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Technology Transition Workshop| *Kristin S. Lowery, Ph.D.*

Ibis™ Assay Workflow Overview

Ibis™ Assay Workflow

1. **Receipt and storage of assay kit components.**
2. **Perform up-front sample processing.**
3. **Register the experiment.**
4. **Set up PCR plate(s).**
5. **Seal the PCR plates.**
6. **Thermocycle PCR plates.**
7. **Prepare reagents for PLEX-ID™.**
8. **Fill reagents on PLEX-ID™ and empty waste.**
9. **Analyze the PCR plates on the PLEX-ID™.**
10. **Review data.**

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Ibis™ Assay Workflow – Step 1

- **Upon receipt, check for assay components**
  - **10 barcoded assay plates**
  - **1 bottle of magnetic beads**
  - **1 reservoir for magnetic beads**
  - **3 cleanup reagents (CR1, CR2 and CR3)**
  - **Instructions sheet**

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Ibis™ Assay Workflow – Step 1

- **Storage: assay plates**
  - **Contain 35 μL per well of PCR master mix**
    - **Primer pairs, modified dNTPs, PCR buffer, and enzyme**
  - **Each column is a separate sample**
    - **Eight wells comprised of three primer pairs per well**
    - **Profile is composite of all eight wells**
  - **User is required to add 5 μL of template to each well of a sample**
  - **Store at -20°C – manual defrost freezer**

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Ibis™ Assay Workflow – Step 1

- **Storage: magnetic bead bottle**
  - **Store at 4° C and in upright position**
  - **DO NOT FREEZE**
- **Storage: magnetic bead reservoir**
  - **Store at room temperature**
- **Storage: cleanup reagents**
  - **Store at 4° C**

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Ibis™ Assay Workflow – Step 2

- **Up-front sample processing**
- **Extract DNA from samples (your choice of method)**
  - **Qiagen® columns**
  - **KingFisher® magnetic bead systems**
  - **Phenol/chloroform**
  - **Others**

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Ibis™ Assay Workflow – Step 3

- **Register experiment**
  - **Import PCR barcodes**
    - **Use import wizard**
    - **Barcode file sent on CD with shipment**

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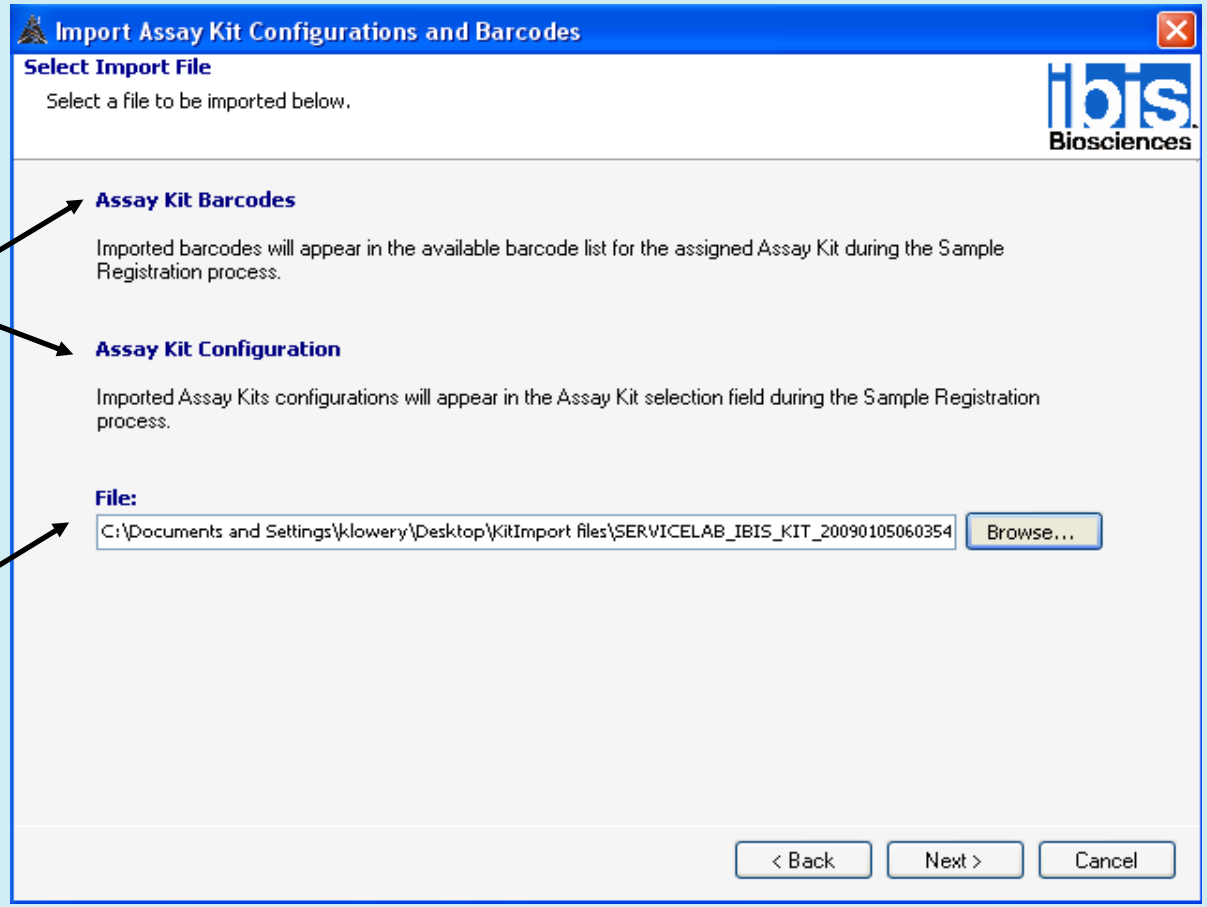
Import Wizard Example

Use to import barcodes or assay plans

Type of import selected based on file



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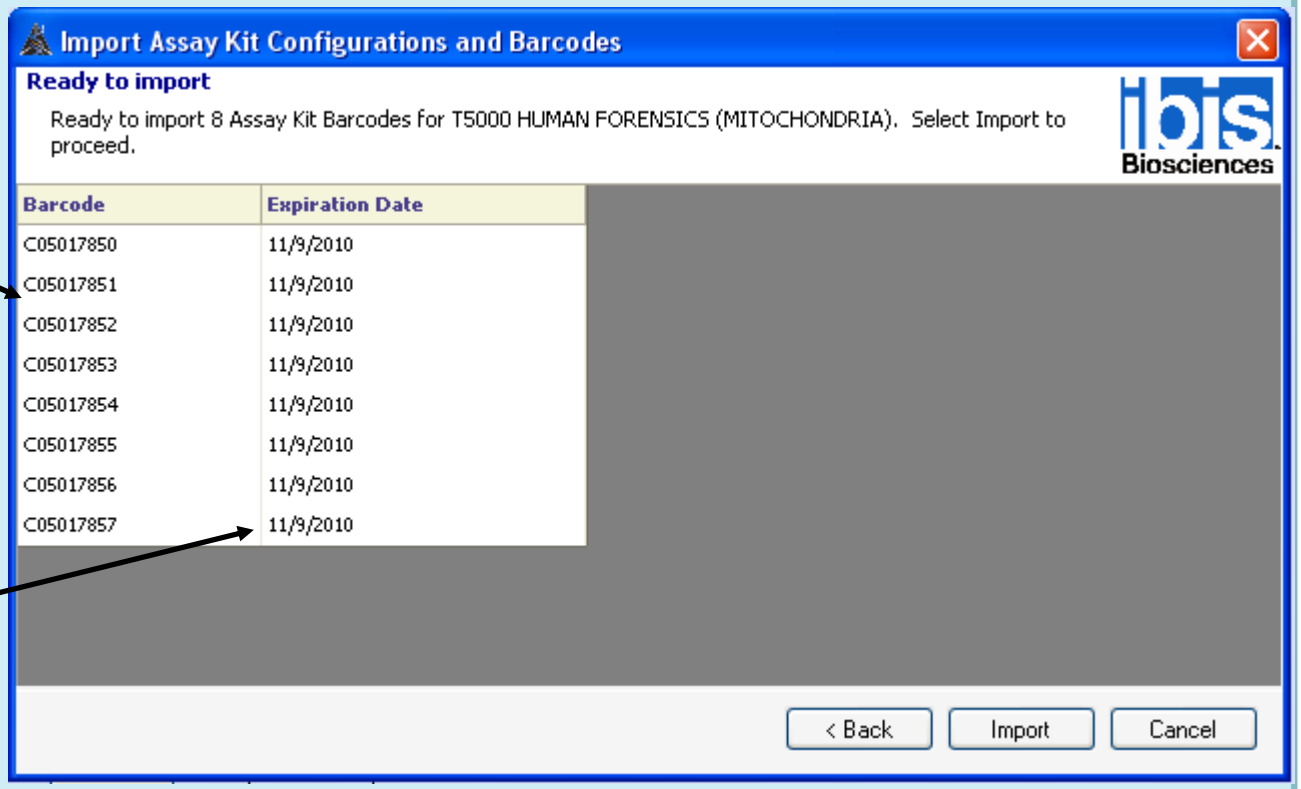
Import Wizard Example

Barcodes to be imported

Expiration date



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

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Ibis™ Assay Workflow – Step 3

- **Register experiment (continued)**
  - **For data analysis to work correctly, the plate must contain a positive control and a negative control**
    - **10 samples can be run on the plate**
  - **Use different wizards depending on number of samples**
    - **Requires sample list – Microsoft® Excel spreadsheet**
    - **Can use tubes or plates for setup**
  - **Use control layouts**
    - **Define positions for negative and positive PCR controls**
    - **Do not need to put controls in sample list**

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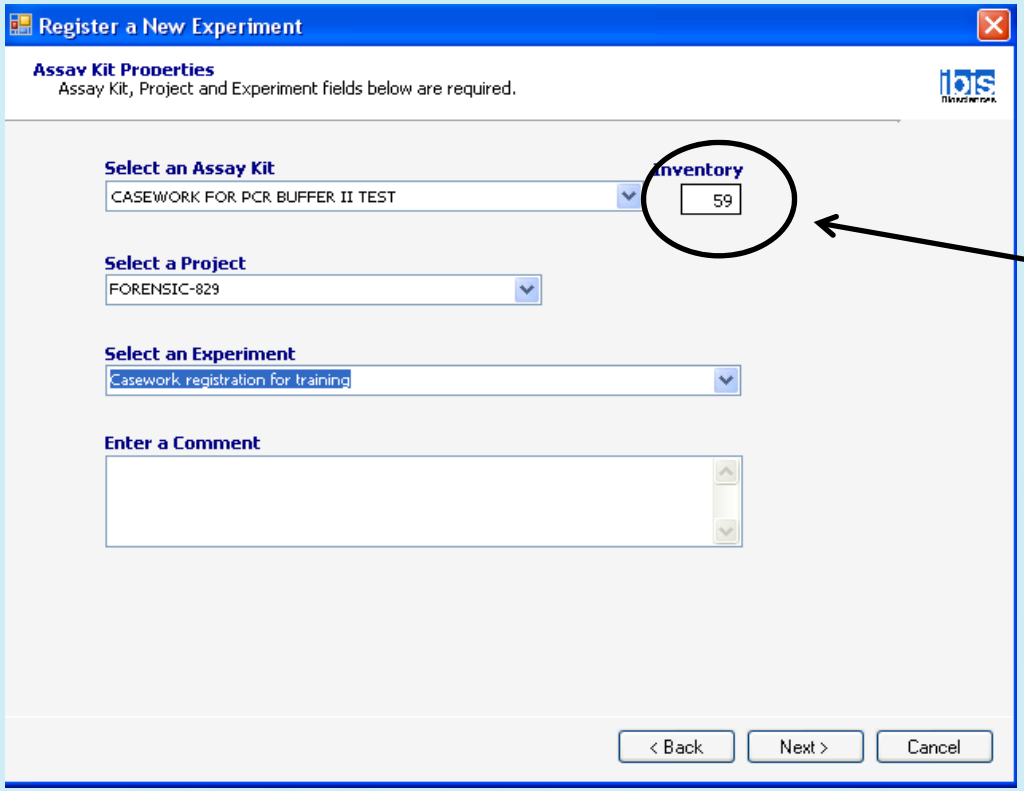
Casework Wizard



Indicates number of barcodes available

- **If "0" barcodes in inventory, import necessary assay barcodes before continuing**

- Select an assay kit from the list
- Select a project from the list
- Select or enter an experiment title
- Click **Next**

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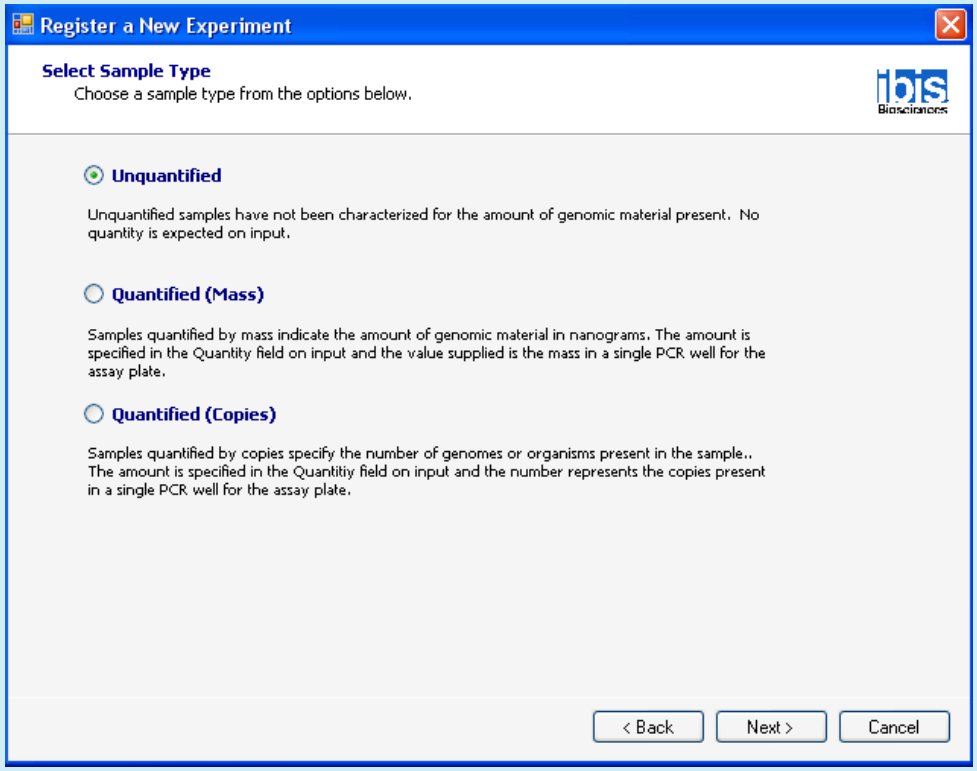
Casework Wizard



- Select sample type
  - Unquantified (enter in dilution factor (DF) for PCR plate)
  - Quantified (enter in picograms or copies for PCR plate)
- Click **Next**

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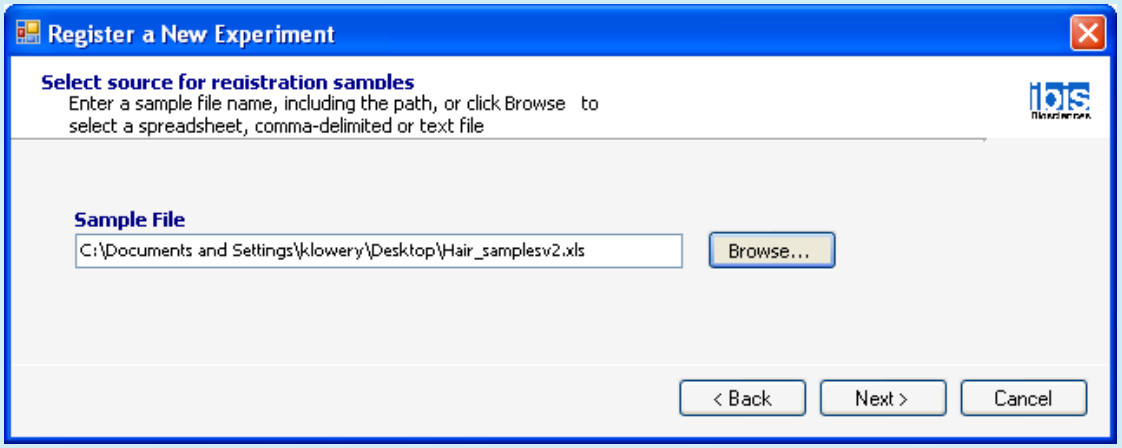
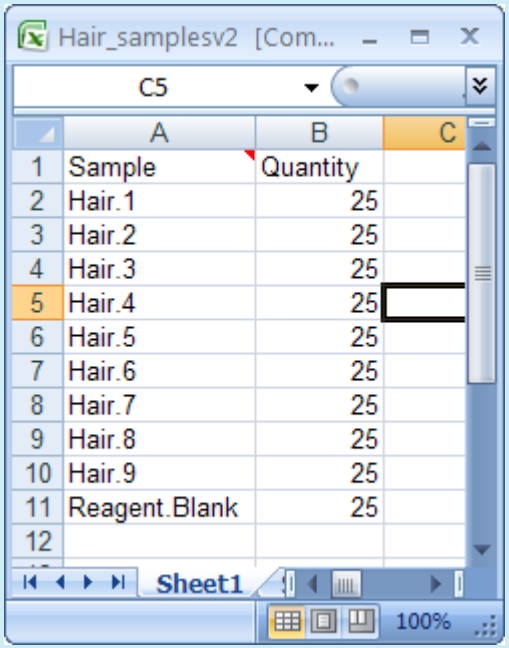
Casework Wizard





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

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Casework Wizard

Select control layout

Automatically interleaves controls with samples based on control layout

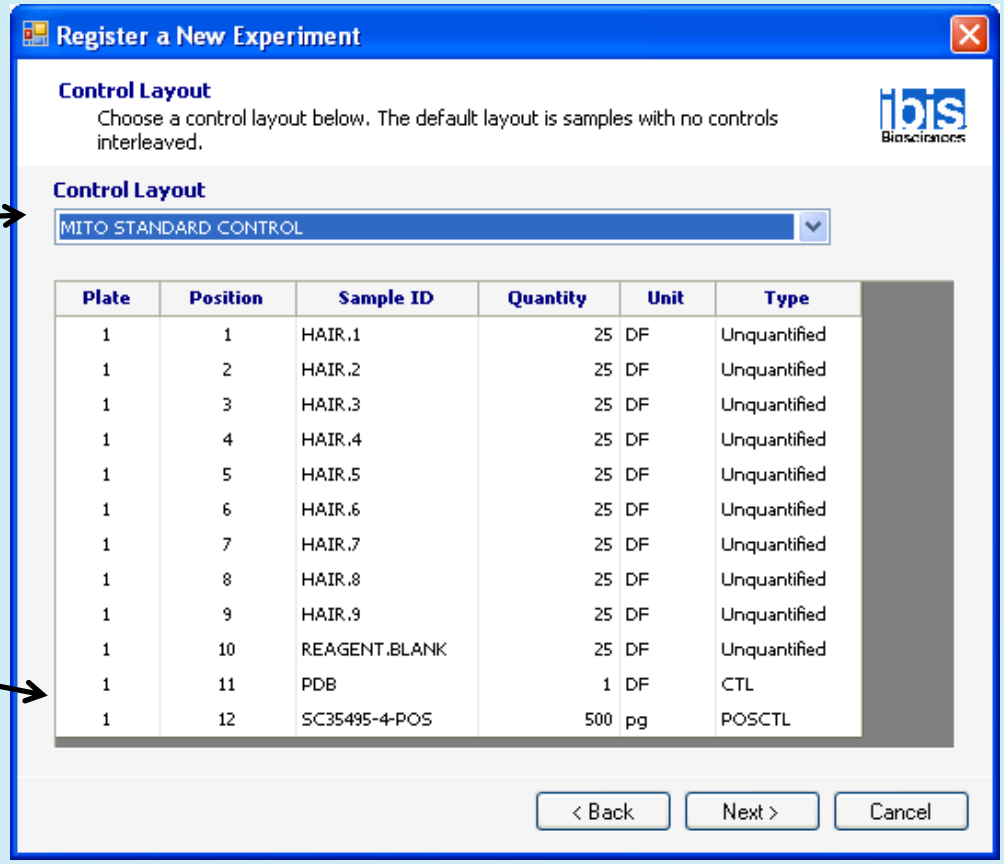

Plate	Position	Sample ID	Quantity	Unit	Type
1	1	HAIR.1	25	DF	Unquantified
1	2	HAIR.2	25	DF	Unquantified
1	3	HAIR.3	25	DF	Unquantified
1	4	HAIR.4	25	DF	Unquantified
1	5	HAIR.5	25	DF	Unquantified
1	6	HAIR.6	25	DF	Unquantified
1	7	HAIR.7	25	DF	Unquantified
1	8	HAIR.8	25	DF	Unquantified
1	9	HAIR.9	25	DF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

- Select control layout – predefined in a previous step
- Click **Next**

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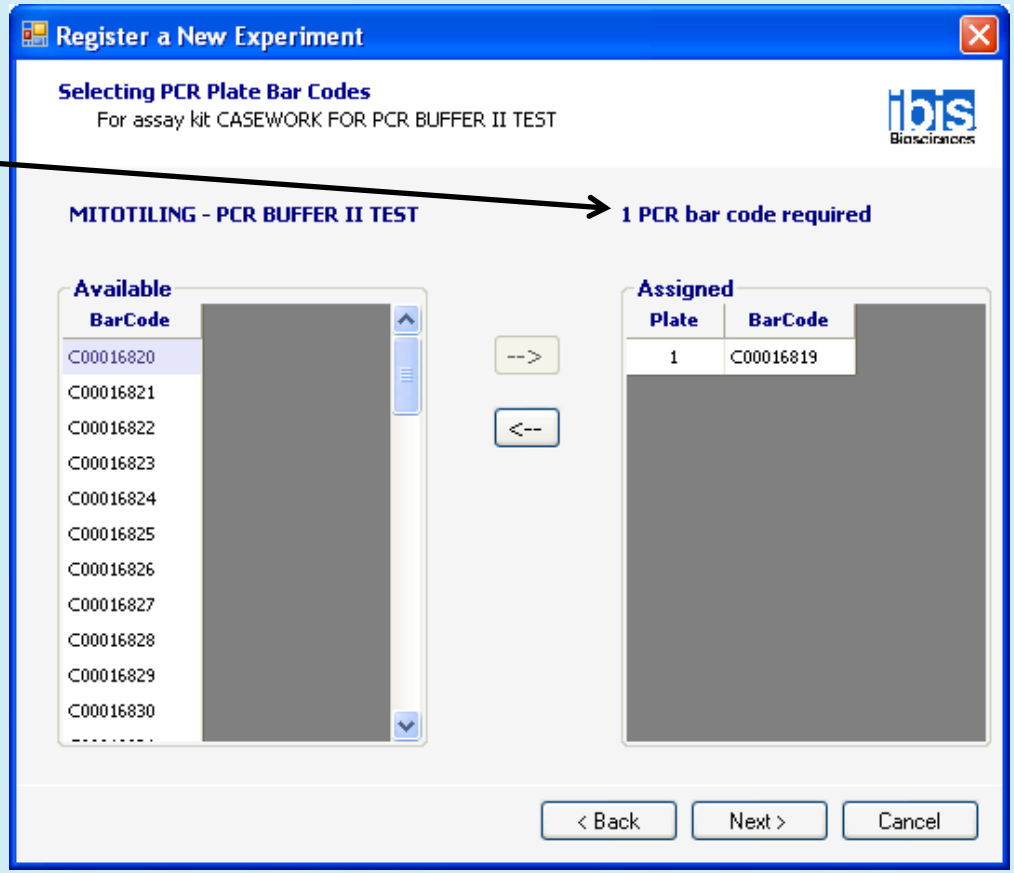
Casework Wizard

Number of barcodes required



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

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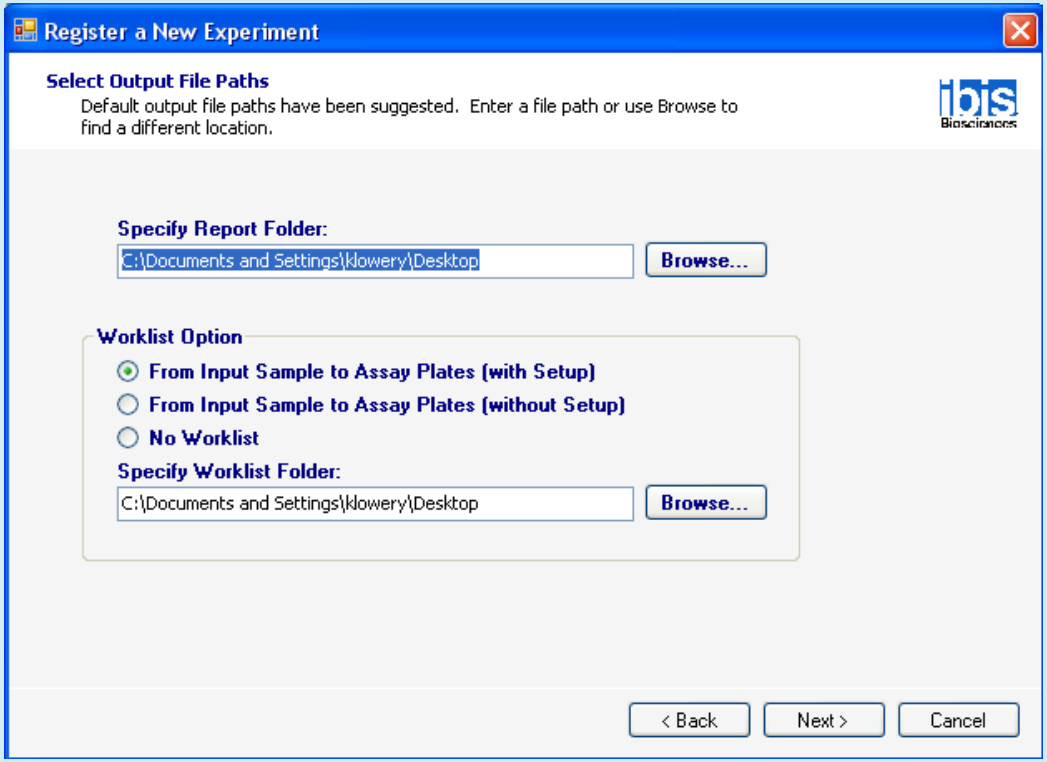
Casework Wizard



- Select a folder for the report and worklist
- Select a worklist option
- Click **Next**

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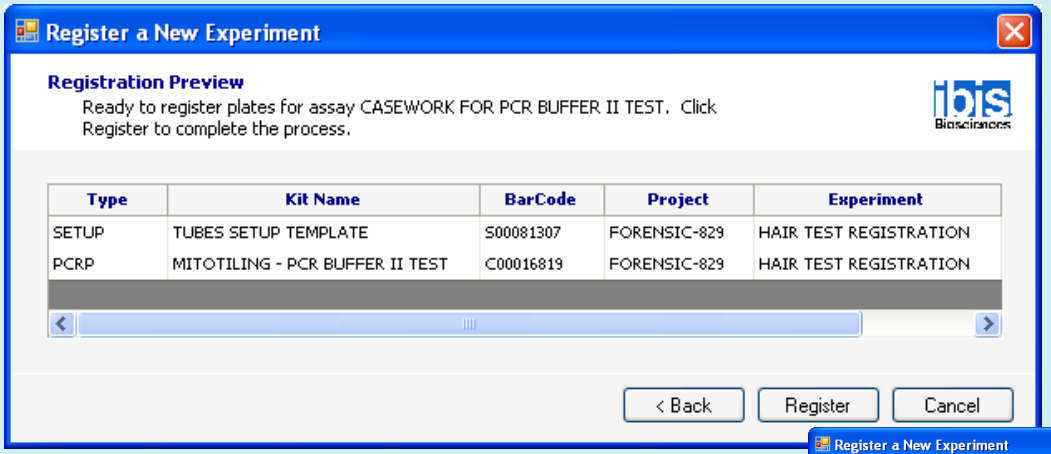
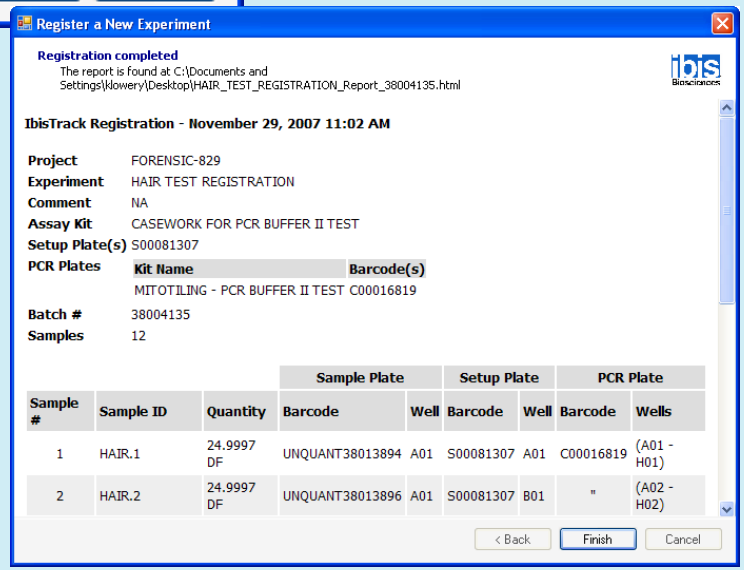
Casework Wizard





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

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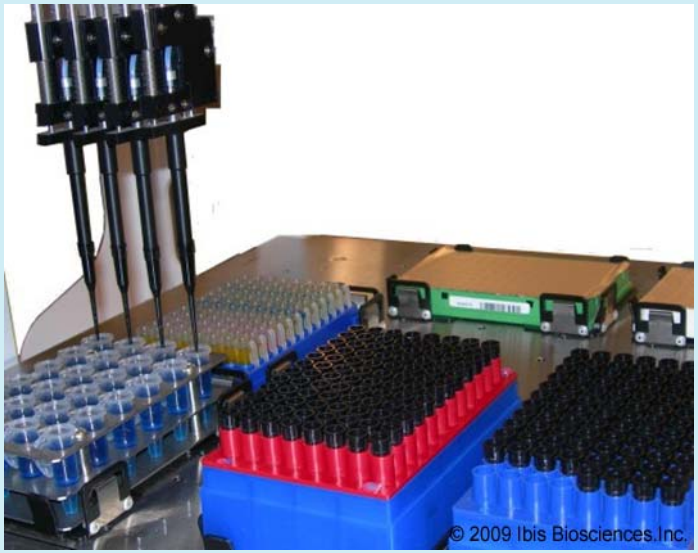
Ibis™ Assay Workflow – Step 4

- **Set up PCR plates either by hand or on reformatting robot**



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Ibis™ Assay Workflow – Step 4

- **Minimize contamination**
  - **Gowning procedure**
  - **Decontamination of PCR hood, pipettes and consumables**
- **5 μL of sample is added per well**
  - **A total of 50 μL is needed for each sample (40 μL required for sample + 10 μL for waste)**
  - **Use a new tip each time**

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Ibis™ Assay Workflow – Step 4

- **Sample dilution**
  - **For quantitated sample, dilute to 500 pg/5 μL with DNA-free water**
  - **For non-quantitated samples, dilute to set volume of 51 μL**
    - **For example, 17 μL stock + 34 μL of PDB**

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Ibis™ Assay Workflow – Step 4

- **Robotic setup**
  - **Barcode validation**
    - **Ensures correct samples placed on plates**
  - **Sort algorithm**
    - **Reduces crossover contamination by ensuring that tips only pass on top of sealed wells**

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Ibis™ Assay Workflow – Step 5

Image courtesy of http://www.thermo.com/com/cda/product/detail/1,,10142764,00.html

- **Heat seal plate**
- **Centrifuge**

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Ibis™ Assay Workflow – Step 6

- **Thermocycle the PCR plates**
- **Programs contain a 10 minute step at 99° C to minimize enzyme activity**
- **After thermocycling, centrifuge the plate for ~15 seconds at ~800 rpm**
- **Plate may be frozen until put on PLEX-ID™**

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Ibis™ Assay Workflow – Step 7

- **Prepare magnetic bead reservoir**
  - **Dispense beads into reservoir**
  - **Seal with tape provided**
- **Prepare clean-up reagents**
  - **Add volume of methanol indicated on bottle**
  - **Use Burdick and Jackson™ HPLC grade**
- **Recommend tracking lot numbers of reagents**

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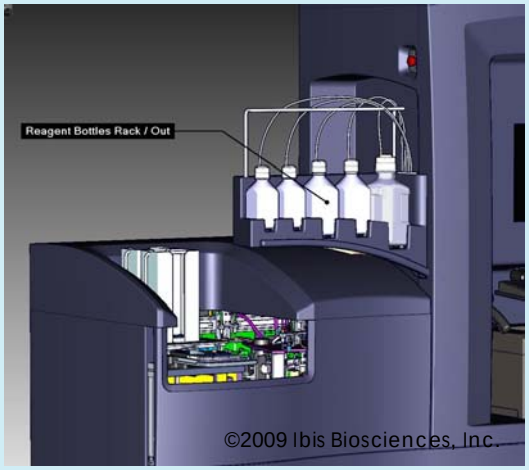
Ibis™ Assay Workflow – Steps 8 and 9

8



Insert magnet bead reservoir, fill reagents and empty waste

9

Run PCR plates on the PLEX-ID™

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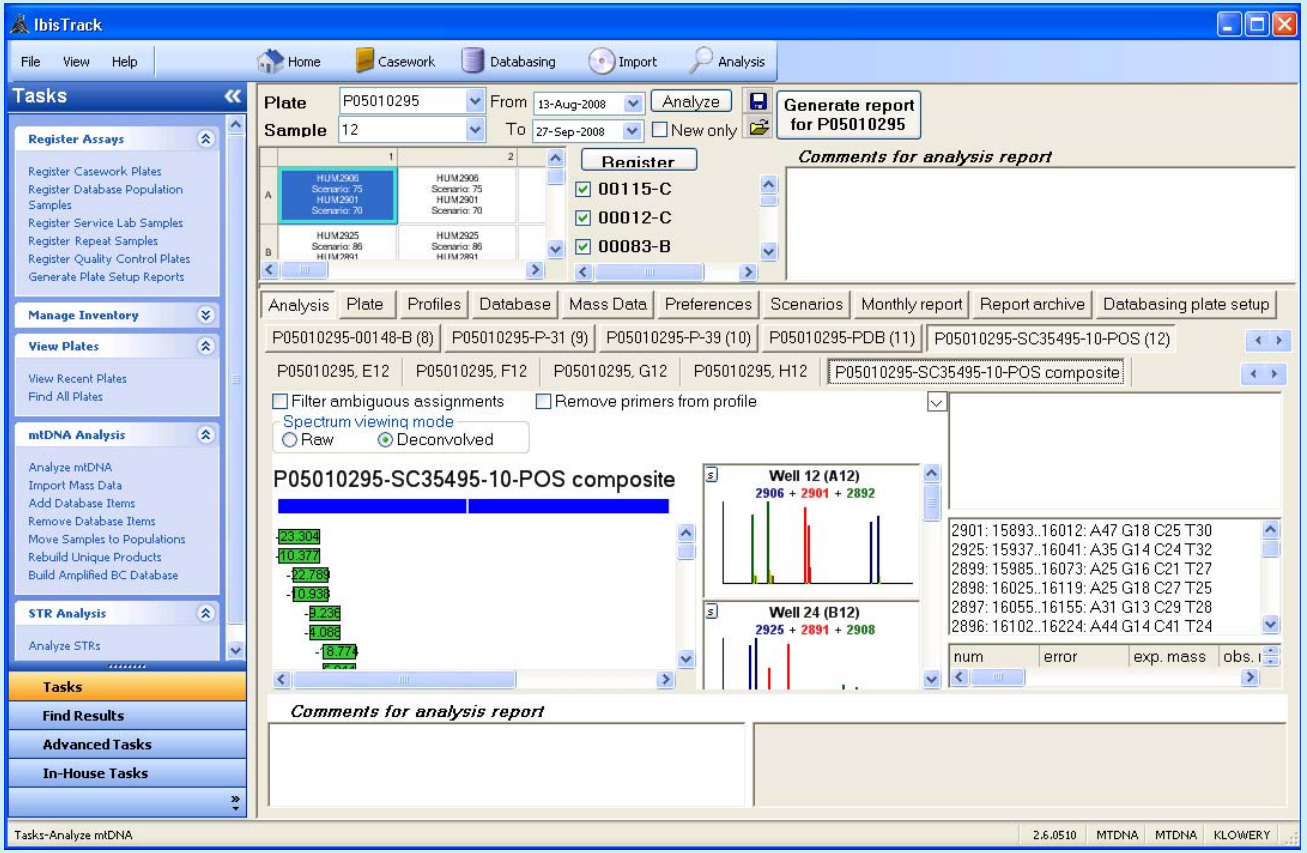
Ibis™ Assay Workflow – Step 10

View results in IbisTrack



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Workflow Timeline

- **Sample preparation**
  - **Isolation: customers are free to use any method they choose**
    - **KingFisher®: 30 minutes setup plus 30 minutes run time (up to 96 samples); setup can be performed manually or on a Tecan Freedom EVO® liquid handler**
    - **Qiagen® columns**
    - **Phenol/chloroform**
- **Plate setup**
  - **Manual: 10 to 20 minutes per plate**
    - **Reproducibility is an issue**
  - **Robotic: 15 minutes per plate**

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Workflow Timeline

- **PCR**
  - **PLEX-ID™ mtDNA Assay – 3.5 hours**
  - **PLEX-ID™ STRs Assay – ~3 hours**
  - **PLEX-ID™ SNPs Assay – 2.5 hours**
- **PLEX-ID™**
  - **Initial flushing and system startup: 20 minutes**
  - **Clean-up: 10 minutes for first well, then one well cleaned every minute**
  - **Spray on TOF: ~50 minutes per plate**
    - **30 seconds per well – 4 minutes per eight well sample**
  - **Data processing: 15 to 20 minutes per plate**

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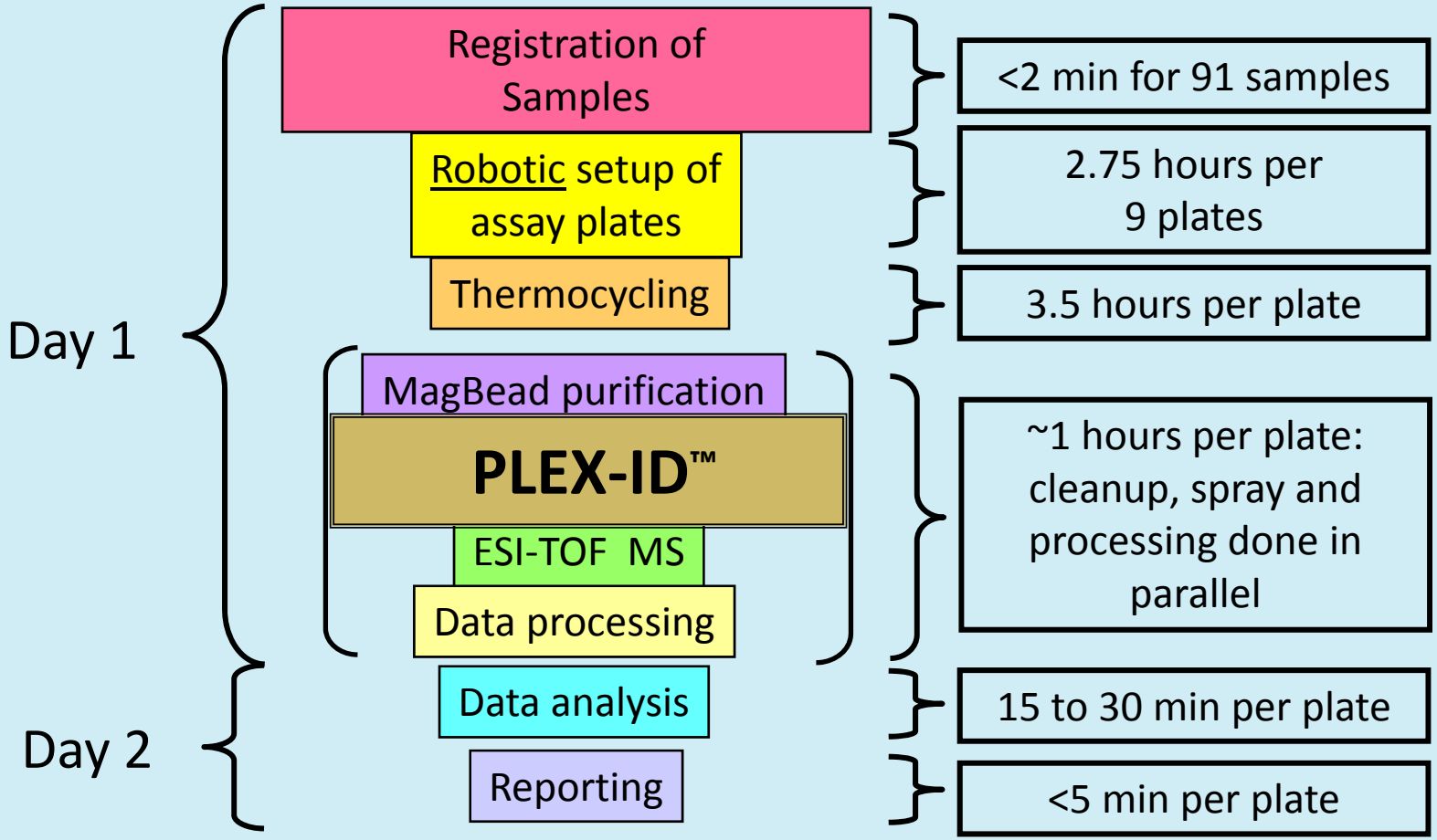
Workflow Timeline



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Workflow Throughput

- **Assuming manual PCR setup and two cyclers, four plates a day**
  - **40 samples per day**
  - **200 samples per week**
  - **10,400 samples per year**
- **Assuming robotic PCR setup and five cyclers, 10 plates a day**
  - **100 samples per day**
  - **500 samples per week**
  - **26,000 samples per year**
- **Limiting factor – number of thermocyclers**

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Questions?

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Contact Information

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