Automation in the Protein Production Laboratory

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P4EU meeting

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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.

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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.

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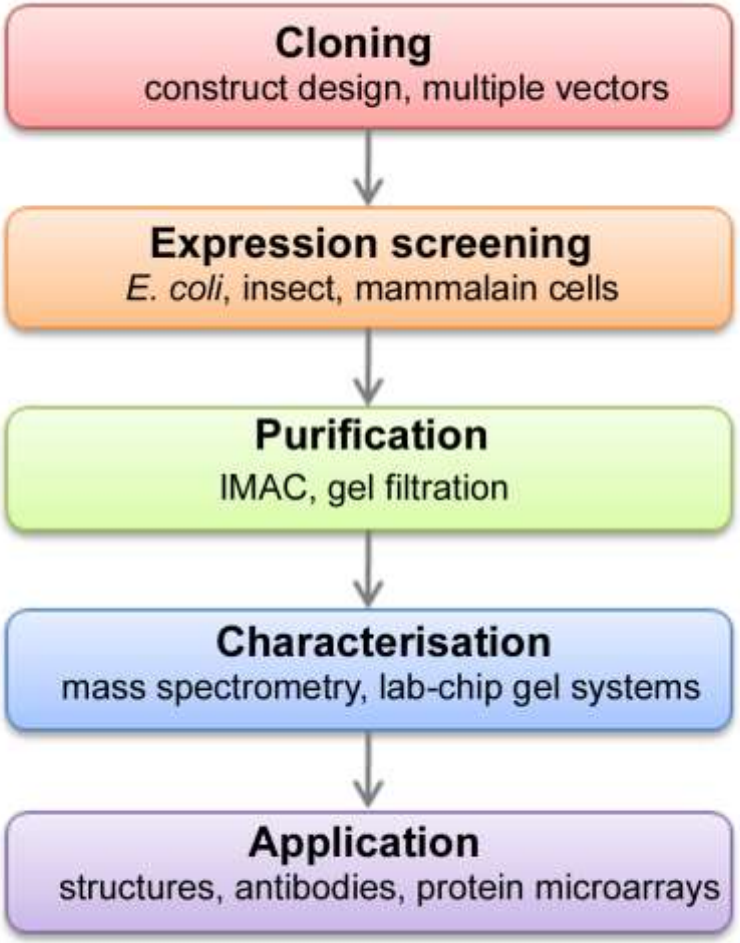
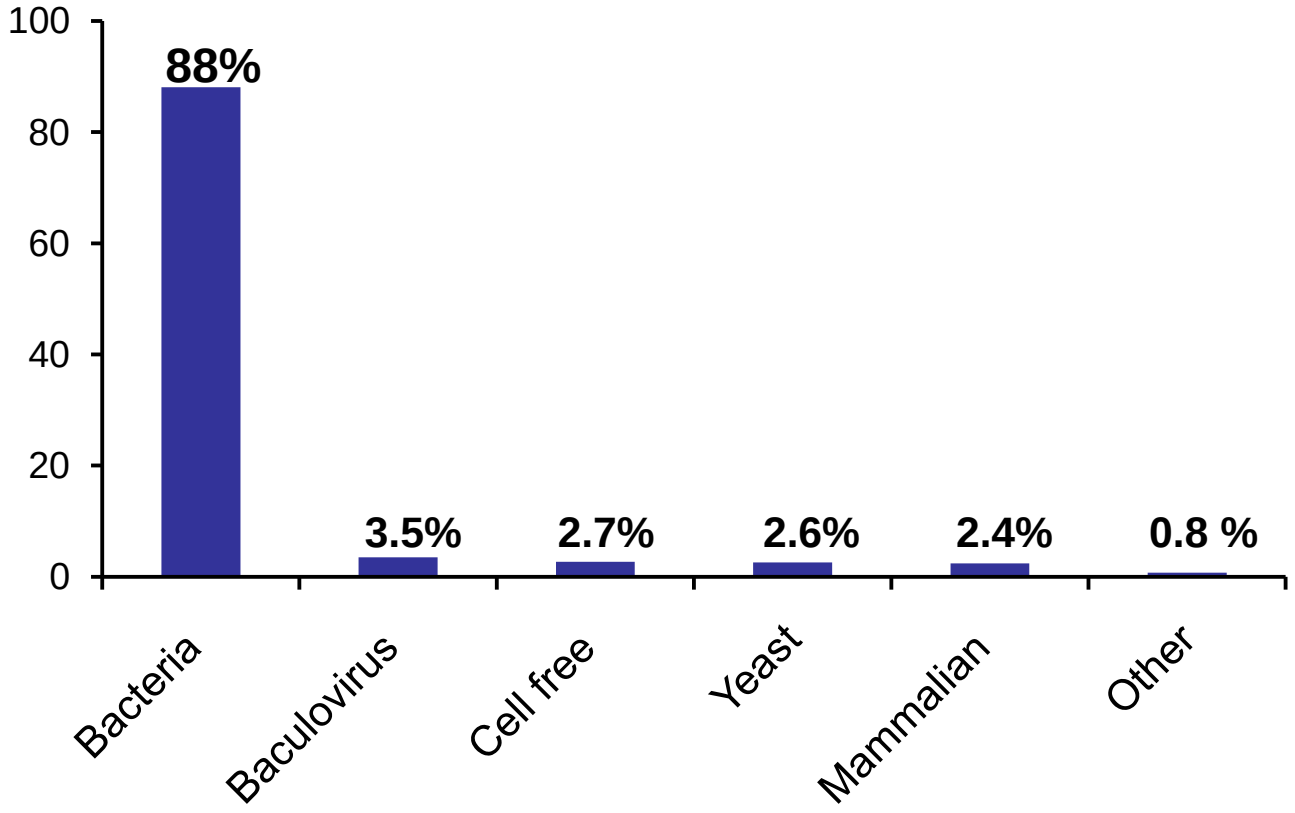
Generic workflow



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.

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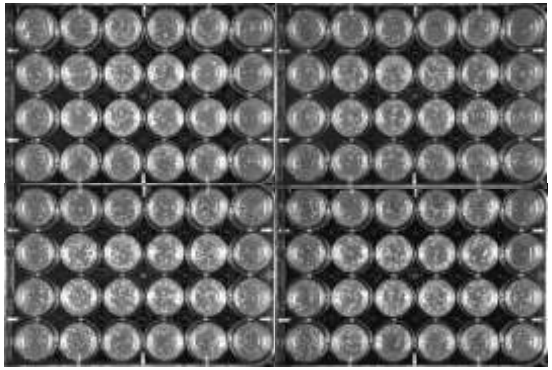
96 Well PCR Cloning

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

Quality assessment.



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

Total elapsed time = 5 days

Miniprep and PCR verification.

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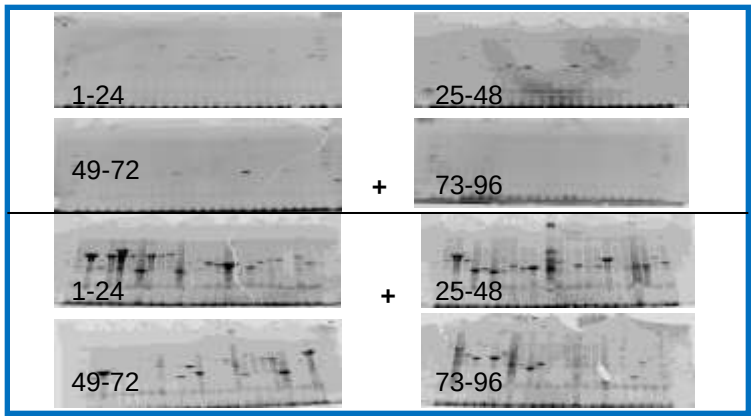
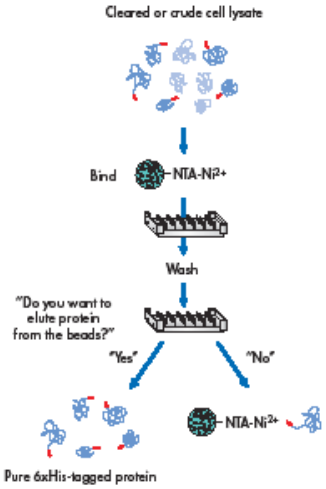
Expression screening in *E.coli*

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

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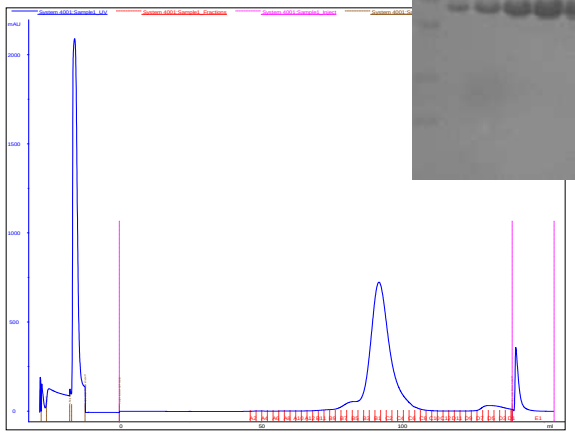
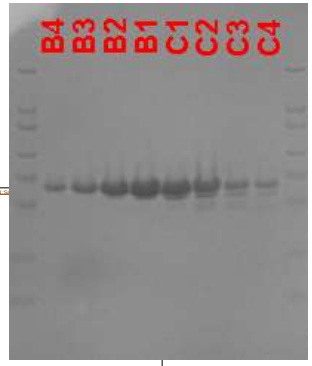
Protein Purification

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).

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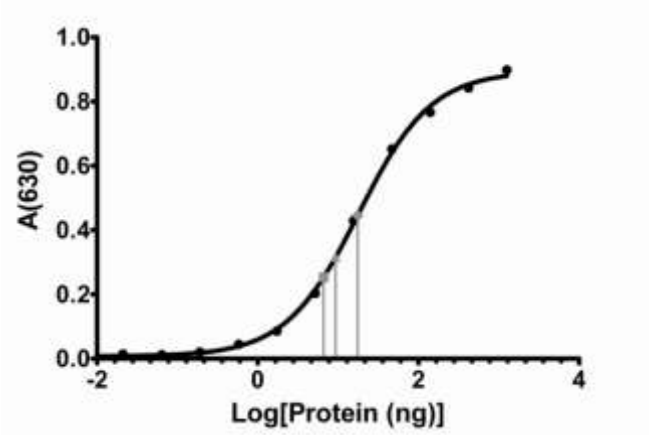
Expression screening in mammalian cells



- Tecan EVO_{75} 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.

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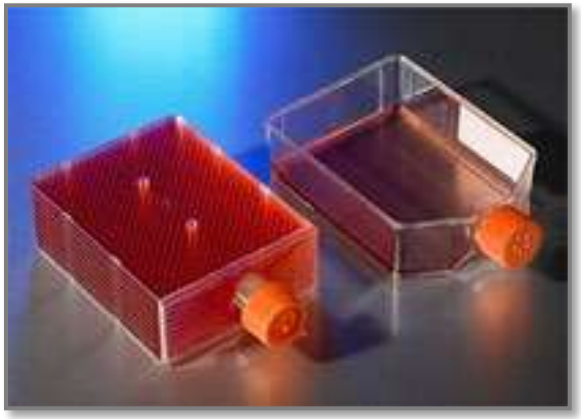
Vizier

CompacT SelecT System

- Ability to grow, passage and transfect multiple cell lines
- Temperature and CO_2 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^{TM} flasks = 1720 cm^2 (cf 2125 cm^2 roller bottle).
- Approx. x 3 cost/ L than roller bottle process.
- Running at approx. 30 L culture/ month



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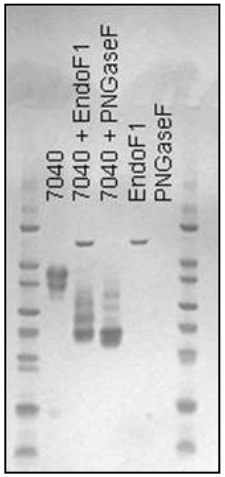
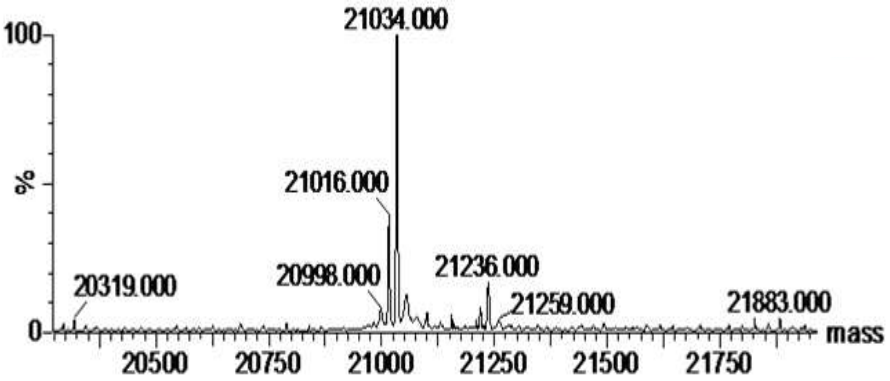
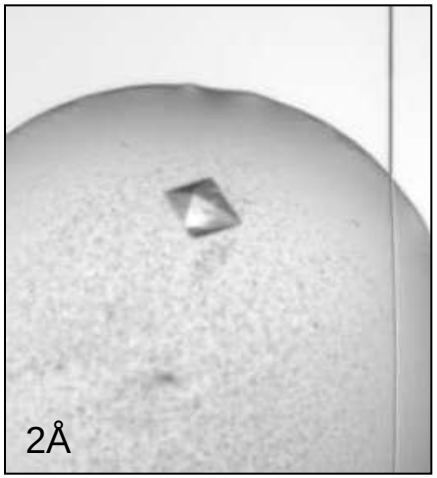
Comparison of Transient Transfection in Roller Bottles and using the SelecT System

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







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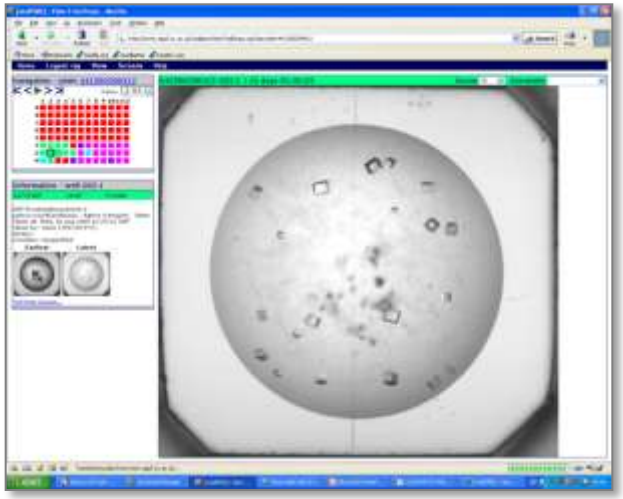
Semi-automated crystallization facility

- 100 + 100 nl sitting drop experiments.
- Automated storage and imaging (incl. UV) 2000 plates at 21^0 C and 1000 plates at 4^0 C.
- In-house software for inspection of plates (XtalPims).



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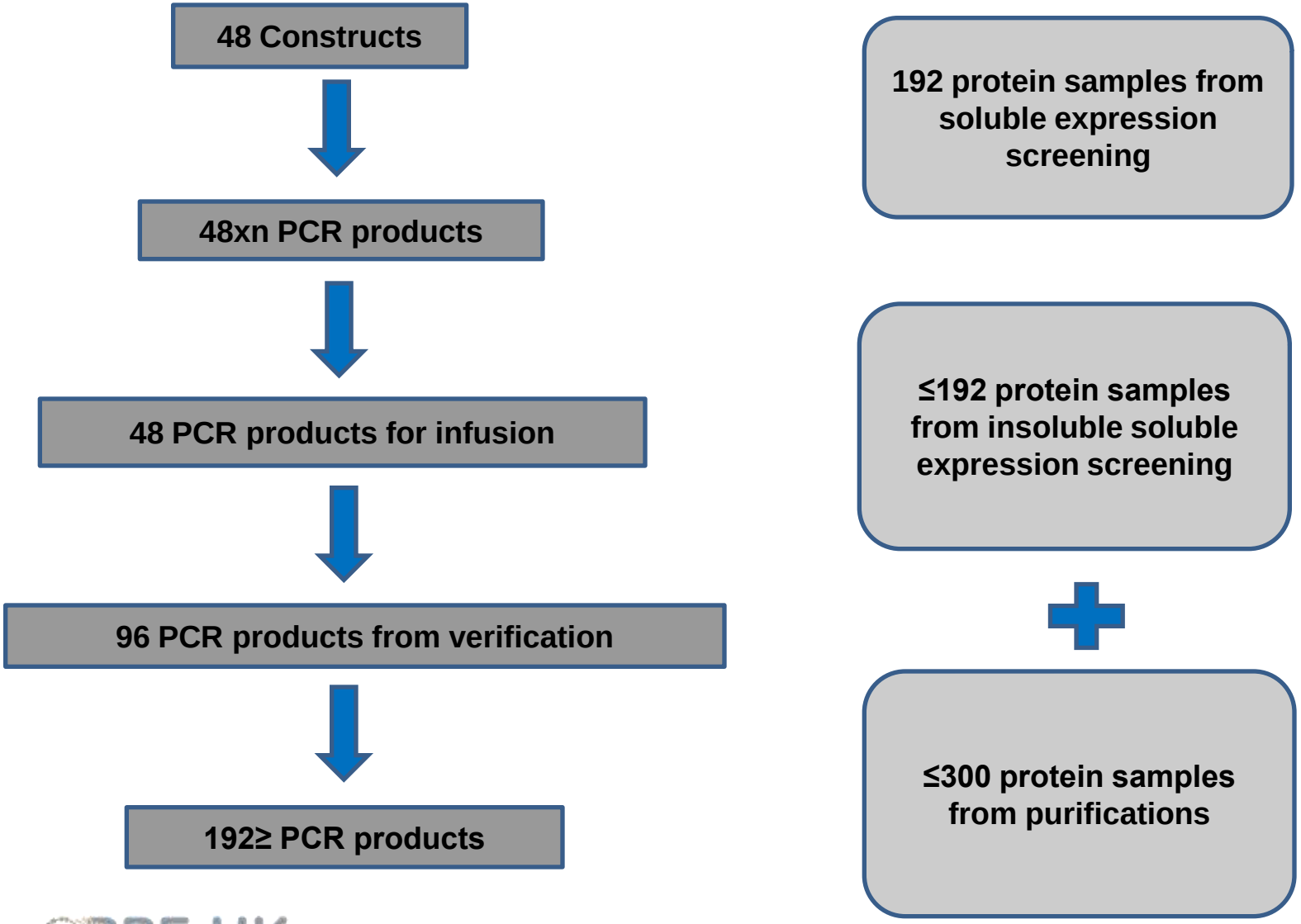
Sample Generation



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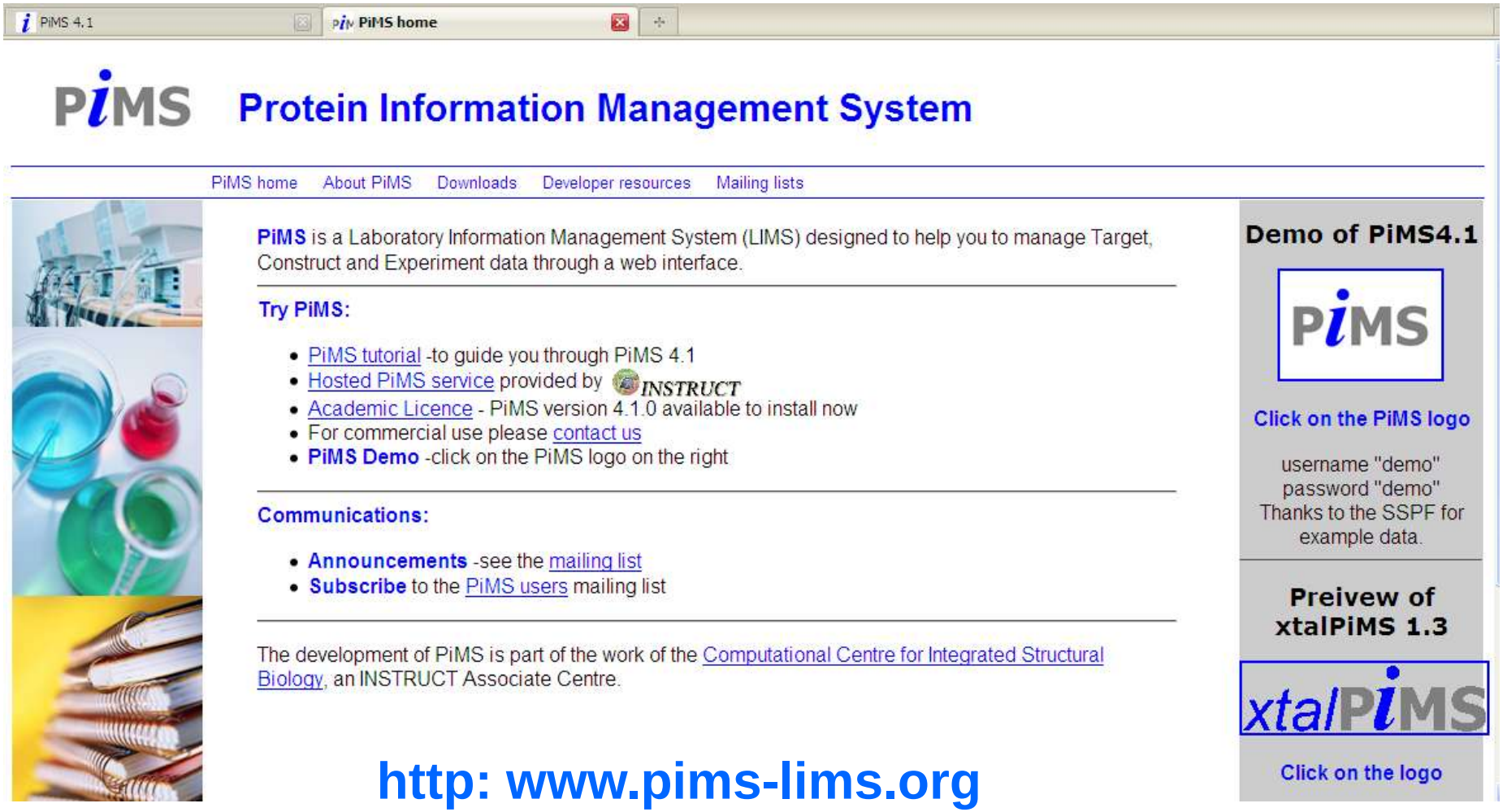
Data and Sample Management: PiMS



http: www.pims-lims.org

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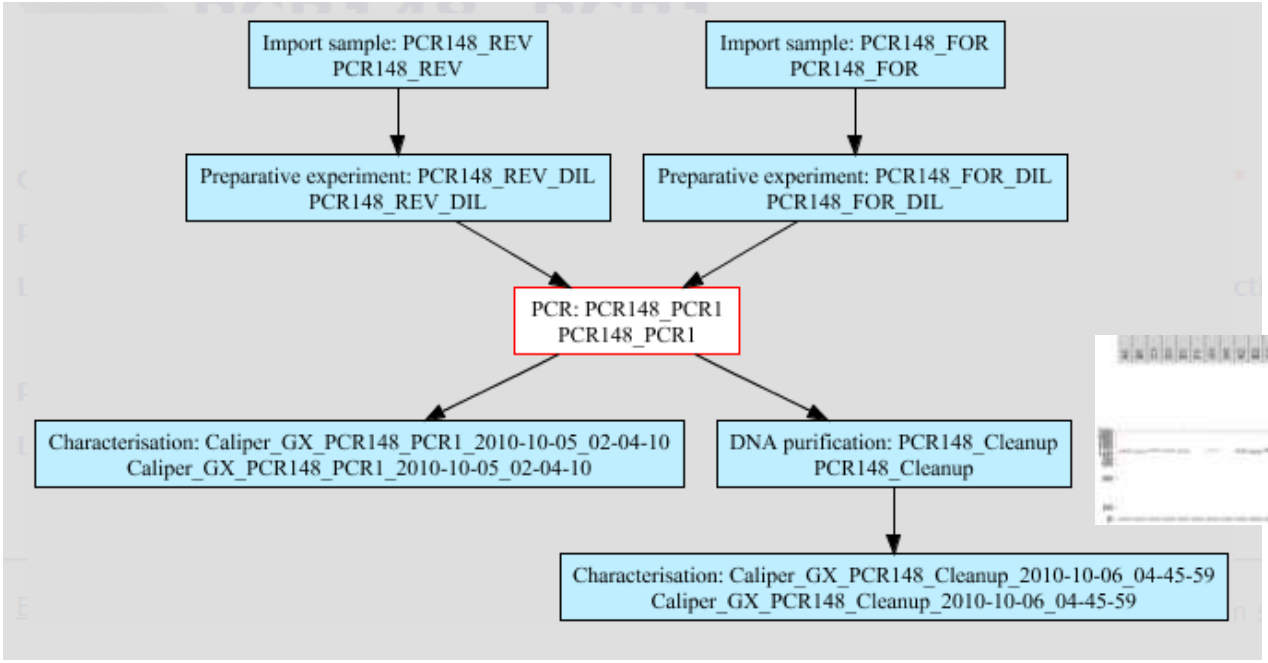
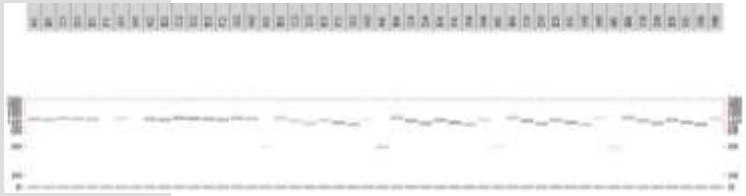
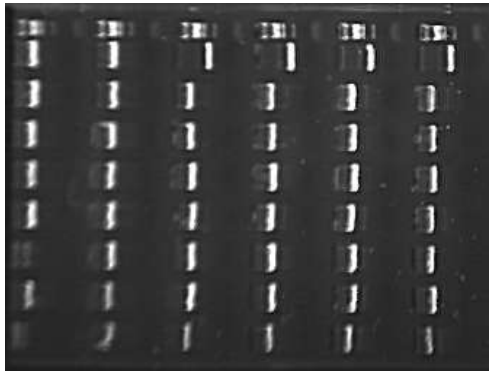
PiMS

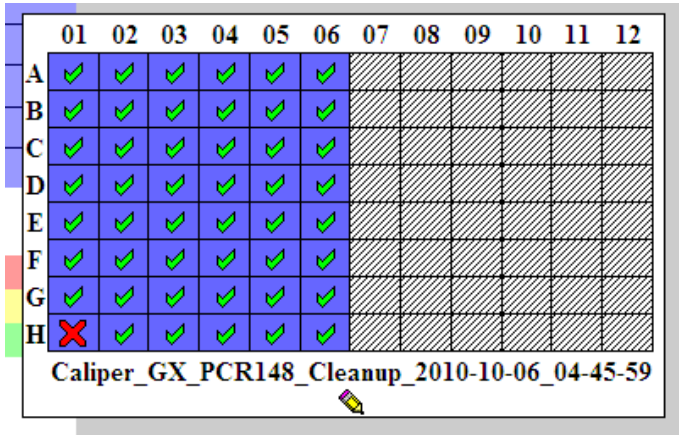
Recording PCR results







Manual assignment



Automated assignment

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The OPPF-UK workflow

Target selection

Source of templates

Design/order primers

PCR cloning

E.coli expression screen

Soluble

Insoluble

Specific re-folding

Soluble

Insect cell (co)-expression screen

Soluble

Mammalian (HEK293T) cell (co)-expression screen

SECRETED

Soluble

Scale-up + Purification, characterisation

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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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