



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

# ***Ibis<sup>TM</sup> 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.**

# ***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**

# ***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**

# ***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

## ***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**

# ***Ibis™ Assay Workflow – Step 3***

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

# Import Wizard Example

Use to  
import  
barcodes or  
assay plans

Type of  
import  
selected  
based on file

**Import Assay Kit Configurations and Barcodes**

**Select Import File**

Select a file to be imported below.

**Assay Kit Barcodes**

Imported barcodes will appear in the available barcode list for the assigned Assay Kit during the Sample Registration process.

**Assay Kit Configuration**

Imported Assay Kits configurations will appear in the Assay Kit selection field during the Sample Registration process.

**File:**

C:\Documents and Settings\klowery\Desktop\KitImport files\SERVICELAB\_IBIS\_KIT\_20090105060354

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# Import Wizard Example

Barcodes to  
be imported

Expiration  
date

Barcode	Expiration Date
C05017850	11/9/2010
C05017851	11/9/2010
C05017852	11/9/2010
C05017853	11/9/2010
C05017854	11/9/2010
C05017855	11/9/2010
C05017856	11/9/2010
C05017857	11/9/2010

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

# ***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

# 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  
Inventory: 59

Select a Project  
FORENSIC-829

Select an Experiment  
Casework registration for training

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

# Casework Wizard

**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.

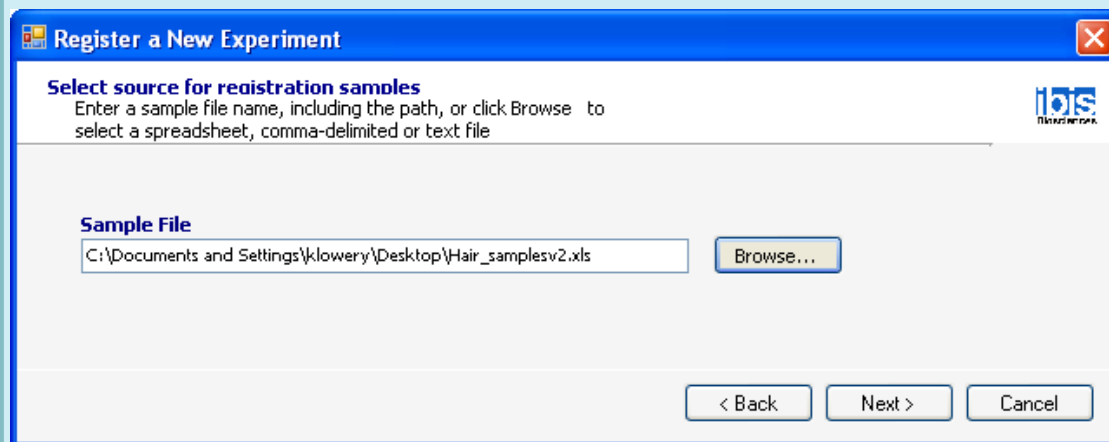
☐ **Quantified (Mass)**  
Samples quantified by mass indicate the amount of genomic material in nanograms. The amount is specified in the Quantity field on input and the value supplied is the mass in a single PCR well for the assay plate.

☐ **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 dilution factor (DF) for PCR plate)
  - Quantified (enter in picograms or copies for PCR plate)
- Click **Next**

# Casework Wizard



	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**
- Sample file is validated
- Click **Next**

# 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	POSCTL

< Back   Next >   Cancel

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

# Casework Wizard

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**
- Click **Next**

# Casework Wizard

**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

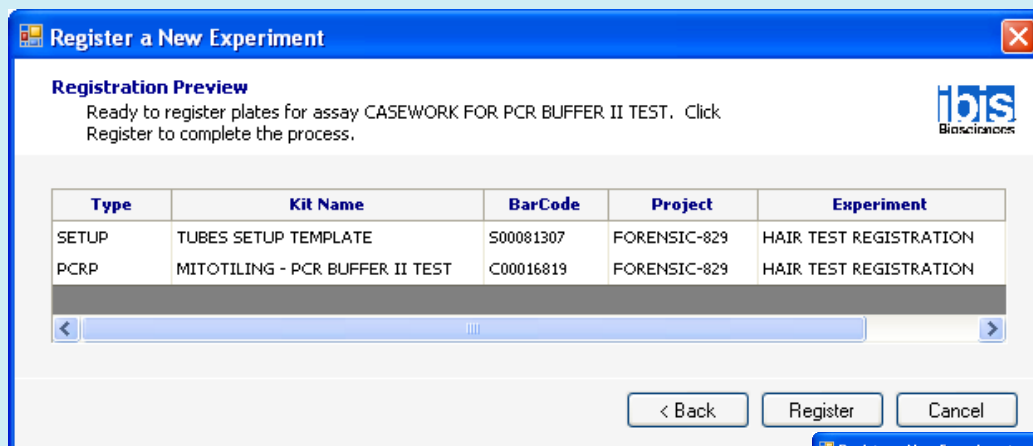
**Specify Worklist Folder:**  
C:\Documents and Settings\klowery\Desktop **Browse...**

**< Back** **Next >** **Cancel**

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



# Casework Wizard



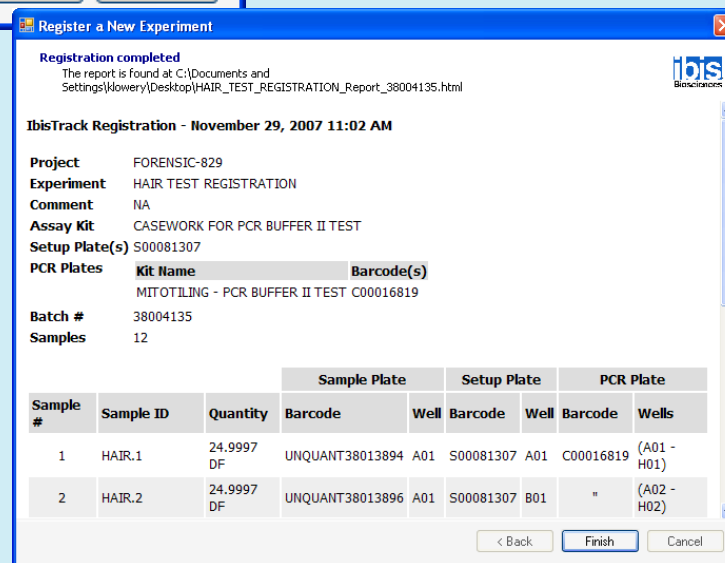
**Register a New Experiment**

**Registration Preview**  
Ready to register plates for assay CASEWORK FOR PCR BUFFER II TEST. Click Register to complete the process.

Type	Kit Name	BarCode	Project	Experiment
SETUP	TUBES SETUP TEMPLATE	S00081307	FORENSIC-829	HAIR TEST REGISTRATION
PCRP	MITOTILING - PCR BUFFER II TEST	C00016819	FORENSIC-829	HAIR TEST REGISTRATION

< Back Register Cancel

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



**Register a New Experiment**

**Registration completed**  
The report is found at C:\Documents and Settings\klowery\Desktop\HAIR\_TEST\_REGISTRATION\_Report\_38004135.html

**IbisTrack Registration - November 29, 2007 11:02 AM**

**Project** FORENSIC-829  
**Experiment** HAIR TEST REGISTRATION  
**Comment** NA  
**Assay Kit** CASEWORK FOR PCR BUFFER II TEST  
**Setup Plate(s)** S00081307  
**PCR Plates**

Kit Name	Barcode(s)
MITOTILING - PCR BUFFER II TEST	C00016819

**Batch #** 38004135  
**Samples** 12

Sample #	Sample ID	Quantity	Sample Plate		Setup Plate		PCR Plate	
			Barcode	Well	Barcode	Well	Barcode	Wells
1	HAIR.1	24.9997 DF	UNQUANT38013894	A01	S00081307	A01	C00016819	(A01 - H01)
2	HAIR.2	24.9997 DF	UNQUANT38013896	A01	S00081307	B01	*	(A02 - H02)

< Back Finish Cancel

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# ***Ibis™ Assay Workflow – Step 4***

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



# ***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

# ***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

# ***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



# ***Ibis™ Assay Workflow – Step 5***



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

- Heat seal plate
- Centrifuge

## ***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™

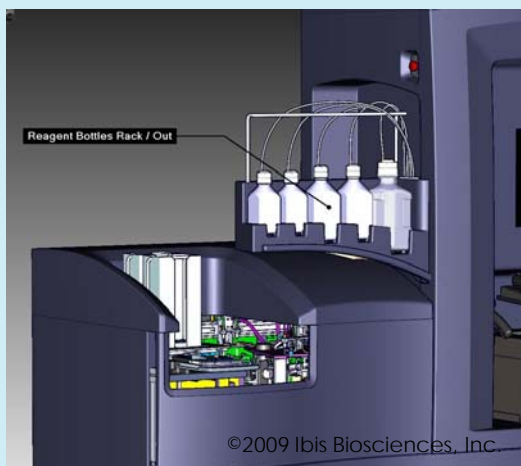
# ***Ibis™ Assay Workflow – Step 7***

- **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**



# *Ibis™ Assay Workflow – Steps 8 and 9*

8



**Insert magnet  
bead reservoir, fill  
reagents and  
empty waste**

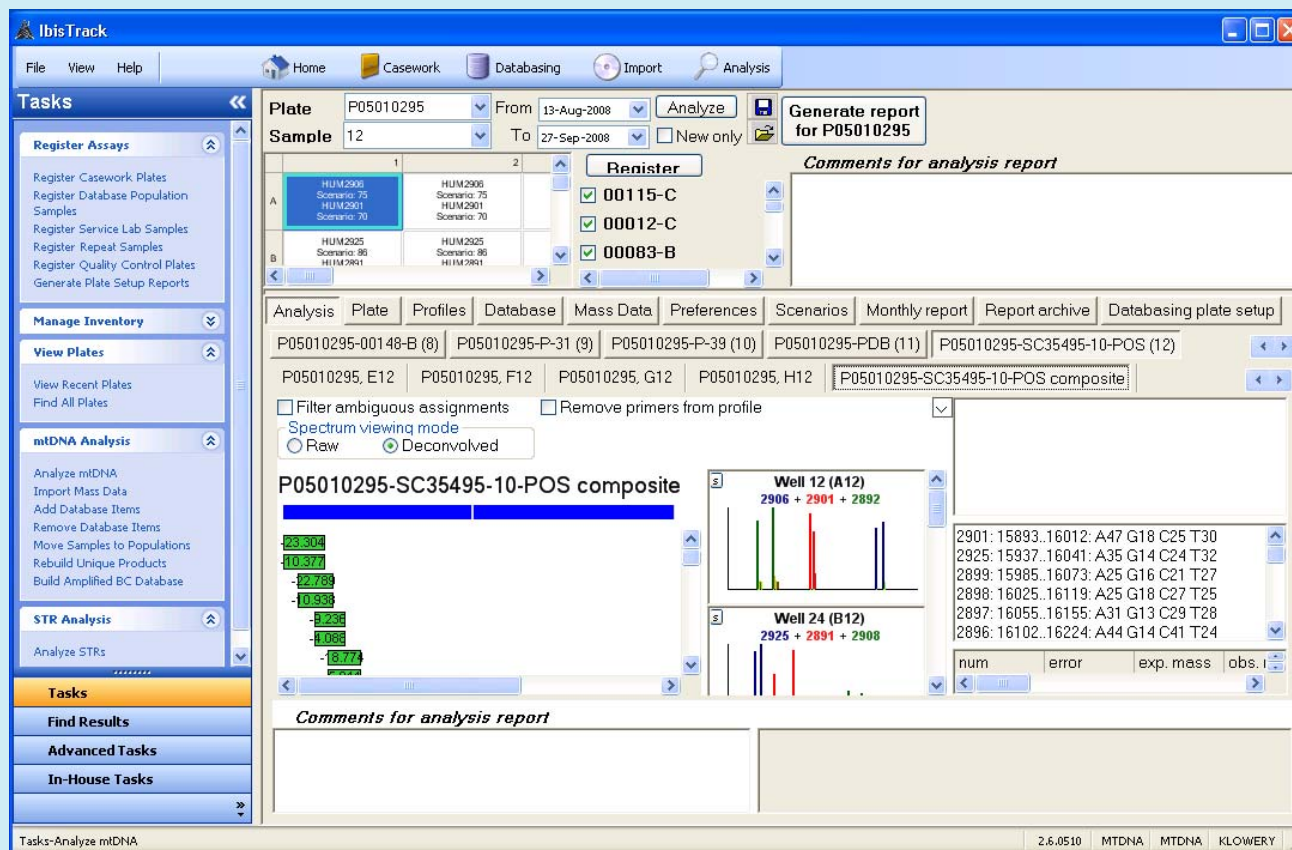
9



**Run PCR plates on the  
PLEX-ID™**

# Ibis™ Assay Workflow – Step 10

View results  
in IbisTrack



# ***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**

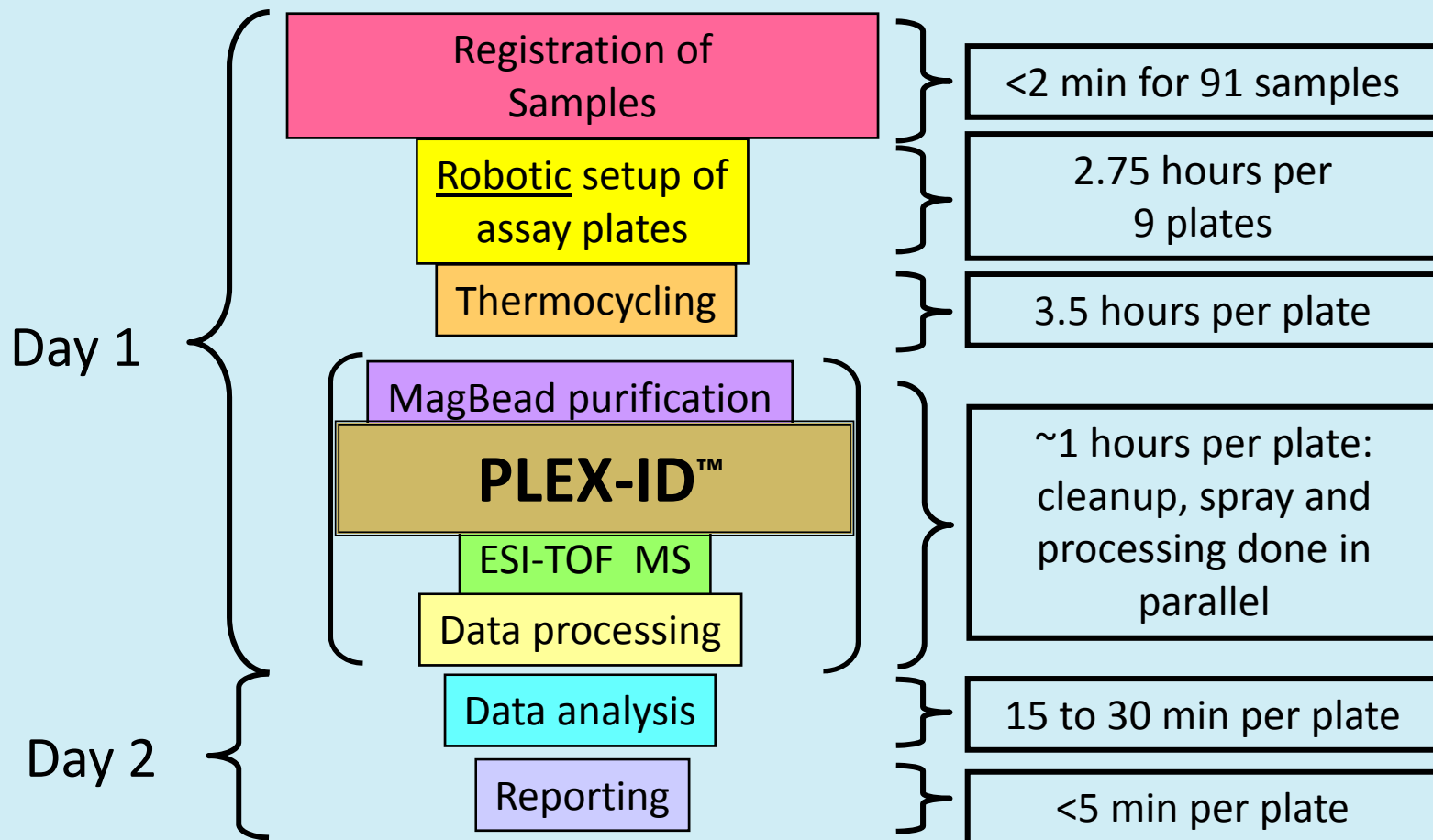
# ***Workflow Timeline***

- **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



# ***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**

# *Questions?*

# ***Contact Information***

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***Note:*** All images are courtesy of Dr. Kristin S. Lowery unless otherwise noted.