

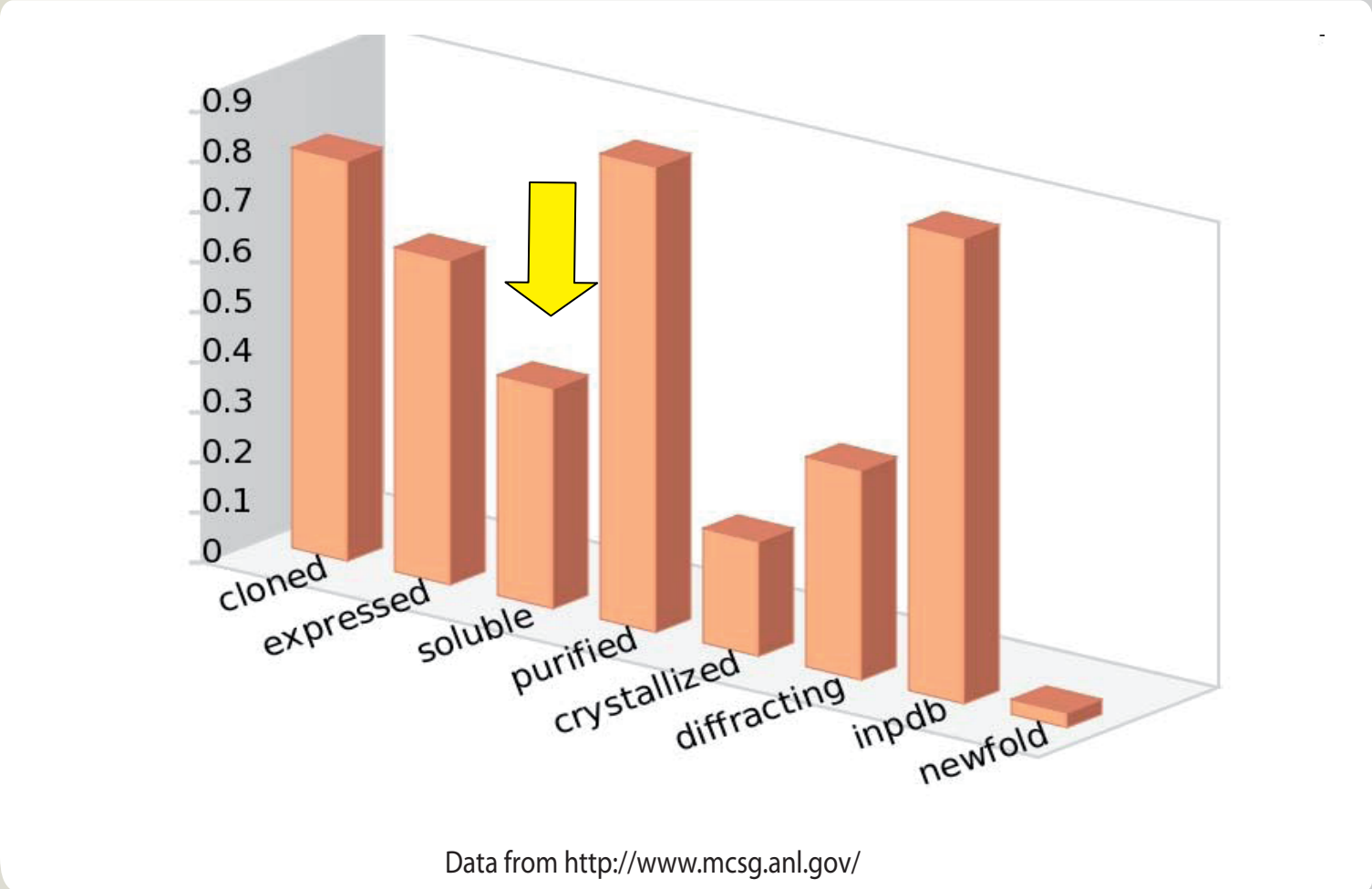
Strategies for expression and solubility analysis.

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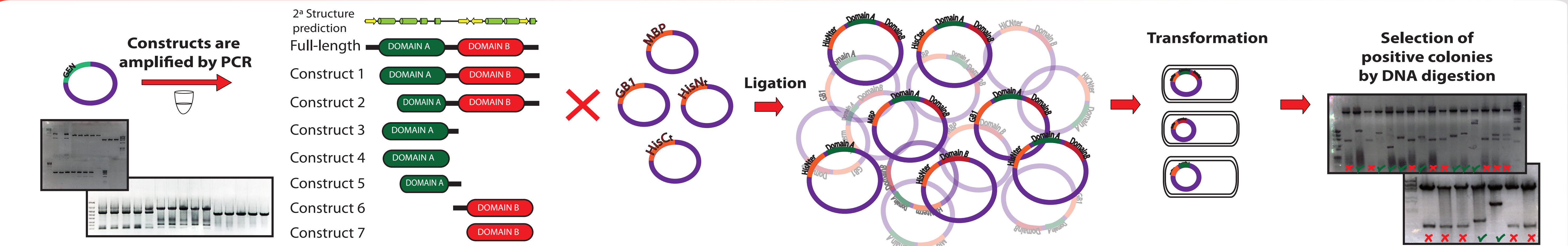
Introduction

Production of soluble protein is one of the major bottlenecks that precede crystallographic studies. During the last years several techniques and strategies have been developed to address this problem. However, many of them imply an economical cost and technologies that are not always available. We will describe a general plan for protein solubility analysis by using a combination of four different but complementary strategies. In this plan, different constructs of a protein interest are designed and cloned in a library of expression vectors. All constructs are subsequently tested for bacterial overexpression using different strains at different culture conditions. The last step consists of a solubility screening for each construct tested for a specific strain and expression condition, by using different buffers designed according to a semi-rational approach.



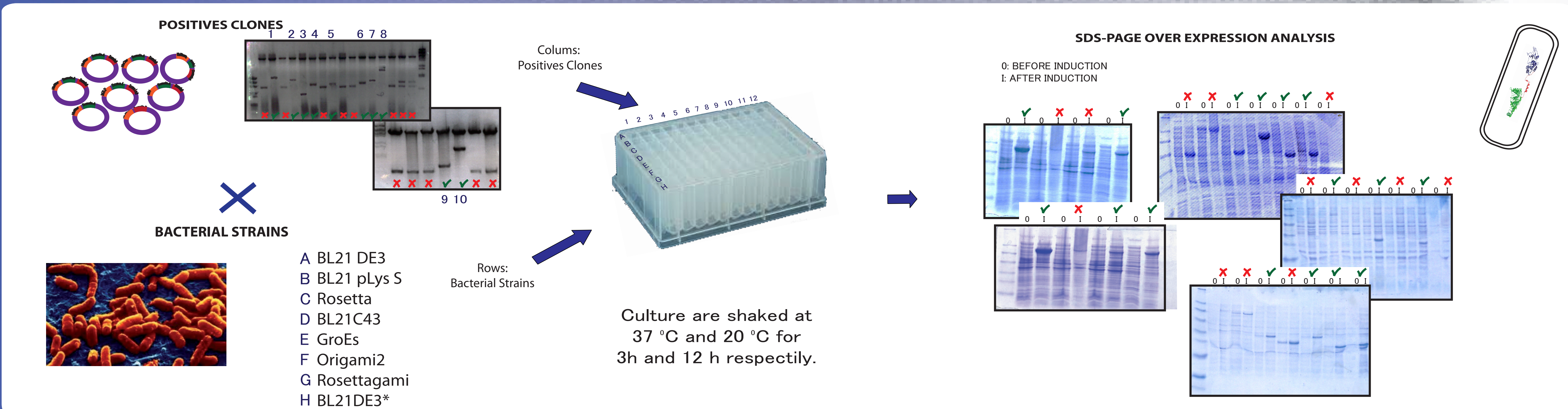
Cloning

To produce protein the first step is cloning. Different constructs of a protein of interest are designed and amplified by PCR introducing in all of them the same double restriction site present in a library of expression vectors, which will add different tags (MBP,GB1, His-tag at the N- or C- terminus) to each construct. Partial constructs are designed taking account the of homology or secondary structure prediction of the protein, so that interruption of secondary structure elements is avoided. The plan is divided in three steps:
1) Amplification the consctructs by PCR.
2) Digestion vectors and inssert with only two restriction enzymes, ligation and transformation into *E.coli*
3) Selection positives clones by plasmid digestion.



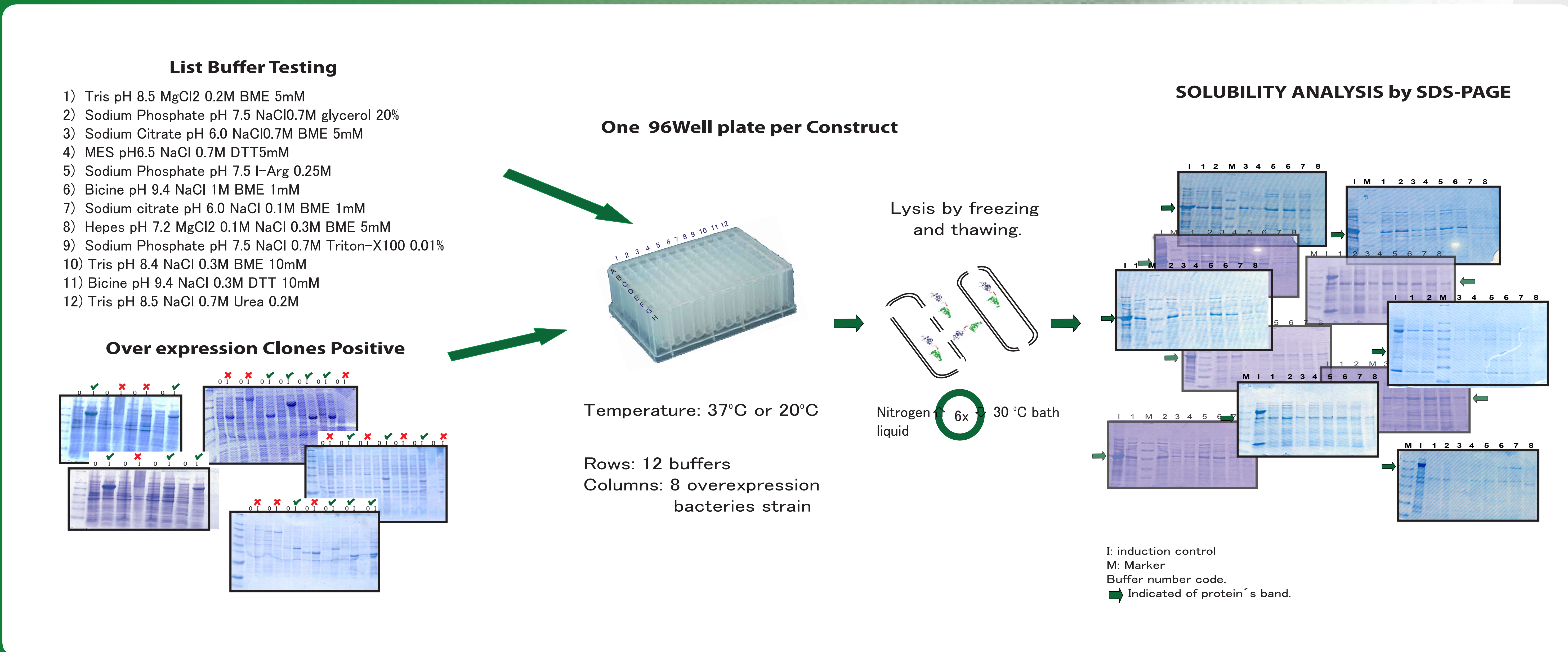
Expression

The second part is the analysis of the expression of all postives clones. First, eight different bacterial strains are trasnformed by one clone and grown in 96 deep-well plate. In this plates 12 cosntructs (X-axis) are tested agains + 8 bacterial strains (Y-axis). Expression test are performed in duplitates to test the induction at two temperature, 37°C and 20°C, both induced with 1mM IPTG. Analysis the results is performed by SDSPAGE where a sample of the culture is compared before and after induction.



Solubility

The third step the solubility analysis is the most critical point. Once known which conctruct is expressed in a bacterial strain, the critical point is establish the optimal chemical conditions for protein solubility. The strategy consist in growing each positive clon a 96well plate, in rows are dispensed the different strains, and, after centrifugation, the pellet is resuspended in 12 different buffers in the Y direction of grid. Bacteriaes are broken by freezing and thawing: 96-well plate is immersed in liquid nitrogen and melted in a 30°C water bath, and vortexed; this process is repeated 6 times. After lysis the sobluble and insoluble fractions are separated by centrifugation, both are loaded in a SDSPAGE for analysis.



Conclusions

The design of different conctructs cloned in vectors with different tags allows us to have a large test battery to hunt the most stable soluble fragment of our protein of interest. The analysis of multiple bacterial strains of bacteria in an orderly fashion with each positive clone and the subsequent combination with a screening of system buffers offers a way to map the solubility quickly and effectively. In addition, the simple system of 96 wells plate using 2ml culture and analysis by SDS-PAGE and makes it affordable for any structural biology laboratory.