Development of an Automated Platform for HT Cloning and Expression

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Introduction

➢ More and more frequently, research projects in fields like structural biology, protein-protein interaction and vaccinology require cloning, expression and purification of a high number of proteins.
➢In the Cloning and Expression Unit at Novartis V&D in Siena we have long been involved in Reverse Vaccinology projects, which requires the production and screening of hundreds of antigens with the aim to select the best vaccine candidates (Rinaudo et al., 2009). Then we decided to exploit automation in order to speed up and potentiate our high throughput (HT) procedures, even under the consideration of the significant advances made in the field over the latter years and successfully implemented to molecular biology techniques (Lien and Lee, 2010; Hughes et al., 2011).

Materials and experimental design

➢ Here, we report the successful set up of HT molecular biology procedures on an automated platform comprising two Hamilton liquid handler workstations (Fig. 1), whose technical features were designed and implemented to include all cloning steps.

➢ The adopted recombinant DNA procedure was the enzyme-free Polymerase Incomplete Primer Extension (PIPE) method (Fig. 2) (Klock et al., 2008), because its simplicity (mix together insert PCR and vector PCR and use the resulting mixture to transform *Escherichia coli* cells) and soundness make it particularly amenable for automation.

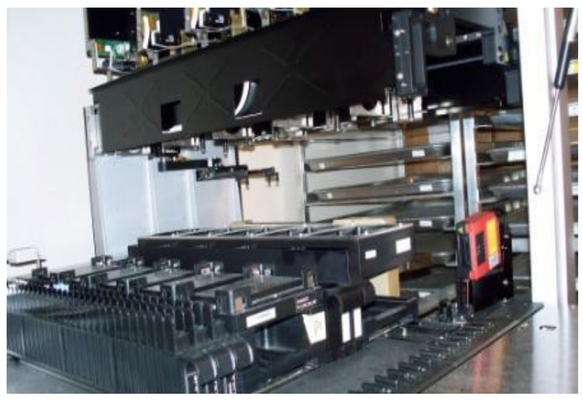

Figure 1. The Hamilton Microlab Star automated workstations

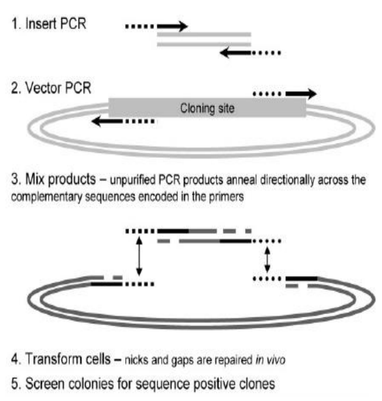

Figure 2. The PIPE Cloning method (image by Klock et al., 2008).

➢ Several vectors, such as pET15, pGex, pMal, pCold and speedET, were adapted to the PIPE method to clone the same PCR product (target gene) in all of them in a single step, allowing simultaneous expression in different systems or in different protein configurations. The lethal *ccdB* gene was introduced in all these expression vectors to limit the number of background colonies.

	Biomolecular protocols of the PIPE cloning method
1	PCR of the genes to be cloned (I-PCR) and agarose gel analysis (Fig. 3)
2	Mixing I-PCRs with V-PCRs, transformation (*E. coli* HK100 strain), plating
3	Colony picking and growth in 96-well microwell plates (Fig. 4)
4	Growth of PCR positive clones in 96-well deep well plates
5	Plasmid DNA purification and quantification
6	Preparation of glycerol batches of HK100 clones
7	Assembly of sequencing reactions in 96-well microwell plates
8	Transformation in expression strains BL21(DE3), BLR(DE3) or T7 Express
9	Small scale inductions in 96-well microwell plates
10	Preparation of both total and soluble protein extracts for SDS-PAGE (Fig. 5)
11	Preparation of glycerol batches of expressing clones

Table 1. Biomolecular protocols covering the whole PIPE cloning method implemented in HT automation.

Results

➢ The whole cloning process was adapted to automation (Tab. 1).

➢ Robotic protocols also cover high time spending-procedures, such as sample loading of agarose gels (Fig. 3) and colony picking and growth (Fig. 4).

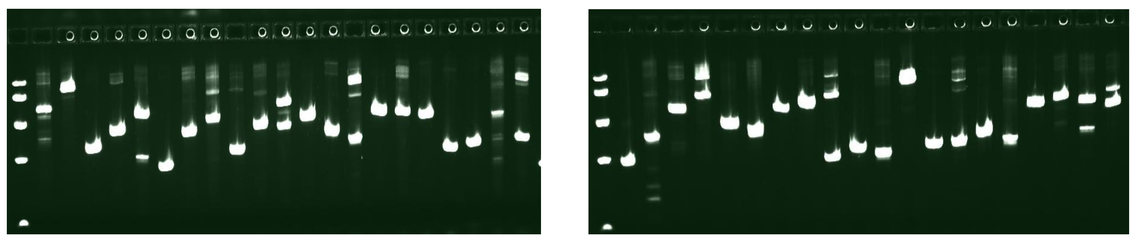

Figure 3. Agarose gel analysis of I-PCR amplicons obtained with bacterial genomic DNA as template.

➢ Achieved cloning efficiency averaged 84% while 64% of the protein antigens were successfully expressed (Tab. 2 and Fig. 5), speeding up the cloning workflow and optimizing quality, consistency and efficiency of the protocols.

➢ The PCR-screening of positive clones was omitted; since the analysis of two transformant colonies gave a 99% probability to get at least one PCR-positive clone, the entire cloning process was accelerated by directly sequencing the plasmids of two transformant clones and selecting the correct ones.

➢ Optimization of procedures for plasmidic minipreps leds to obtain DNA yields 5-fold higher than with standard protocols and to an increase in both plasmid quality and transformation efficiency.

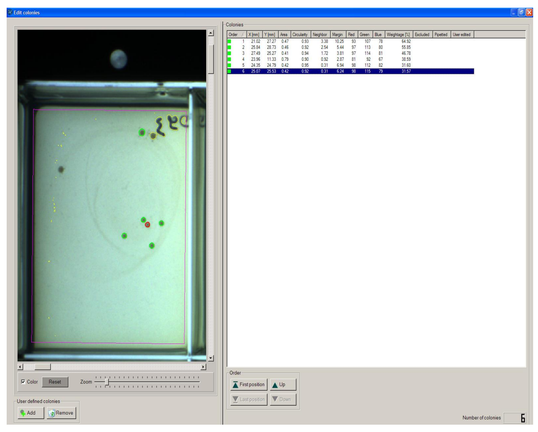

Figure 4. Software for colony picking and growth in 96-well microwell plates. Among colonies identified by the workstation platform and evaluated as “pickable” (green) (“non-pickable” colonies are in yellow), selection for picking and subsequent inoculation in 96-well grown (red) may be performed by either manual or automated fashion.

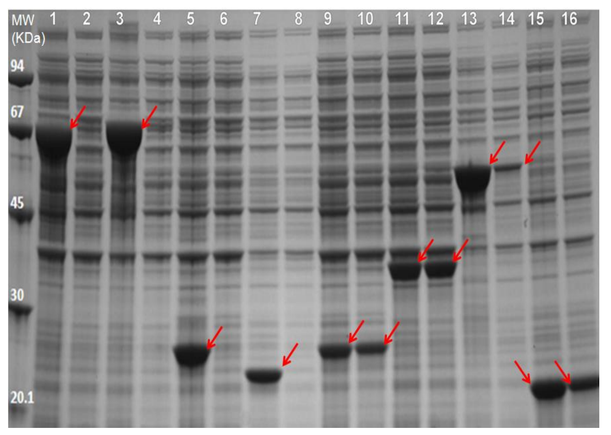

Figure 5. SDS-PAGE analysis of total (odd wells) and soluble (even) protein extracts obtained from small scale inductions. Bands of recombinant proteins successfully expressed are pointed out by red arrows.

	Success Rate
Genes cloned in HK100 cells	84.0%
Genes subcloned and expressed in BL21(DE3), BLR(DE3) or T7 Express cells	82.4%
Clones expressing the expected protein	64.2%

Table 2. Cloning genes in HK100 cells and subcloning in BLR(DE3), BL21(DE3) or T7 Express cells: Success Rate of Automated HT Procedures

Conclusions and Outlook

- **Biomolecular protocols covering the whole cloning process were implemented in liquid handler robots.**
- **Achieved success rates were comparable to the ones usually obtained using manual procedures.**
- **In comparison with the manual approach, automation significantly speeds up HT cloning and expression workflow by optimizing consistency, robustness and efficiency of the process.**

References

Hughes SR et al. *Journal of Laboratory Automation* 16: 17-37. - Klock HE, Koesema EJ, Knuth MW, Lesley SA. *Proteins*. 2008; 71(2): 982-94. Combining the polymerase incomplete primer extension method for cloning and mutagenesis with microscreening to accelerate structural genomics efforts. - Lien KY, Lee GB. *Analyst* 2010; 135 (7), 1499-1518. Miniaturization of molecular biological techniques for gene assay. - Rinaudo CD, Telford JL, Rappuoli R, Seib KL. Vaccinology in the genome era. *J Clin Invest.* 2009; 119(9): 2515-25.

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