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

Ibis™ Assay Workflow Overview

Ibis™ Assay Workflow

1. **Receive and store 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 and store as indicated**
  - **10 barcoded assay plates**
    - **Store @ -20° C – manual defrost freezer**
  - **1 bottle of magnetic beads**
    - **Store @ 4° C and in upright position**
  - **1 reservoir for magnetic beads**
    - **Store @ room temperature**
  - **3 cleanup reagents (CR1, CR2 and CR3)**
    - **Store @ 4° C**
  - **Instructions sheet**

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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**
  - **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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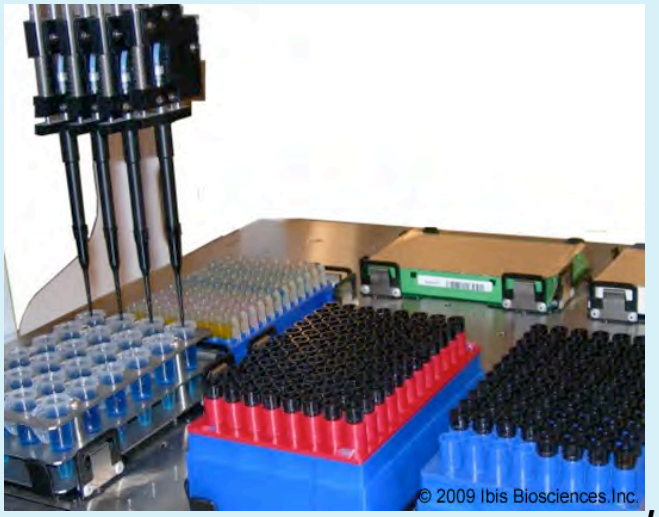
Ibis™ Assay Workflow – Step 4

- **Set up PCR plates either by hand or on reformatting robot**
- **5 μL of sample is added per well**
  - **A total of 50 μL is needed for each sample**



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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 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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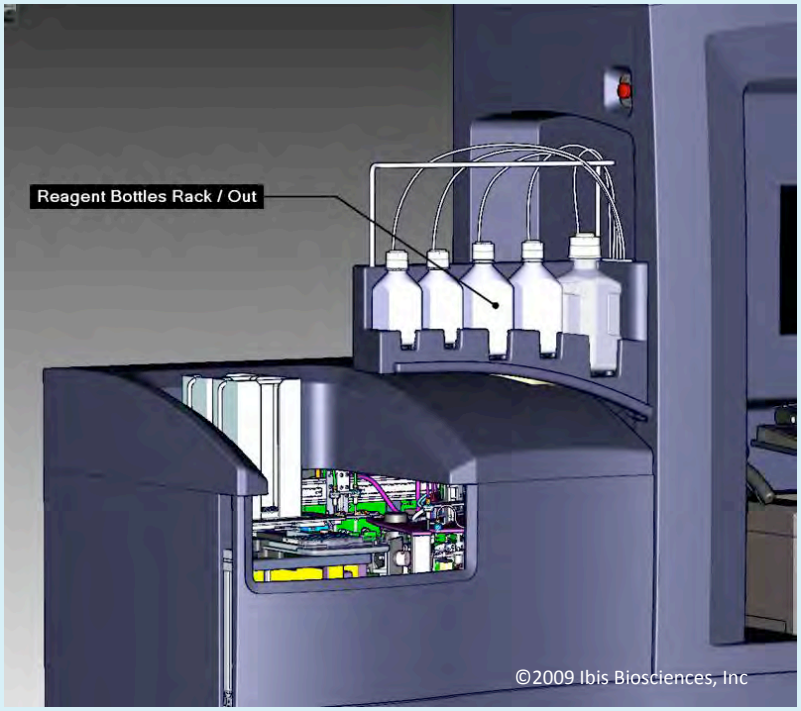
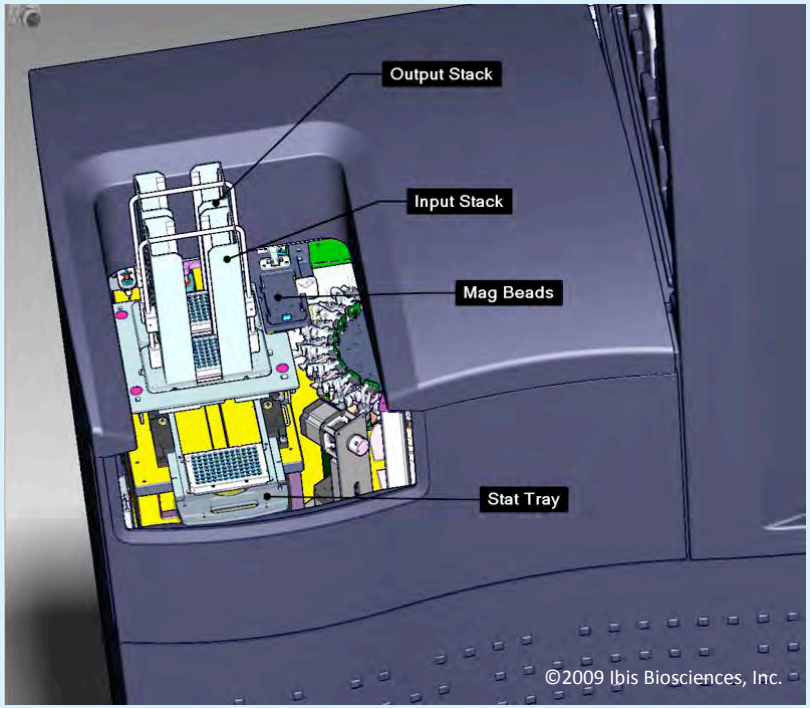
Ibis™ Assay Workflow – Step 8



Fill reagents and empty waste



Insert magnetic bead reservoir and plates

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Ibis™ Assay Workflow – Step 9

Run PCR plates on the PLEX-ID™

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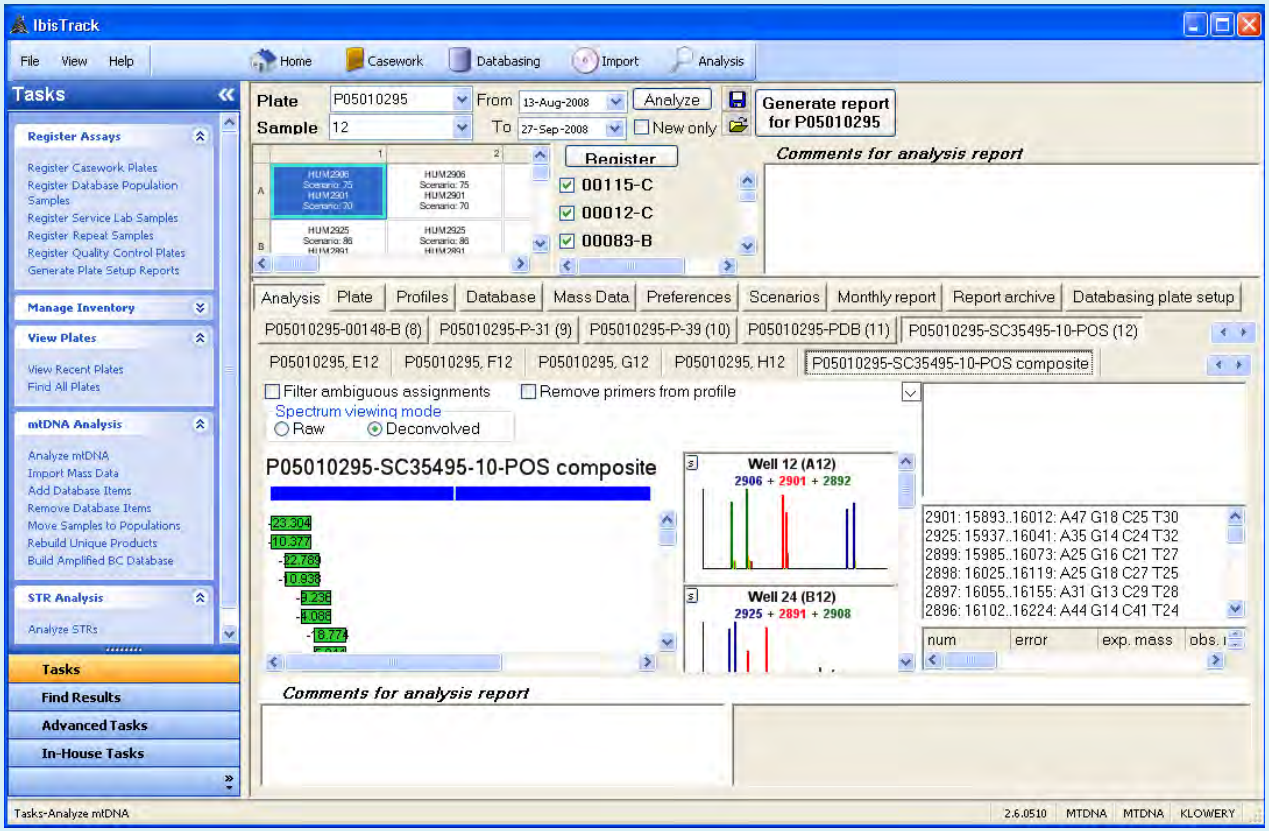
Ibis™ Assay Workflow – Step 10

- **View results in IbisTrack**



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Workflow Timeline

- **Sample Prep**
  - **Depending on method 30 to 60 minutes**
- **Plate Setup**
  - **Manual: 10 to 20 minutes per plate**
    - **Reproducibility is an issue**
  - **Robotic: 15 minutes per plate**
- **PCR**
  - **PLEX-ID™ STR Assay – ~3 hours**
  - **PLEX-ID™ mtDNA Assay – 3.5 hours**
  - **PLEX-ID™ SNP Assay – 2.5 hours**

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Workflow Timeline

- **PLEX-ID™**
  - **Initial flushing and system startup: 20 minutes**
  - **Clean-up: 10 minutes for first well, then one well cleaned every 30 seconds**
  - **Spray on TOF: ~50 minutes per plate**
    - **30 seconds per well – 4 minutes per 8 well sample**
  - **Data processing: 15 to 20 minutes per plate**

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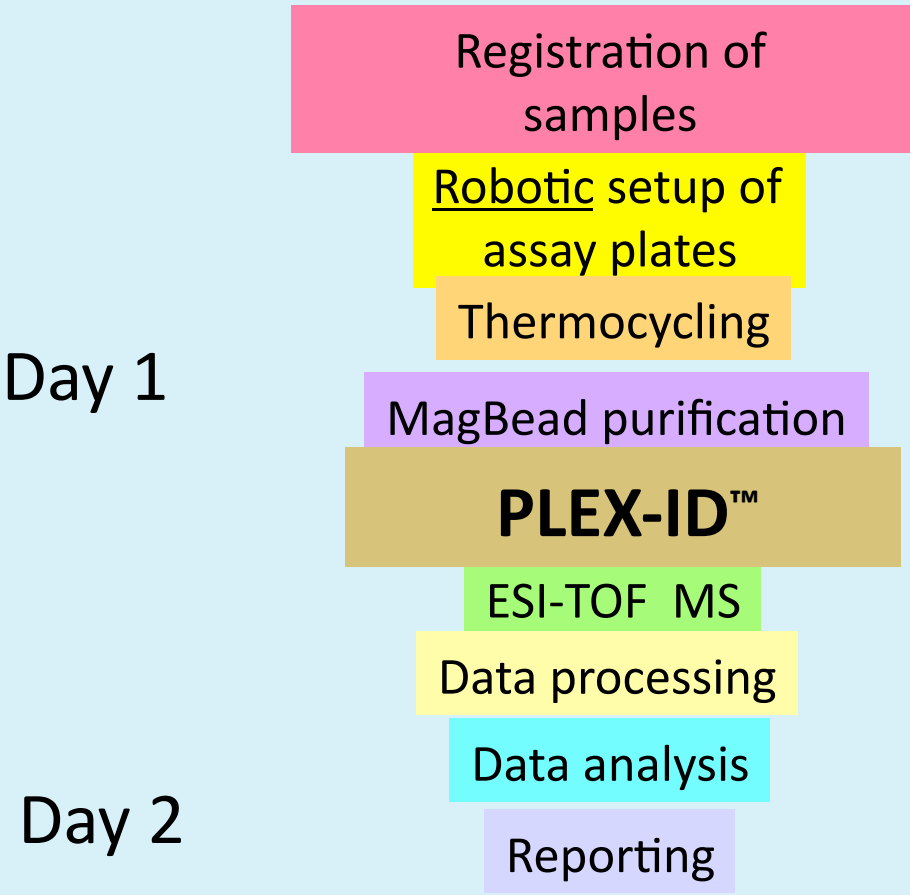
Workflow Timeline



<2 min for 91 samples

2.75 hours per 9 plates

3.5 hours per plate

~50 minutes per plate: clean-up, spray, and processing done in parallel

15 - 30 min per plate

<5 min per plate

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Workflow Throughput

- **Assuming manual PCR setup and 2 cyclers, 4 plates a day**
  - **40 samples per day**
  - **200 samples per week**
  - **10,400 samples per year**
- **Assuming robotic PCR setup and 5 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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Note: *All images are courtesy of Dr. Kristin S. Lowery unless otherwise noted.*

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