



Technology Transition Workshop

Ibis Assay System Workflow Overview

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Ibis T5000™ Workflow

1. Receipt and storage of Assay kit components.
2. Import kits into IbisTrack inventory.
3. Perform up-front sample processing.
4. Register the experiment in IbisTrack.
5. Set up PCR plate(s).
6. Seal the PCR plates.
7. Thermocycle PCR plates.
8. Fill reagents on Ibis T5000™ and empty waste.
9. Analyze the PCR plates on the Ibis T5000™.
10. Review data in IbisTrack.



Ibis T5000™ Workflow – Step 1

- Upon receipt, check for Assay Components
 - 10 Barcoded Assay plates
 - 10 Barcoded Magnetic bead plates
 - 10 Barcoded Elution plates
 - 3 Cleanup reagents (CR1, CR2 and CR3)
 - Instruction sheet
 - CD with import file(s)



Ibis T5000™ Workflow – Step 1

- Receipt and 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
 - 8 wells comprised of 3 primer pairs per well
 - Profile is composite of all 8 wells
 - User is required to add 5 μL of template to each well of a sample
 - Store @ -20 C – manual defrost freezer



Ibis T5000™ Workflow – Step 1

- Magnetic bead plates
 - Upon receipt, centrifuge for 15 seconds @ 800 rpm
 - Store @ 4 C and in upright position
 - DO NOT FREEZE
- Elution plates
 - Store @ room temperature
- Cleanup reagents
 - Store @ 4 C
 - Before use, add methanol
 - Volume to add on label
 - Burdick and Jackson™ HPLC grade
 - Recommend tracking lot numbers of reagents



Ibis T5000™ Workflow – Step 2

- Import files sent on CD with shipment
- Use Import Wizard
- Plan files
 - Usually sent once or as needed
 - Have “Plan” in the title



Ibis T5000™ Workflow – Step 2

- Barcode files
 - Sent with each shipment
 - Have “Ibis Kit” in the title
 - Assay plates, magnetic bead and elution plate barcode may be in individual files or all together



Ibis T5000™ Workflow – Step 3

- Up-front sample processing, if necessary — extract DNA from samples (your choice of method)
 - Qiagen® columns
 - KingFisher® magnetic bead systems
 - Phenol/Chloroform
 - Others



Ibis T5000™ Workflow – Step 4

- Register experiment
 - 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

Ibis T5000™ Workflow – Step 5

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



Ibis T5000™ Workflow – Steps 6 - 9

6



Image courtesy of
<http://www.kbiosystems.com/pdfs/Wasp%202006.pdf>

Heat seal the PCR plates

7



Image courtesy of K. S. Lowery, Ph.D.

Thermocycle the PCR plates

8



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Fill reagents and empty waste as needed

9



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Run PCR plates on the T5000

Ibis T5000™ Workflow – Step 10

- View Results in IbisTrack

The screenshot displays the IbisTrack software interface. The main window shows a plate grid for 'Plate P05010295' and 'Sample 12'. The grid contains samples from scenarios 75 and 86. A 'Register' button is visible. Below the grid, there are tabs for 'Analysis', 'Plate', 'Profiles', 'Database', 'Mass Data', 'Preferences', 'Scenarios', 'Monthly report', 'Report archive', and 'Databasing plate setup'. The 'Analysis' tab is active, showing a list of analysis results for 'P05010295-SC35495-10-POS composite'. The spectrum viewer shows two wells: 'Well 12 (A12)' with peaks at 2906, 2901, and 2892; and 'Well 24 (B12)' with peaks at 2925, 2891, and 2908. A table of results is visible on the right side of the spectrum viewer.

num	error	exp. mass	obs. i
2901	15893..16012	A47 G18 C25 T30	
2925	15937..16041	A35 G14 C24 T32	
2899	15985..16073	A25 G16 C21 T27	
2898	16025..16119	A25 G18 C27 T25	
2897	16055..16155	A31 G13 C29 T28	
2896	16102..16224	A44 G14 C41 T24	

Image courtesy of K. S. Lowery, Ph.D.



Workflow Timeline

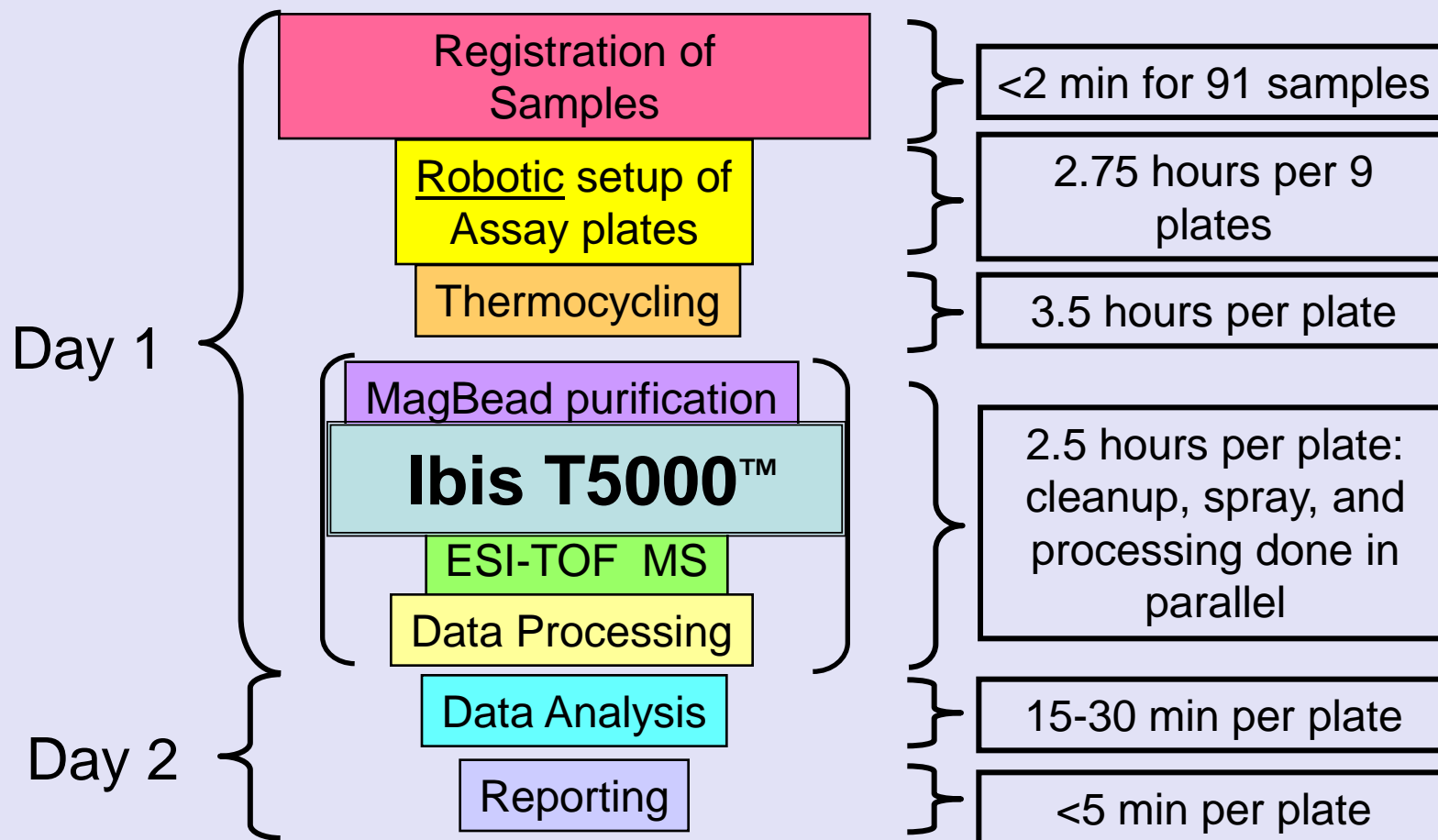
- **Sample Prep**
 - Isolation: With Ibis T5000™, customers are free to use any method they choose
 - KingFisher®: 30 minutes setup plus 30 minutes run time (up to 96 samples); setup can be performed manually or on JANUS®
 - Qiagen® columns
 - Phenol/Chloroform
- **Plate Setup**
 - Manual: 10-20 minutes per plate
 - Reproducibility is an issue
 - PE JANUS®: 15 minutes per plate



Workflow Timeline

- PCR
 - T5000 Human Forensics (Mitochondria) assay – 3.5 hours
- Ibis T5000™
 - Initial flushing and system startup: 20 minutes
 - Clean-up: 45 minutes per plate (all plates after first are done while previous plate is spraying)
 - Spray on TOF: ~90 minutes per plate
 - Data Processing: 15-20 minutes per plate

Workflow Timeline





Workflow Throughput

- Assuming manual PCR setup and 2 cyclers, 4 plates a day
 - 40 samples per day
 - 200 samples per week
 - 10400 samples per year
- Assuming robotic PCR setup and 5 cyclers, 10 plates a day
 - 100 samples per day
 - 500 samples per week
 - 26000 samples per year
- Limiting factor – number of thermocyclers



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