

Automation in the Protein Production Laboratory

A wide-angle photograph of a protein production laboratory. The room is filled with white lab benches and equipment. In the foreground, a blue and white automated workstation is prominent, featuring a computer monitor, keyboard, and mouse. The background shows multiple workstations with various lab supplies, including bottles, containers, and specialized equipment. The lab is well-lit with overhead fluorescent lights and has a clean, organized appearance.

Ray Owens:
P4EU meeting
23rd February 2011

The OPPF-UK



➤ The OPPF project established in 2002 with funding from UK MRC and subsequently renewed in 2005 and 2008 with support from MRC & BBSRC.

➤ We seek to achieve high-throughput production of proteins and protein crystals by automating, parallelizing and miniaturizing all stages of the process involved.

➤ To apply HTP technology to challenging biomedical problems through the development of a portfolio of collaborative projects.

➤ We relocated in January 2010 to establish the OPPF-UK at the Research Complex at Harwell adjacent to the Diamond Light Source.

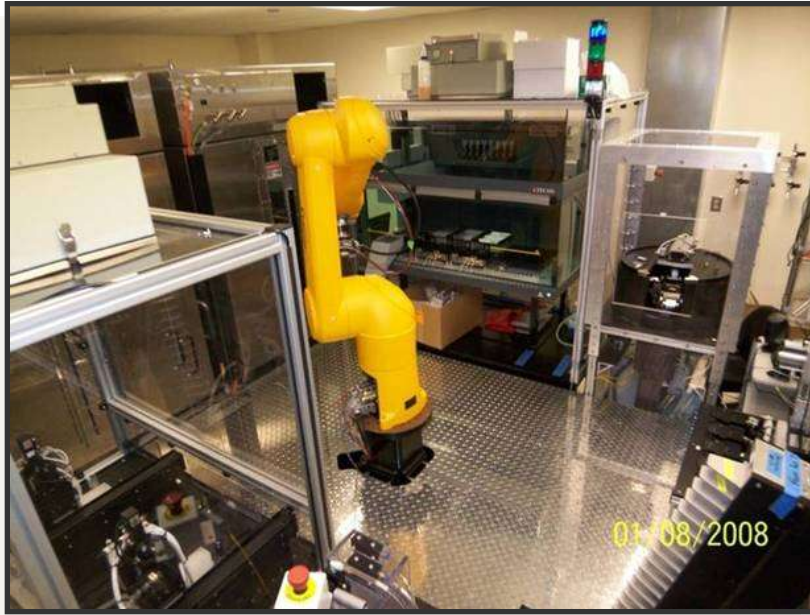
➤ The OPPF-UK is a National Resource Centre for protein production offering free access to its technology platforms for UK academics.

Why automate ?

- **Handle large numbers of samples.**
- **Improve reproducibility of routine tasks.**
- **Sustainability of a process(es).**
- **Free up scientist's time.**
- **Save money (?)**

Issues

- **Specification of the process(es) to be automated.**
- **Stability of process(es) to be automated.**
- **Degree of integration of multiple tasks (modular vs full integration).**



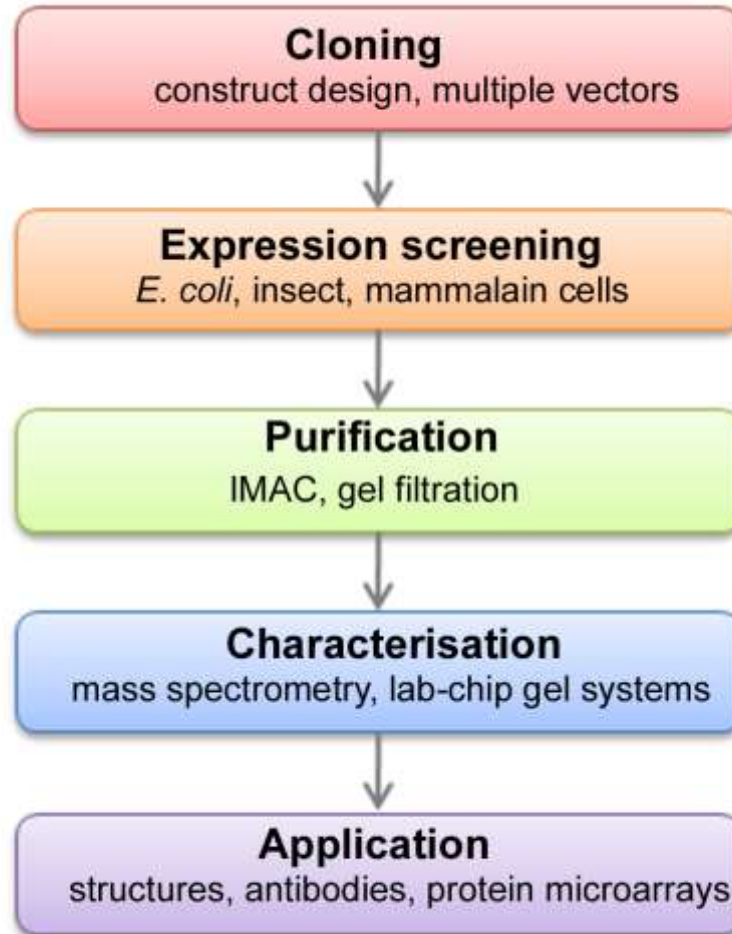
Large motion-controlled systems that perform multiple tasks by integrating several robotic devices.

Vs

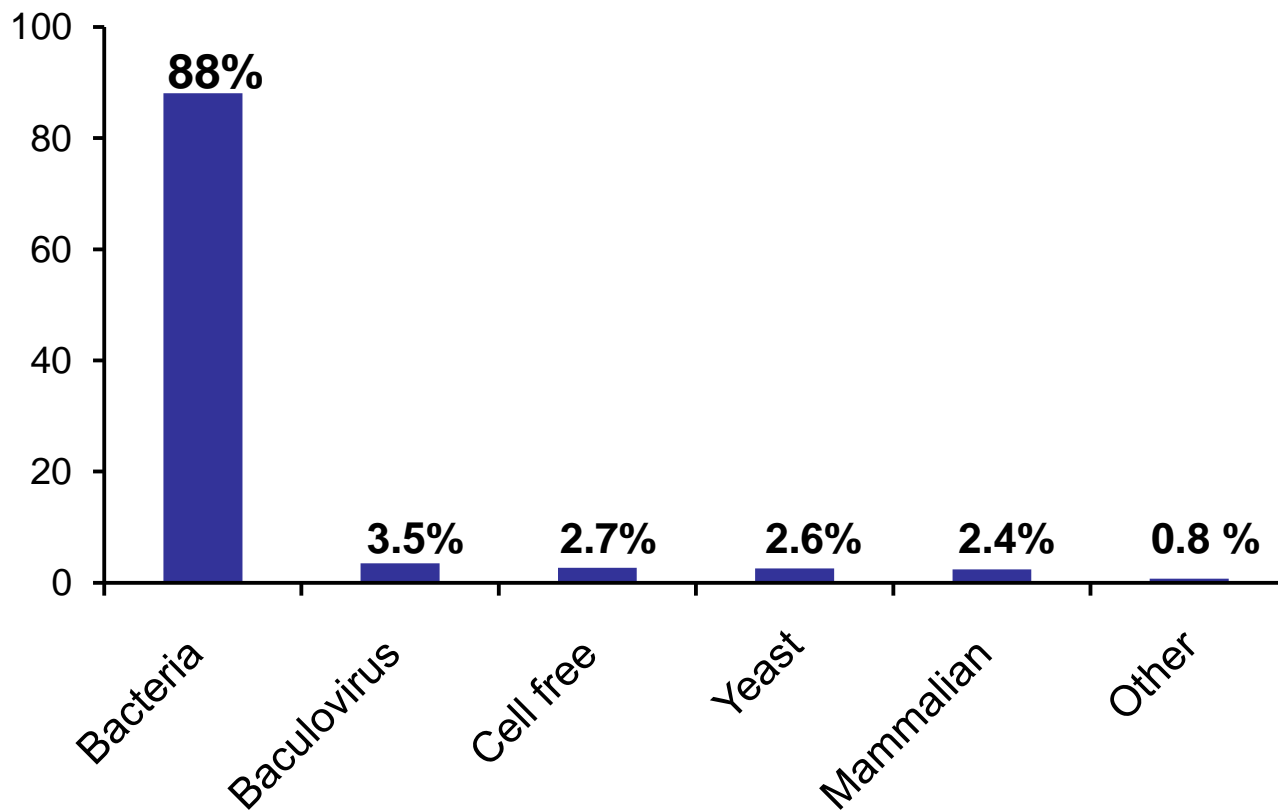
Liquid handling work stations dedicated to specific tasks.



Generic workflow



Structures solved by X-ray crystallography



The number of chains deposited in the PDB by expression system (n =53544), and as a percentage of the total number of chains with an identifiable expression system, as of December 2009.

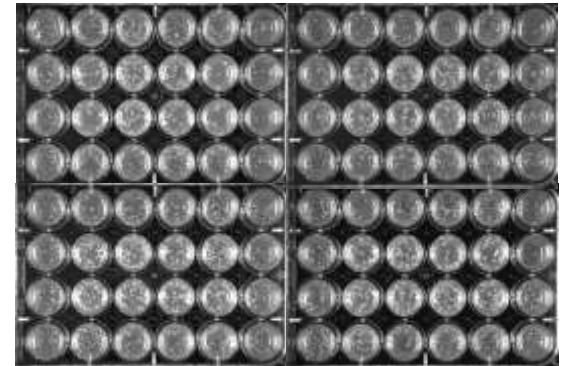
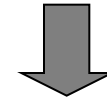
96 Well PCR Cloning



PCR amplification (50ul) in 96 well plate: DpnI treat and purify using magnetic beads (Agencourt AMPure).



Quality assessment.



**Total elapsed time
= 5 days**



Miniprep and PCR verification.

96 well plate ligation independent cloning into expression vector and transformation plating on 4 x 24 well plates.

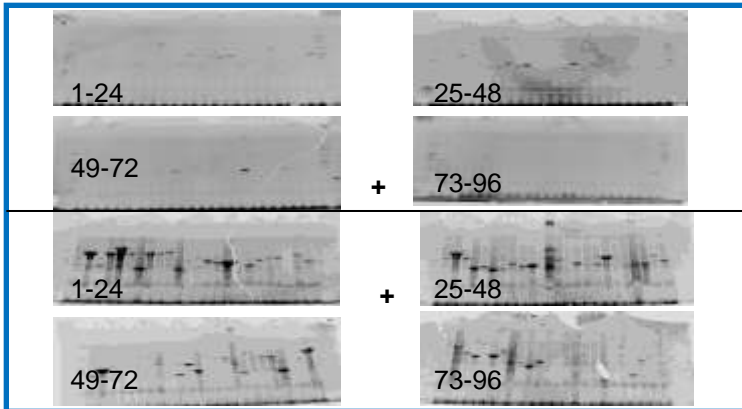
Expression screening in *E.coli*



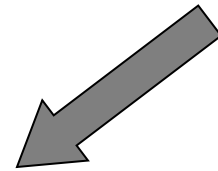
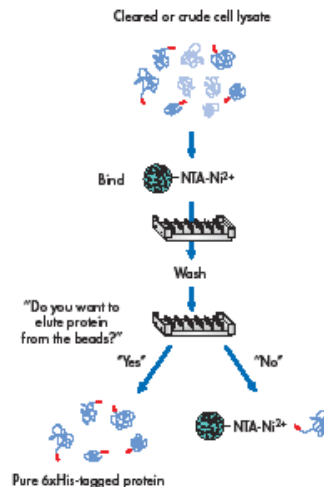
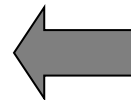
4x24 deep-well plates
Two strains
Two Induction methods



**Total elapsed time
= 5 days**



SDS-PAGE analysis

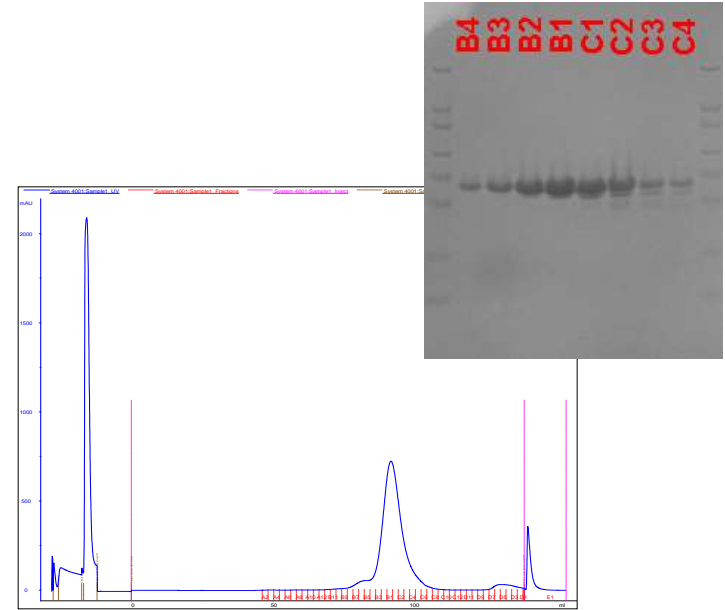


Expression screen
Ni NTA-magnetic beads for soluble
Ni-NTA agarose on a vacuum manifold for insoluble

Protein Purification



Purification of both intracellular and secreted proteins by automated Ni chelation chromatography followed by gel filtration.



Quality Control by LC-ESI-MS (Liquid Chromatography-Electrospray Ionization-Mass Spectrometry).

Expression screening in mammalian cells



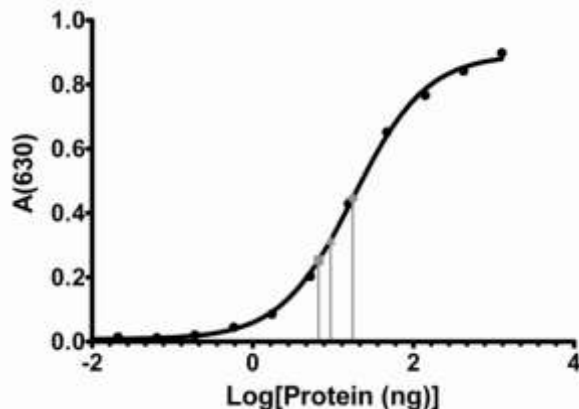
➤ Tecan EVO₇₅ in Class 100 cabinet (~25% of Class II cost).

➤ 1 fixed probe with 5 ml dilutor for large scale and repeat reagent dispensing and media aspiration.

➤ 1 disposable tip cone for 10 µl or 200 µl tips with 500 µl dilutor for smaller volume DNA, transfection reagent and transfection mix dispensing.

➤ Capable of 'non-centric' pipetting to prevent disruption of cell monolayer.

➤ Example: recombinant Fab: 3.2 ± 0.4 µg/ml across plate transfection.

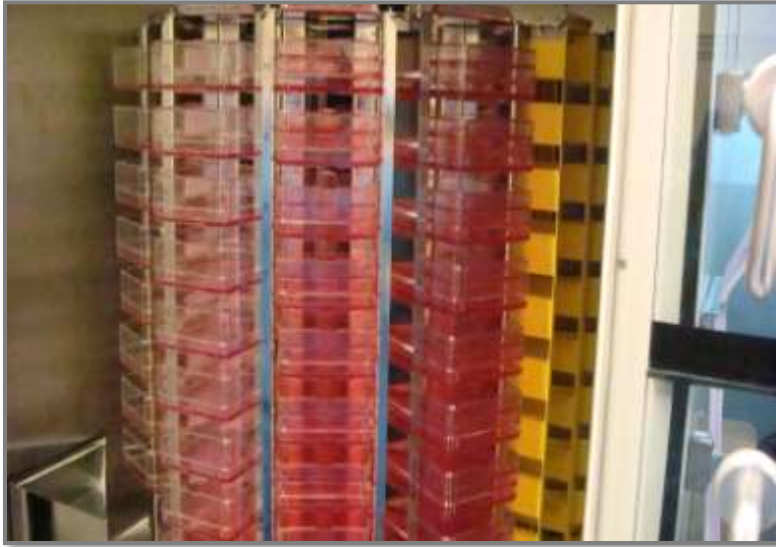


Compact Select System

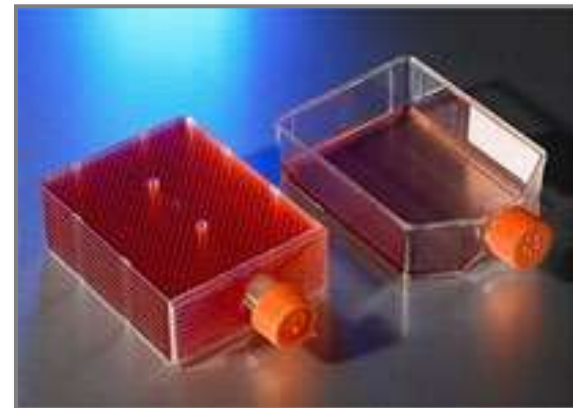


- Ability to grow, passage and transfect multiple cell lines
- Temperature and CO₂ controlled incubator housing rotating flask holder with 130 positions (40 for maintenance, 90 for expression).
- Built-in laminar hood.
- Robotic arm for flask and pipette handling.
- De-capping and waste collection units.
- 12 peristaltic pumps for delivery of media and other reagents.
- Complex software for task design and management.
- All flasks bar-coded, ability to integrate with LIMS.

Automating large-scale transient expression



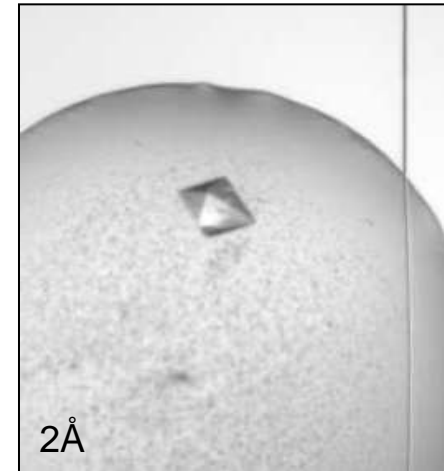
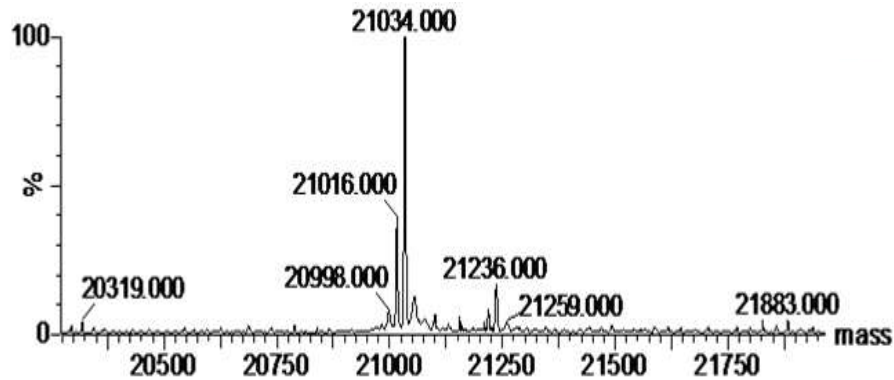
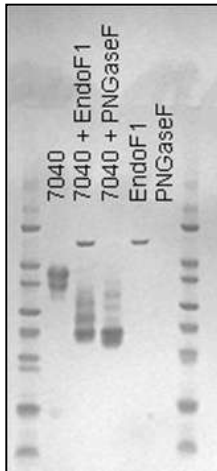
- Cell growth rate and optimal passage time determined for 2 cell lines (HEK293T and 293S GnTI-)
- HYPER™ flasks = 1720 cm² (cf 2125 cm² roller bottle).
- Approx. x 3 cost/ L than roller bottle process.
- Running at approx. 30 L culture/ month



Comparison of Transient Transfection in Roller Bottles and using the SelecT System



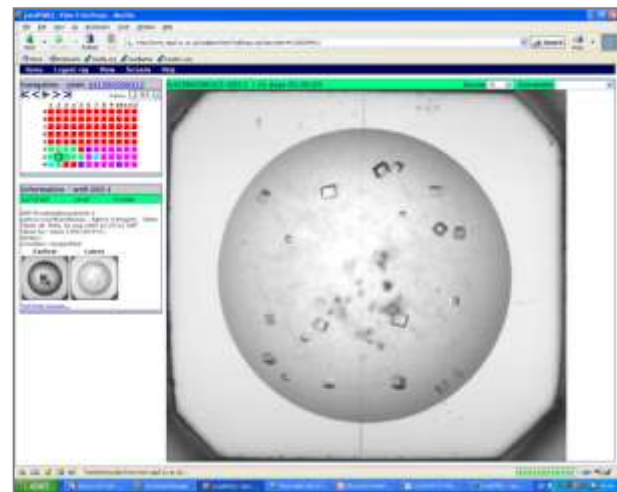
Roller bottles: 1.07 mg/L
SelecT robot: 2.59 mg/L



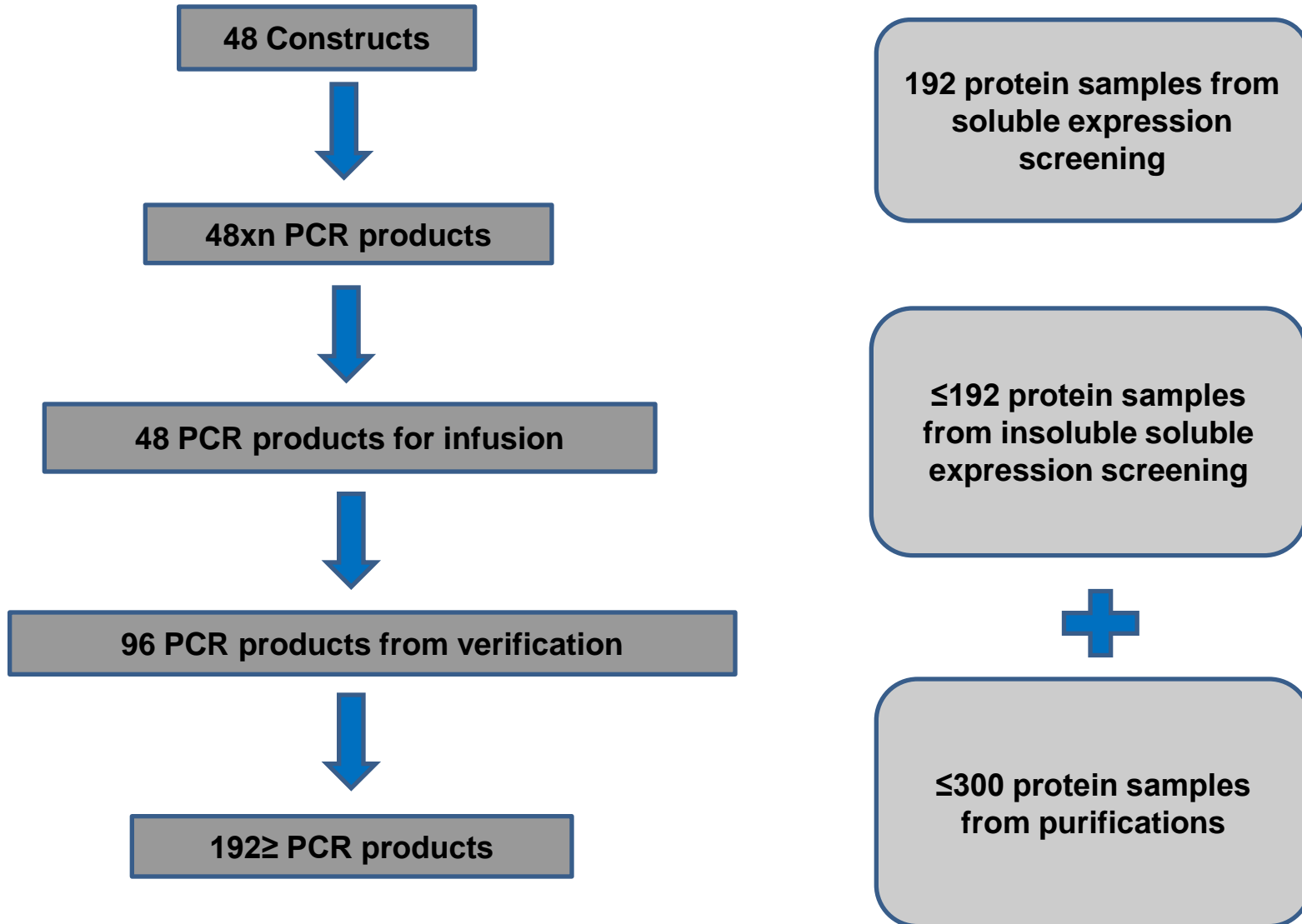
Semi-automated crystallization facility



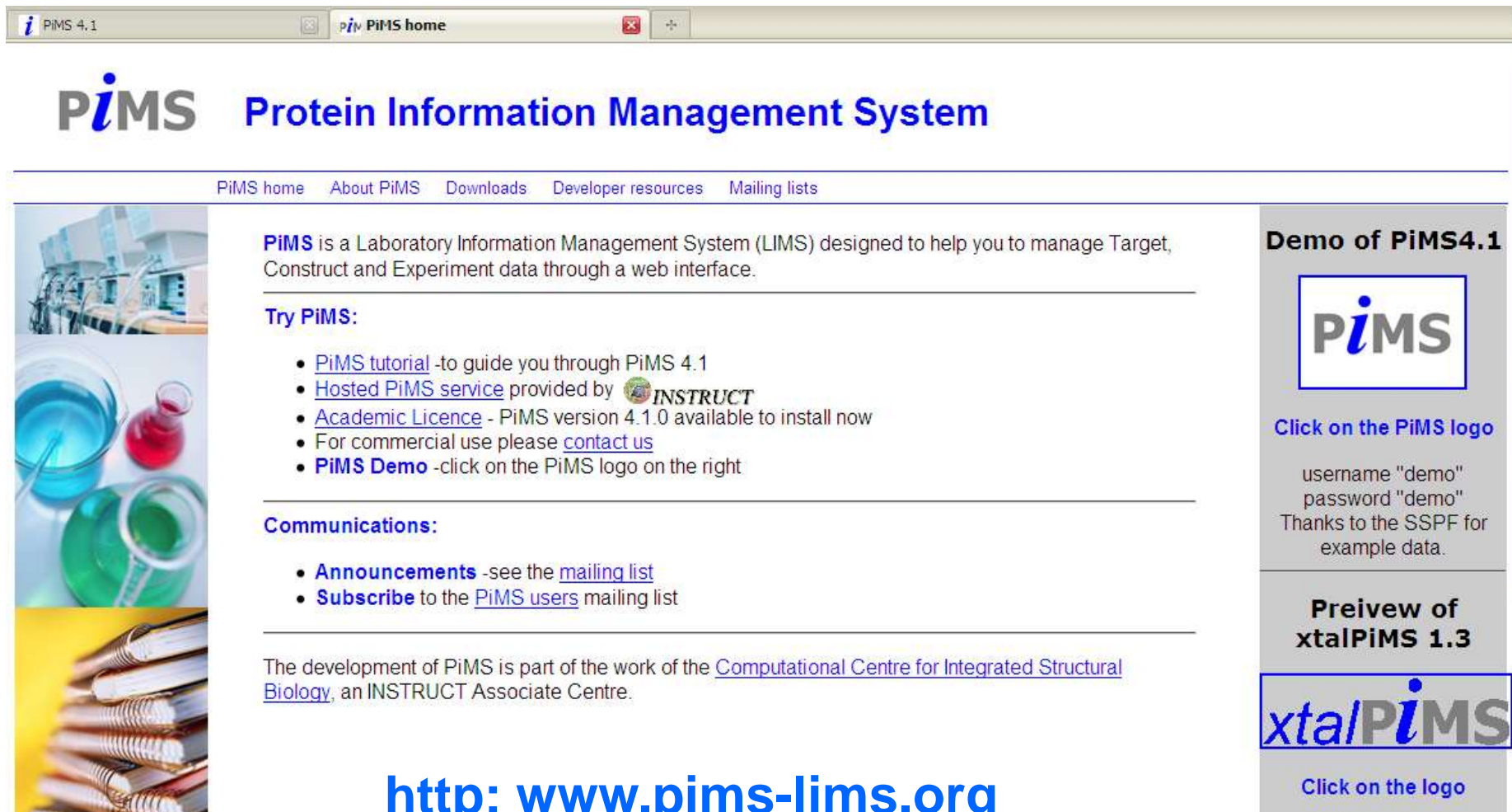
- 100 + 100 nl sitting drop experiments.
- Automated storage and imaging (incl. UV) 2000 plates at 21⁰ C and 1000 plates at 4⁰ C.
- In-house software for inspection of plates (XtalPims).



Sample Generation



Data and Sample Management: PiMS



The screenshot shows a web browser window with the address bar displaying "PiMS 4.1" and "PiMS home". The main heading is "PiMS Protein Information Management System". A navigation menu includes "PiMS home", "About PiMS", "Downloads", "Developer resources", and "Mailing lists". The main content area is divided into three sections: "Try PiMS:" with links to a tutorial, hosted service, academic licence, contact, and demo; "Communications:" with links to announcements and a mailing list; and a paragraph about the development of PiMS by the Computational Centre for Integrated Structural Biology. On the right, there are two promotional boxes: "Demo of PiMS4.1" with a PiMS logo and login details (username "demo", password "demo"), and "Preview of xtalPiMS 1.3" with an xtalPiMS logo.


PiMS 4.1 PiMS home

PiMS Protein Information Management System

PiMS home About PiMS Downloads Developer resources Mailing lists

PiMS is a Laboratory Information Management System (LIMS) designed to help you to manage Target, Construct and Experiment data through a web interface.

Try PiMS:


- [PiMS tutorial](#) -to guide you through PiMS 4.1
- [Hosted PiMS service](#) provided by  **INSTRUCT**
- [Academic Licence](#) - PiMS version 4.1.0 available to install now
- For commercial use please [contact us](#)
- **PiMS Demo** -click on the PiMS logo on the right

Communications:

- **Announcements** -see the [mailing list](#)
- **Subscribe** to the [PiMS users](#) mailing list

The development of PiMS is part of the work of the [Computational Centre for Integrated Structural Biology](#), an INSTRUCT Associate Centre.


Demo of PiMS4.1



[Click on the PiMS logo](#)

username "demo"
password "demo"
Thanks to the SSPF for example data.

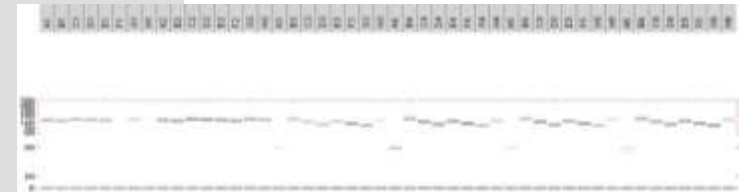
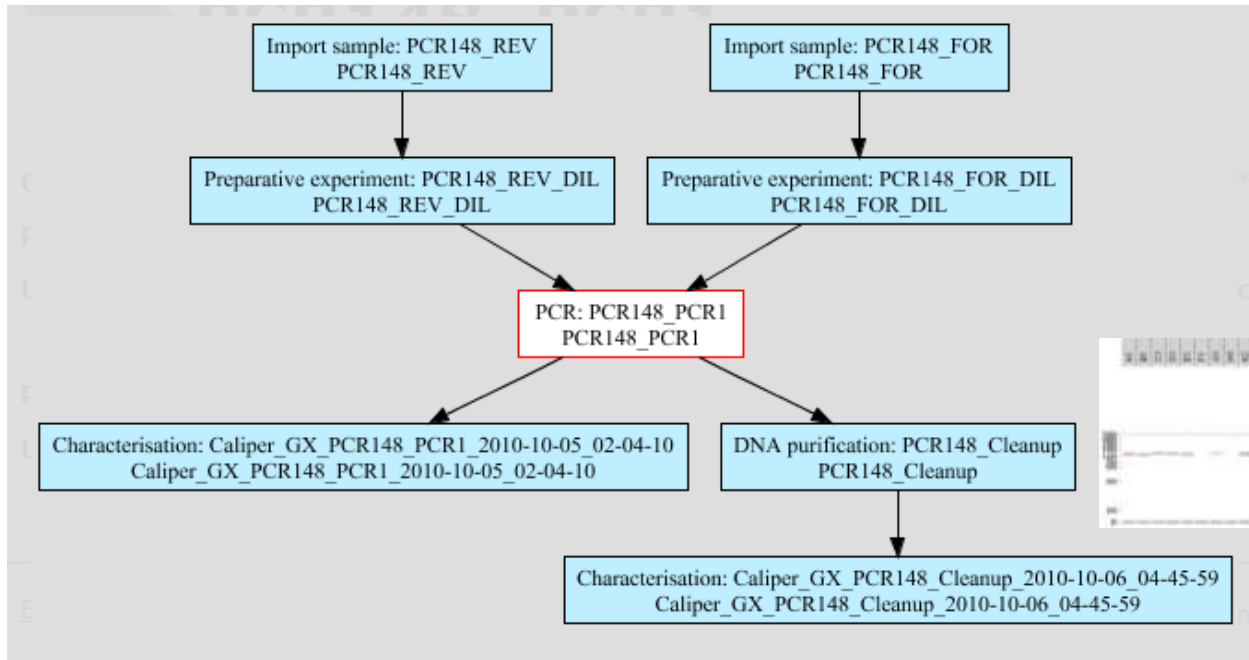
Preview of xtalPiMS 1.3



[Click on the logo](#)

[http: www.pims-lims.org](http://www.pims-lims.org)

Recording PCR results



	01	02	03	04	05	06
A	✓	✓	✓	✓	✓	✓
B	✓	✓	✓	✓	✓	✓
C	✓	✓	✓	✓	✓	✓
D	✓	✓	✓	✓	✓	✓
E	✓	✓	✓	✓	✓	✓
F	?	✓	✓	✓	✓	✓
G	✓	✓	✓	✓	✓	✓
H	✗	✓	✓	✓	✓	✓

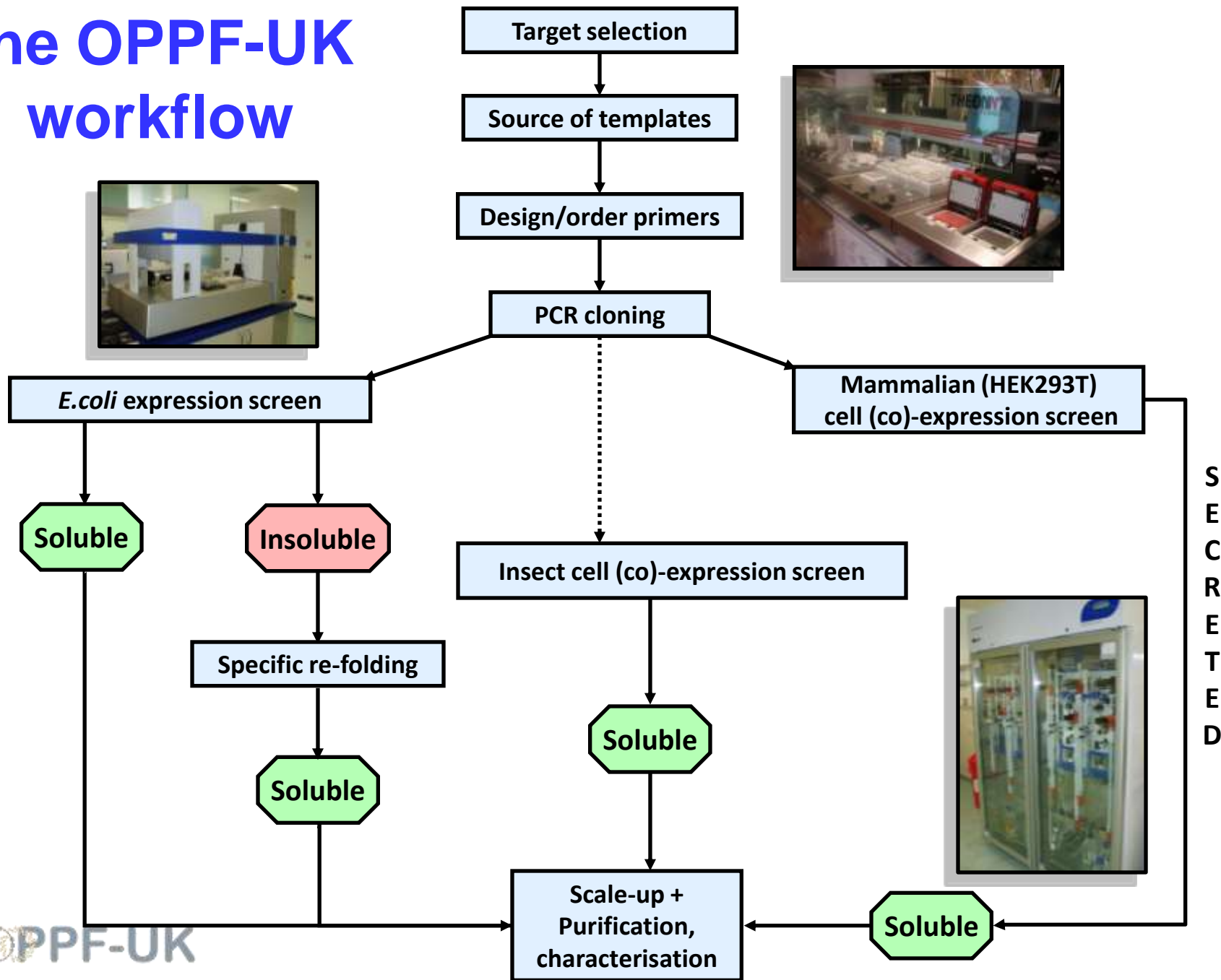
Manual assignment

	01	02	03	04	05	06	07	08	09	10	11	12
A	✓	✓	✓	✓	✓	✓						
B	✓	✓	✓	✓	✓	✓						
C	✓	✓	✓	✓	✓	✓						
D	✓	✓	✓	✓	✓	✓						
E	✓	✓	✓	✓	✓	✓						
F	✓	✓	✓	✓	✓	✓						
G	✓	✓	✓	✓	✓	✓						
H	✗	✓	✓	✓	✓	✓						

Caliper_GX_PCR148_Cleanup_2010-10-06_04-45-59

Automated assignment

The OPPF-UK workflow



S
E
C
R
E
T
E
D

Automation in the OPPF

Process	Automated	Use in pipeline	comment
DNA purification	✓	✓	Plates and tubes
Expression screen	✓	✓	Plates and tubes
HEK transients	✓	✓	Large-scale transients
Purification	✓	✓	Two column process
PCR	✓	✗	Analysis under development
Crystallization	✓	✓	Storage and imaging

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