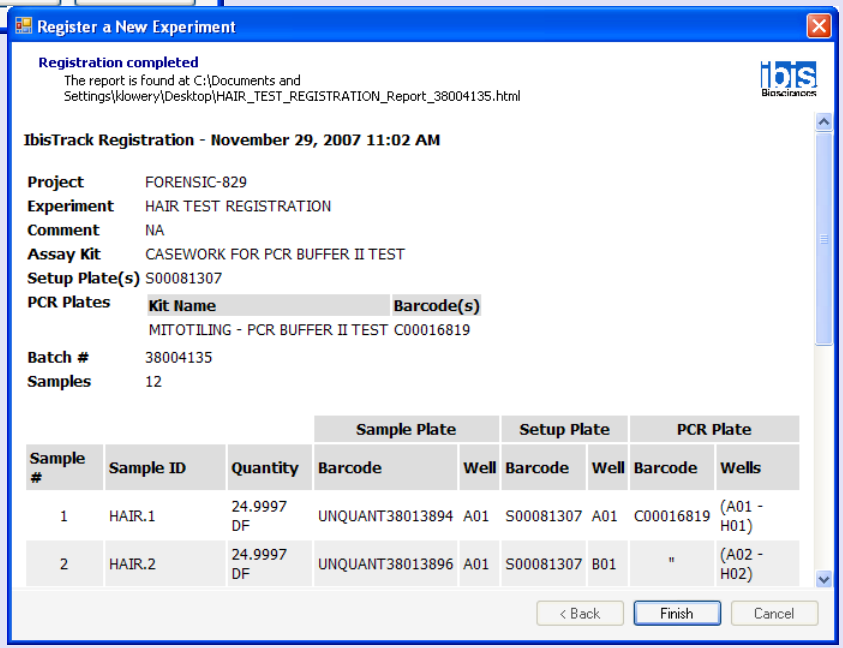
Casework Wizard





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

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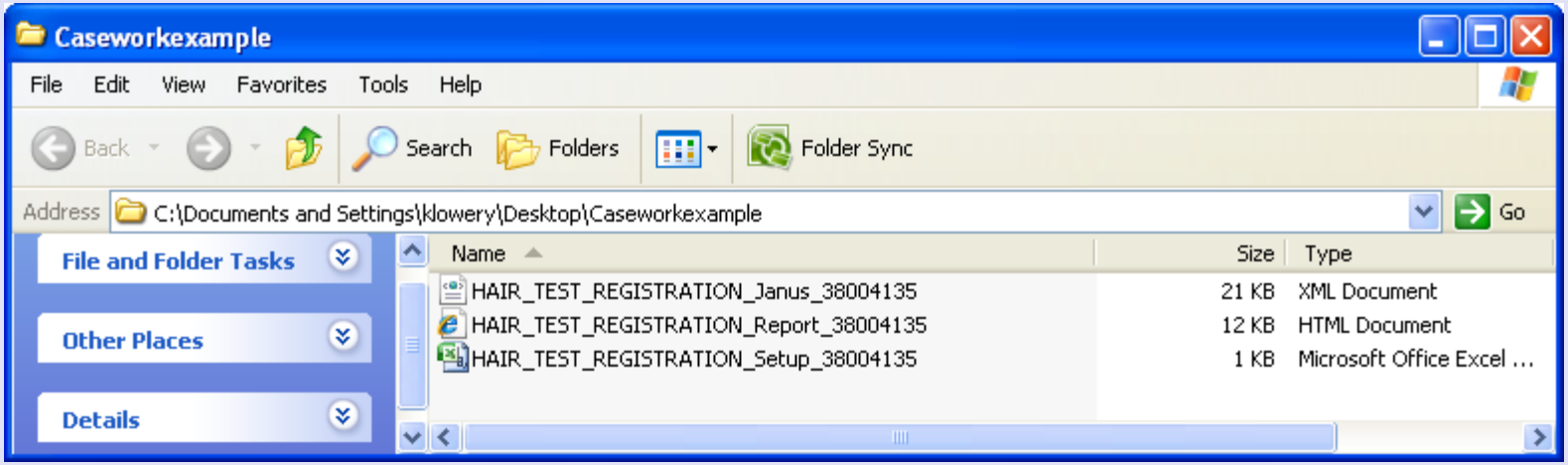
Casework Wizard



- 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®

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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™

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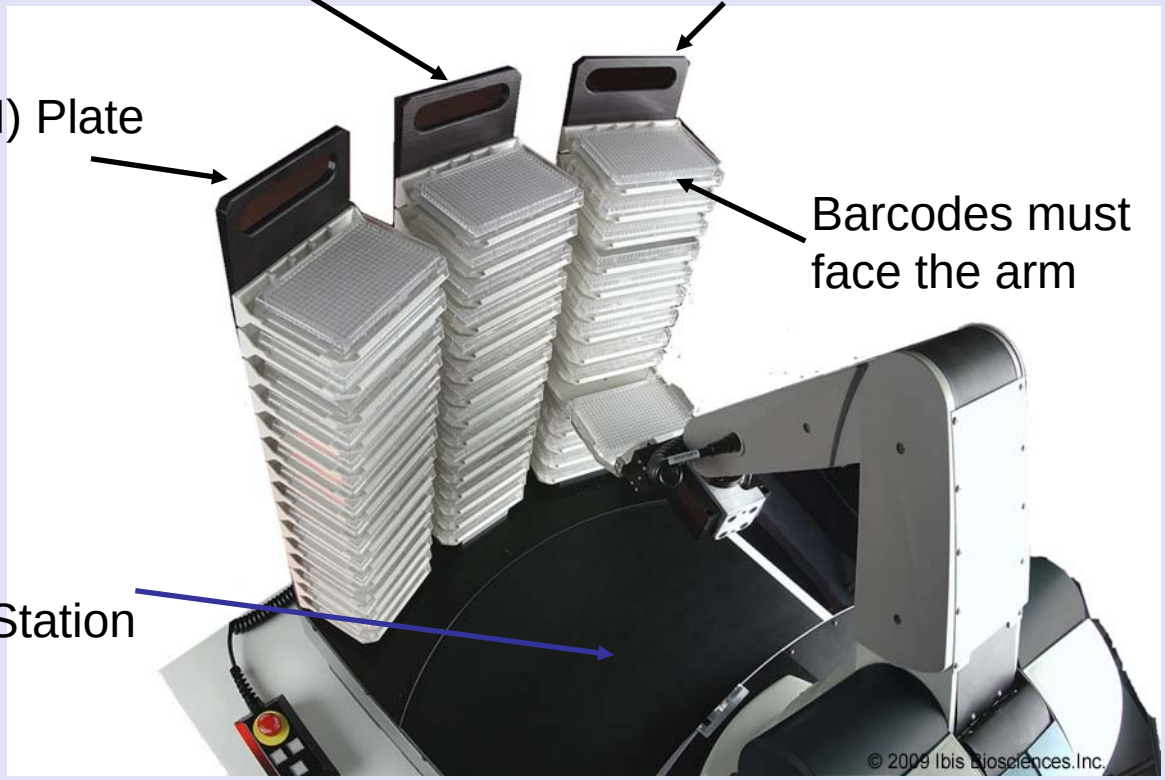
Ibis T5000™ system



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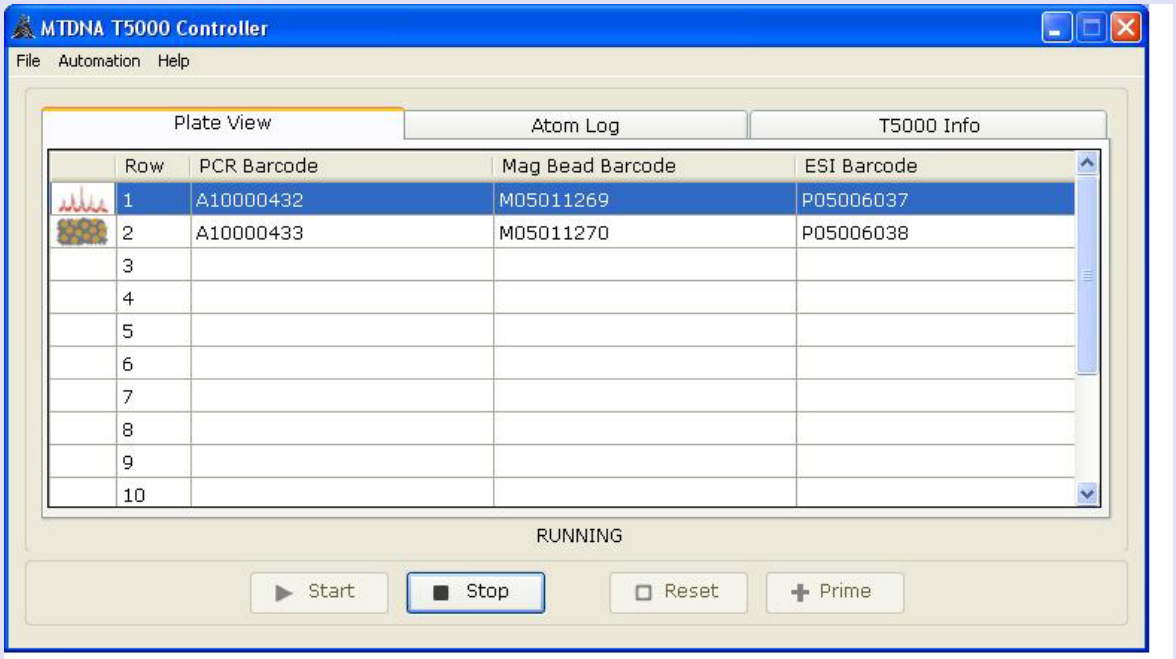
Ibis T5000™ system

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



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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

Contact Information:

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