

# ESPRIT & CoESPRIT: Soluble fragment screening for structural biology

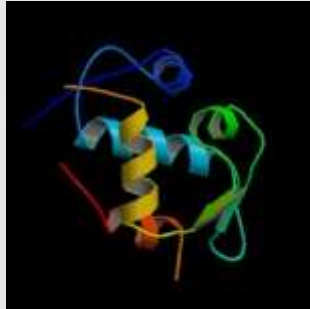
Darren Hart  
Grenoble



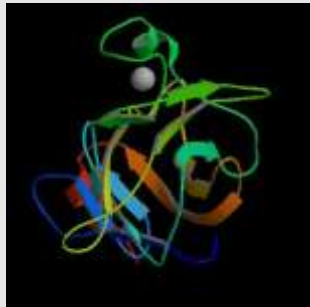
# Introduction

- ESPRIT: Library-based construct screening
  - Domain hunting in poorly understood proteins
  - Construct optimisation for crystallisation
- Applications & developments of ESPRIT method
  - Protein complexes: “CoESPRIT”
  - Intrinsically unstructured proteins
  - The reading frame problem: “ORF selector ESPRIT”

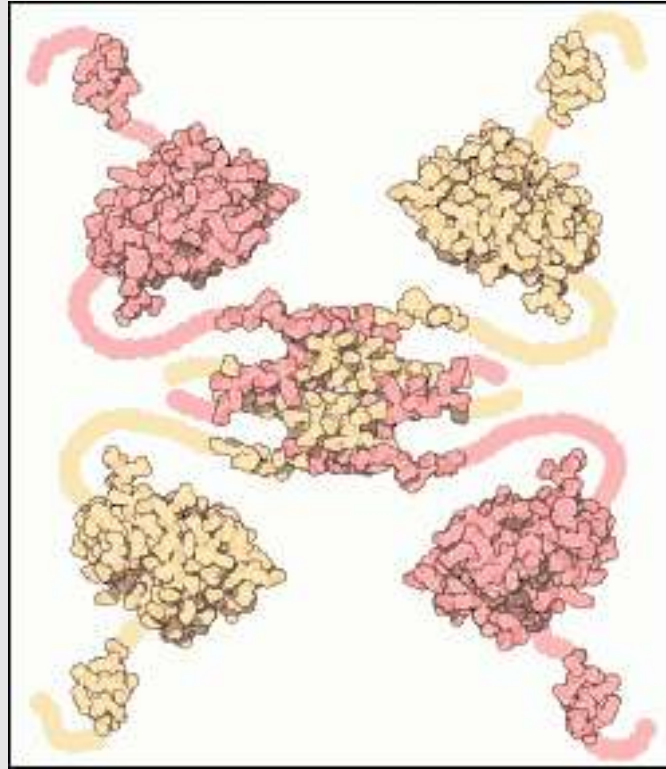
# Large proteins can often be divided into functional domains



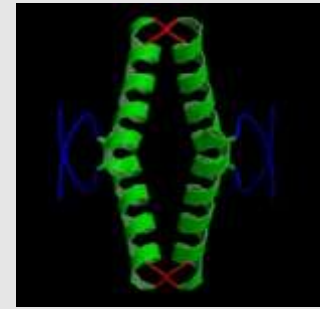
Transactivation domain



DNA-binding domain



p53 tumour suppressor



Tetramerisation domain

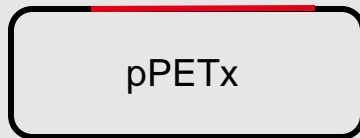
# What we want to replace:

Sequence alignments  
Bioinformatics  
Biochemistry  
Literature

} Hypothesise domain location



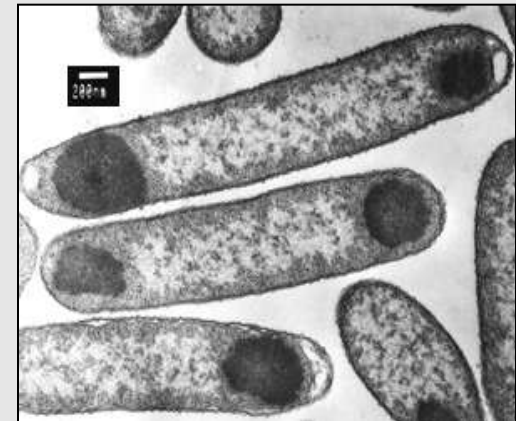
PCR



Cloning



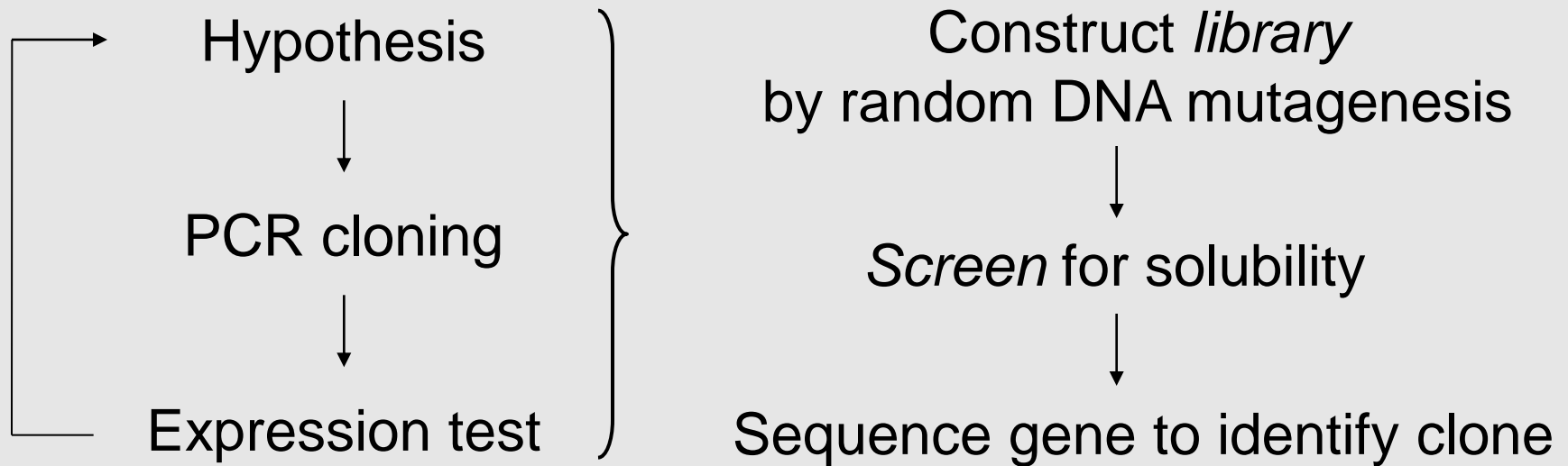
Expression



Requires (1) **Good information** (2) **Good luck** (3) **Time**

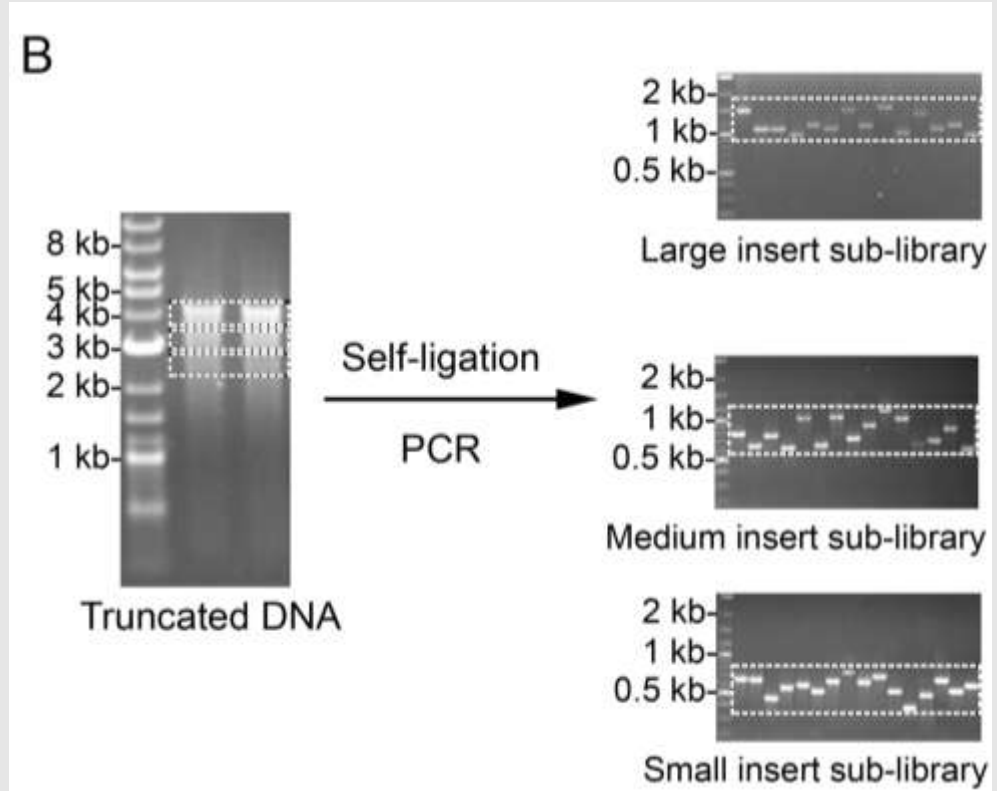
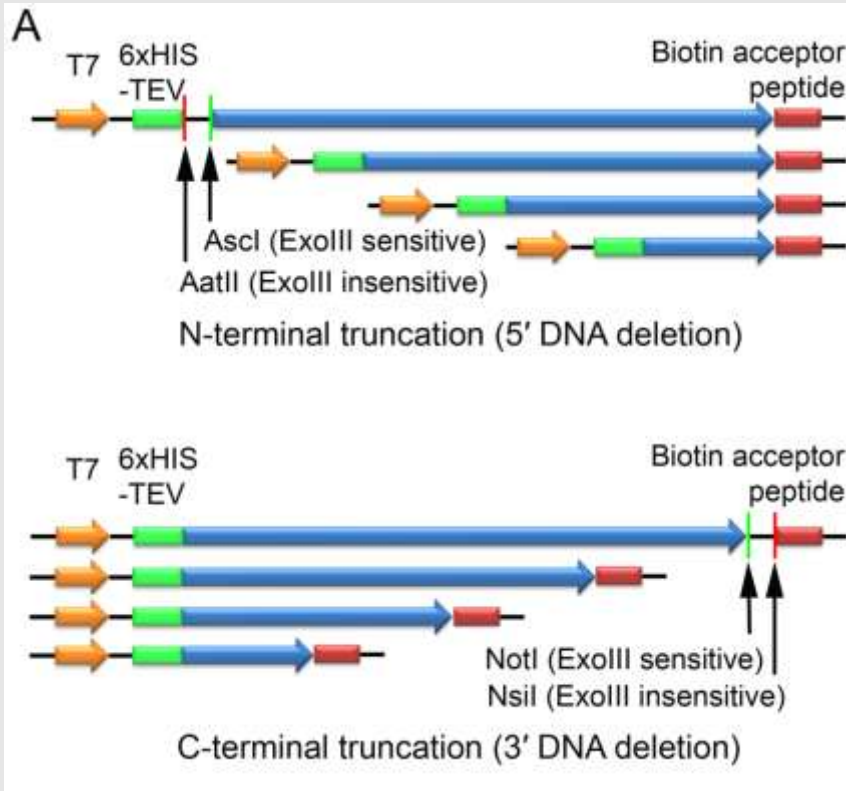
# THE CORE TECHNOLOGY

# Rational design vs screening

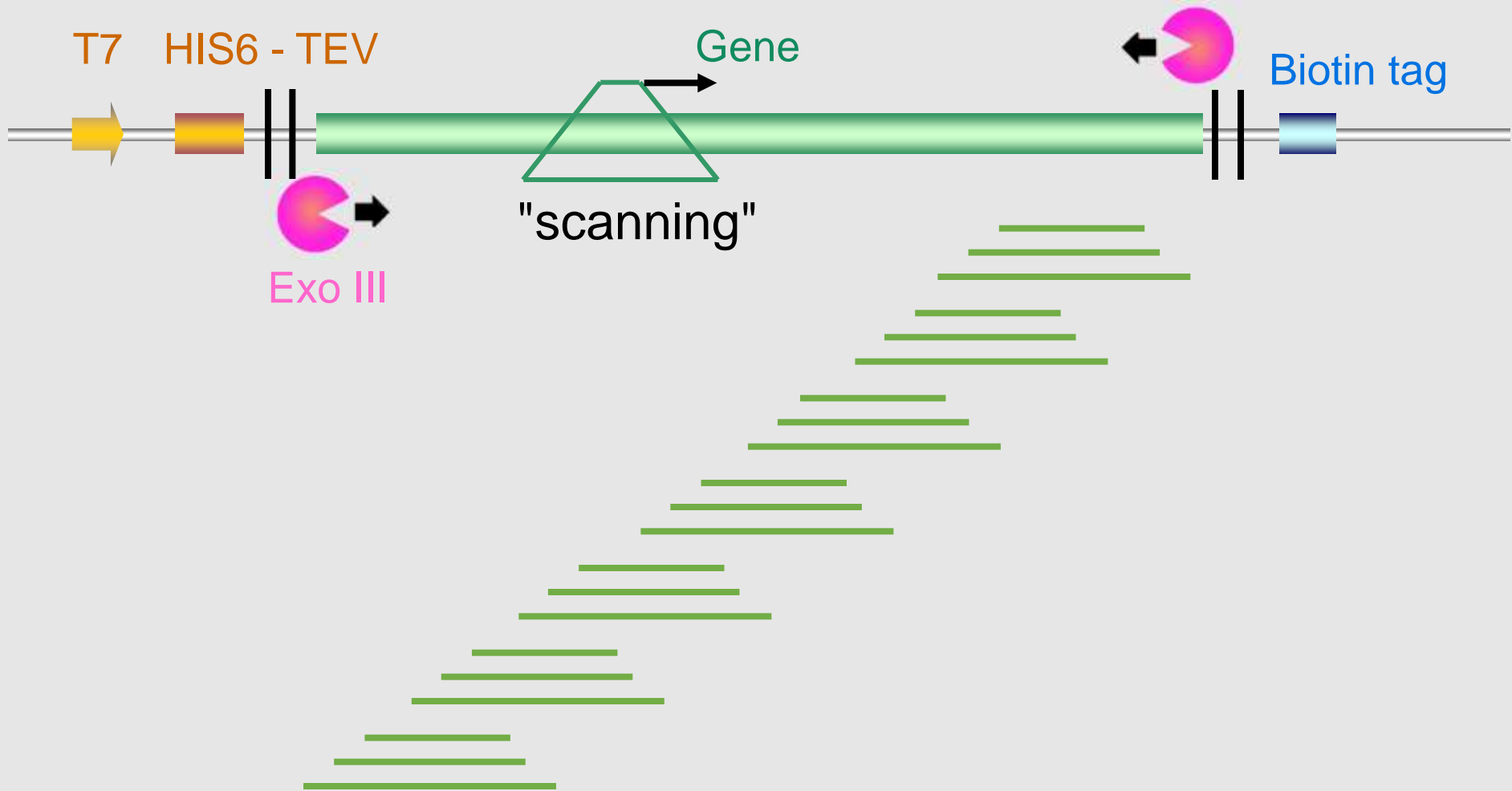


**ESPRIT (*Expression of Soluble Proteins by Random Incremental Truncation*)**

# Random DNA Truncation Libraries



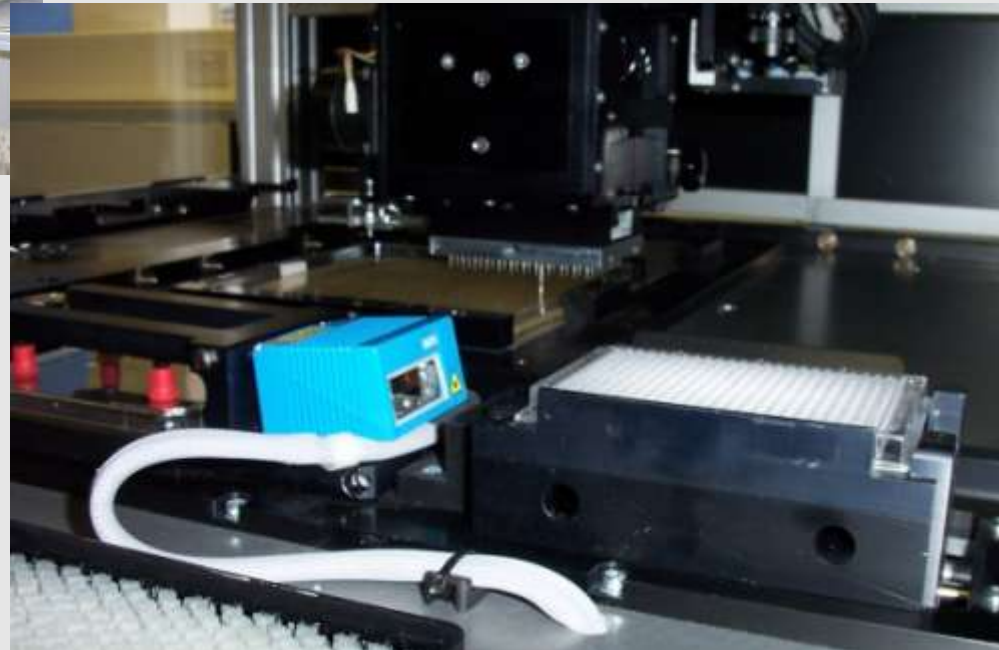
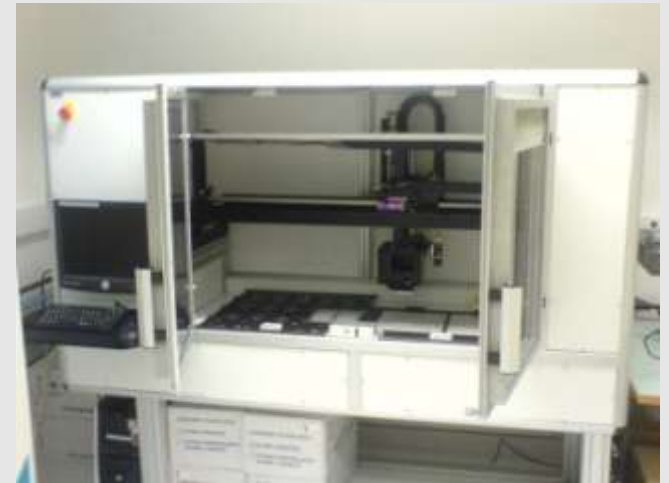
# *Bidirectional truncation: Internal Domains*





# Screening Robots

- Picker-gridder (genome sequencing)
- 4000 colonies h<sup>-1</sup> into 384 well plates
- Prints colony arrays
- Rearrays hits



Plated colonies

Lid lift

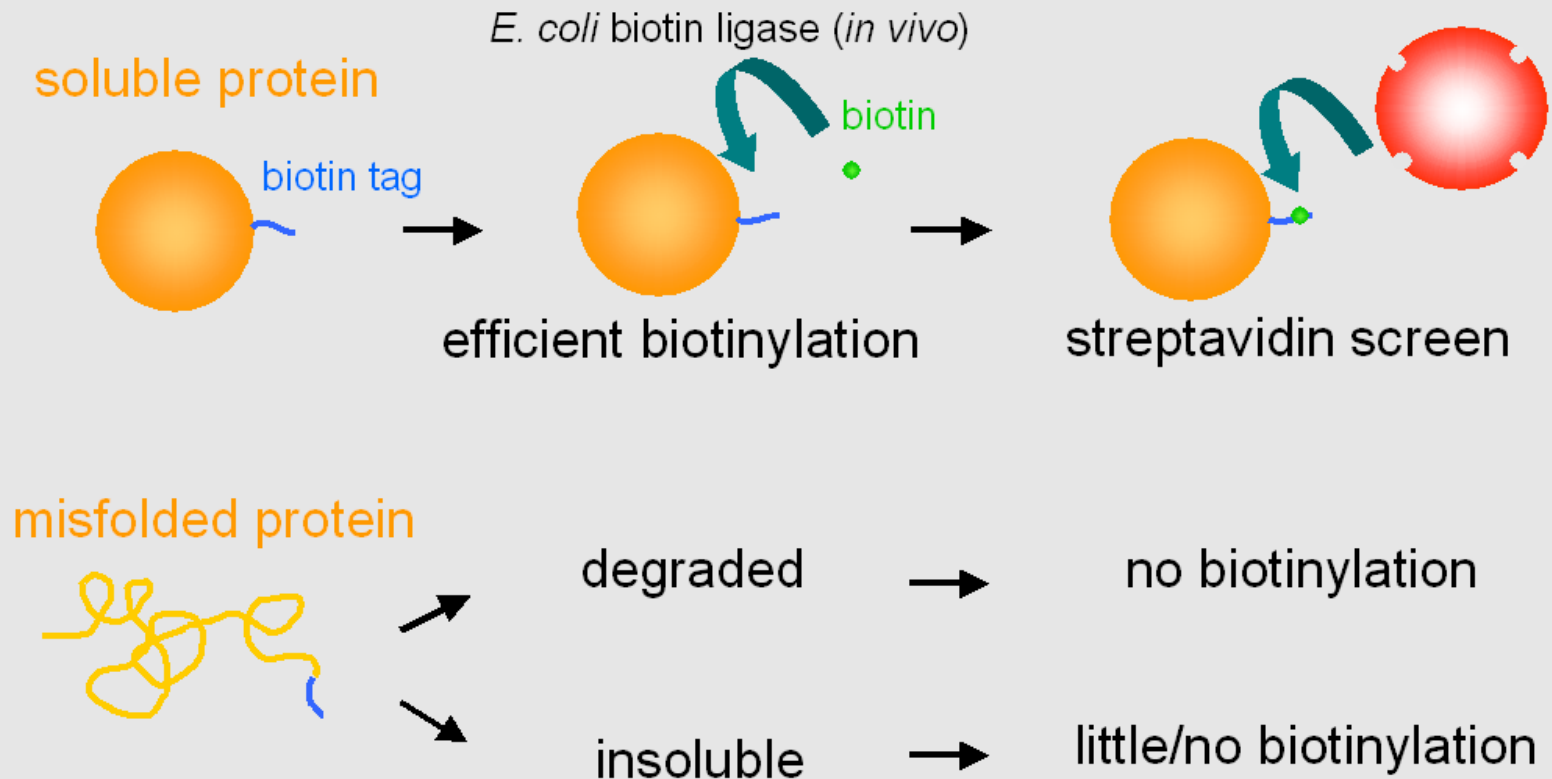
Pin sterilisation

Video camera

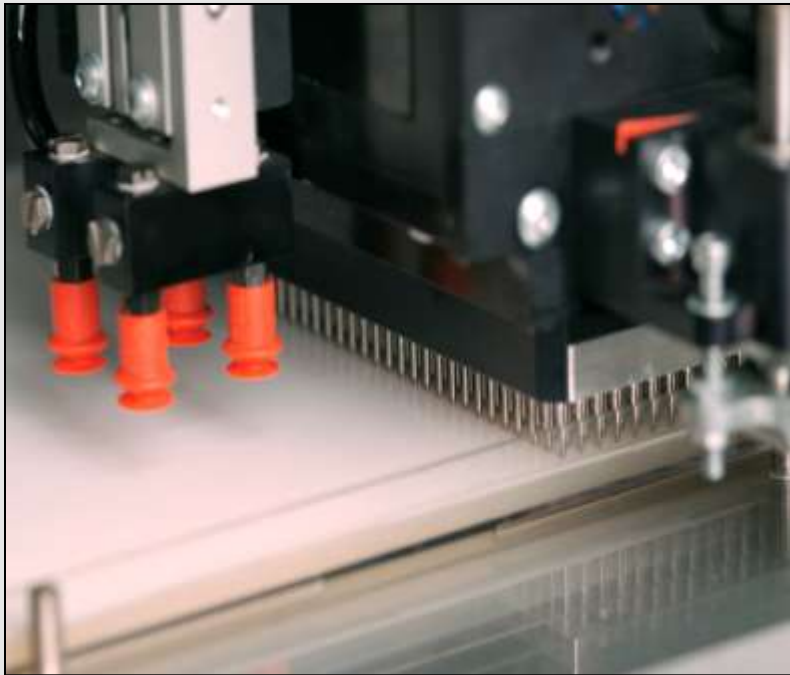
96 pin picking head

384 well plate

# *In vivo* Biotinylation as a Proxy for Solubility



# Test for Protein Expression

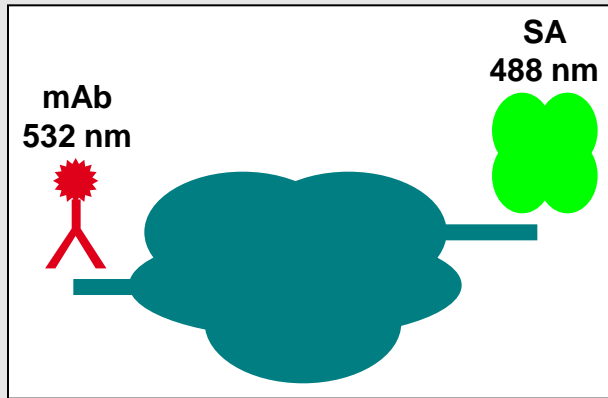


Printing clones on membranes

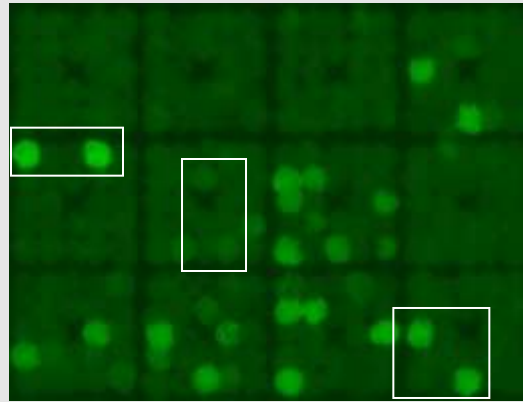


Colony arrays for protein expression  
28,000 genetic constructs

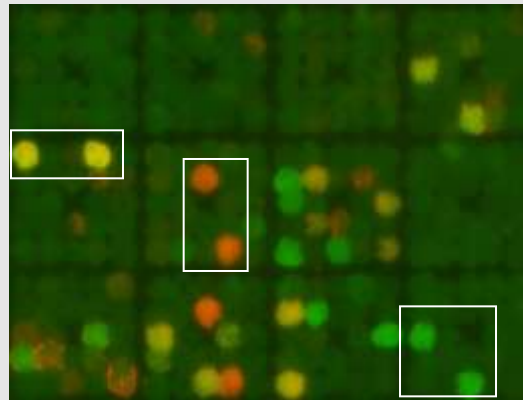
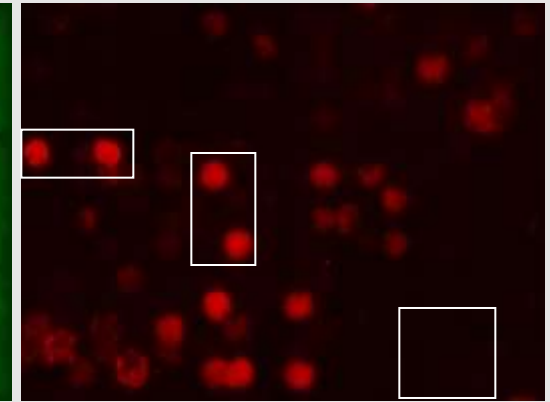
# Array-Based Expression Analysis



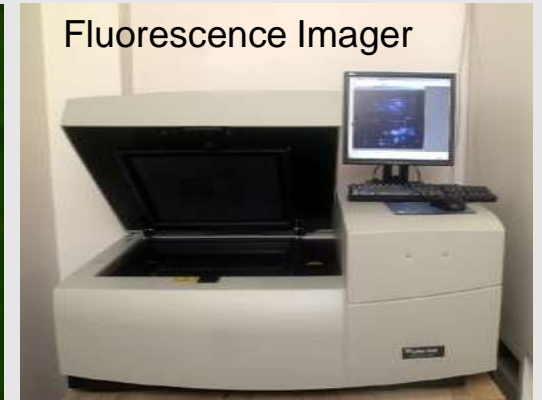
C-ter biotin



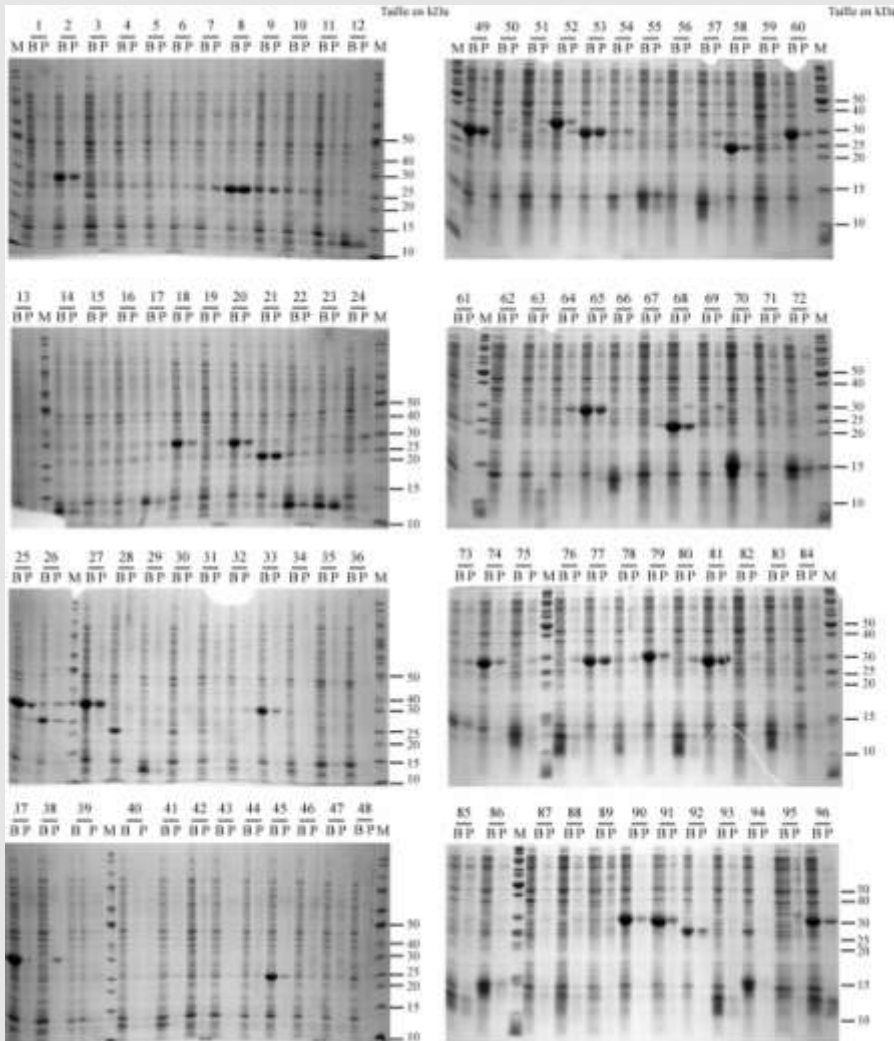
N-ter His tag



Merge

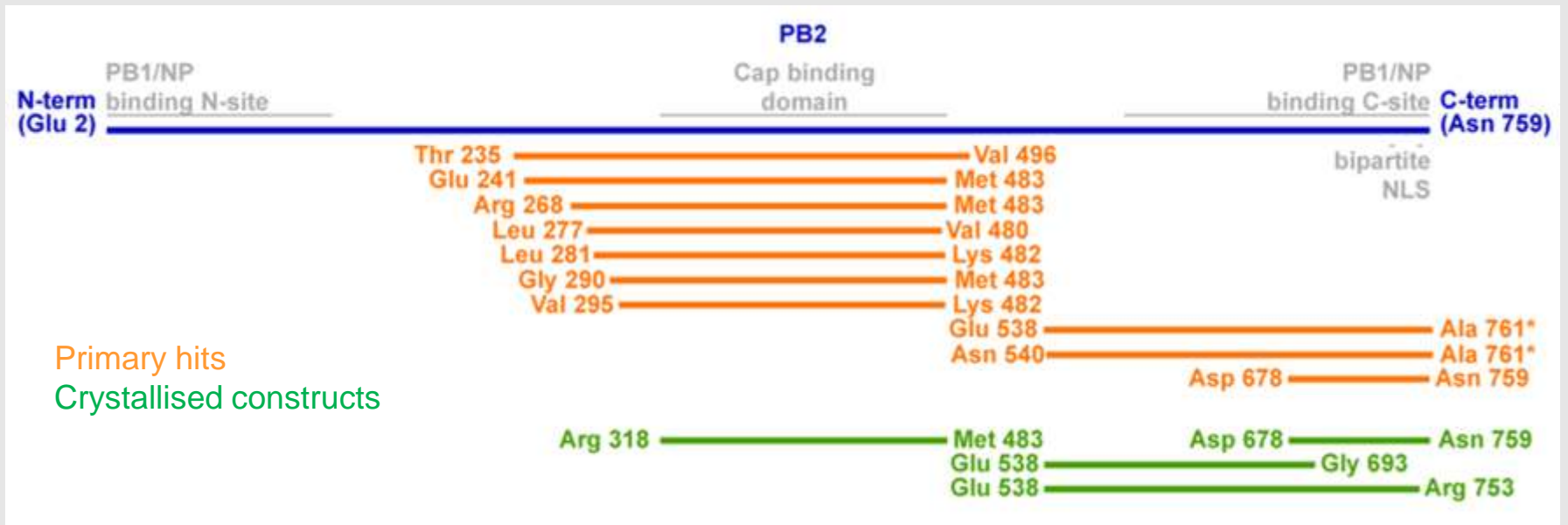


# His tag purif & analysis

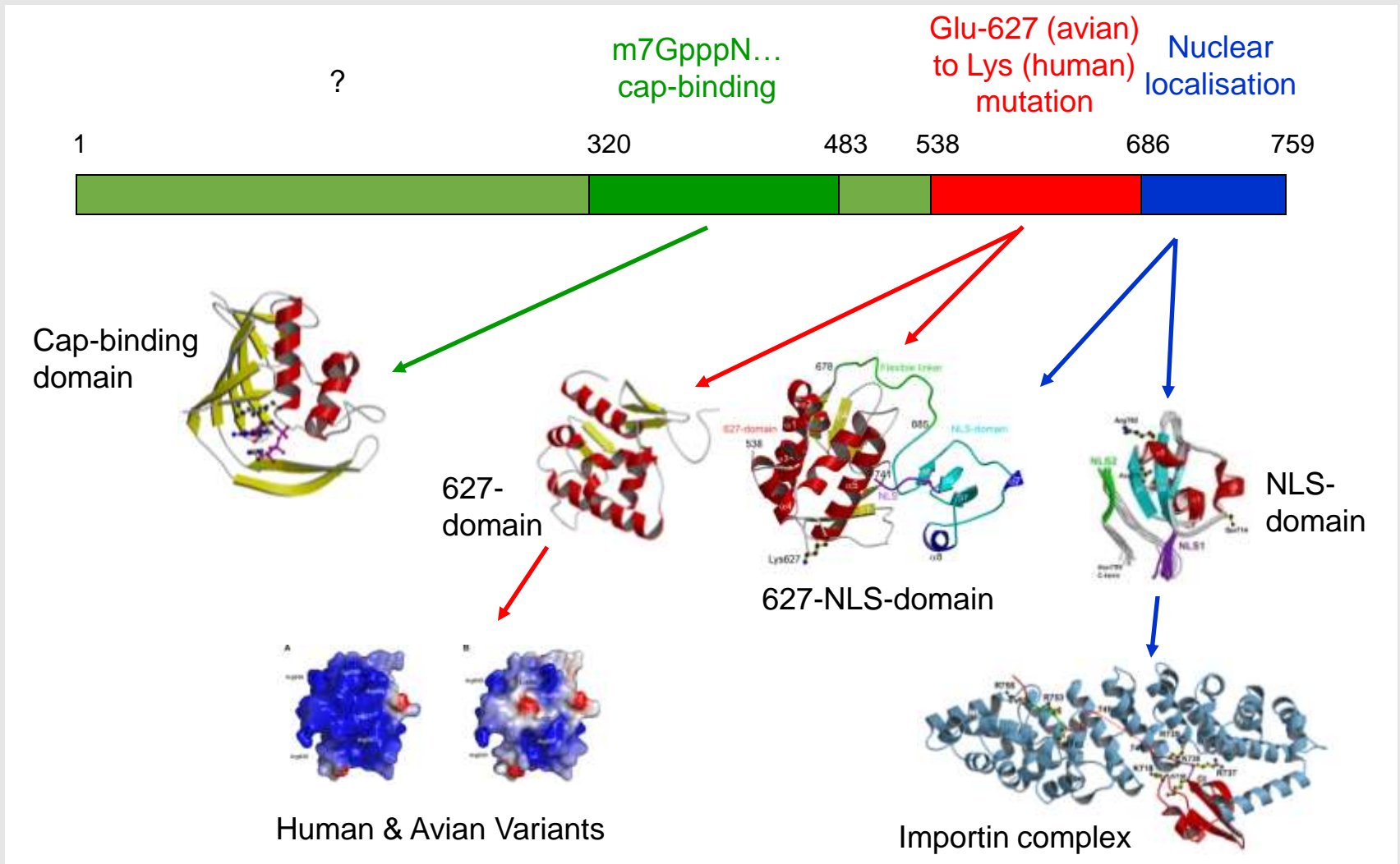


# SINGLE GENE SCREENING

# Unsuspected domains in an unannotated protein: influenza PB2

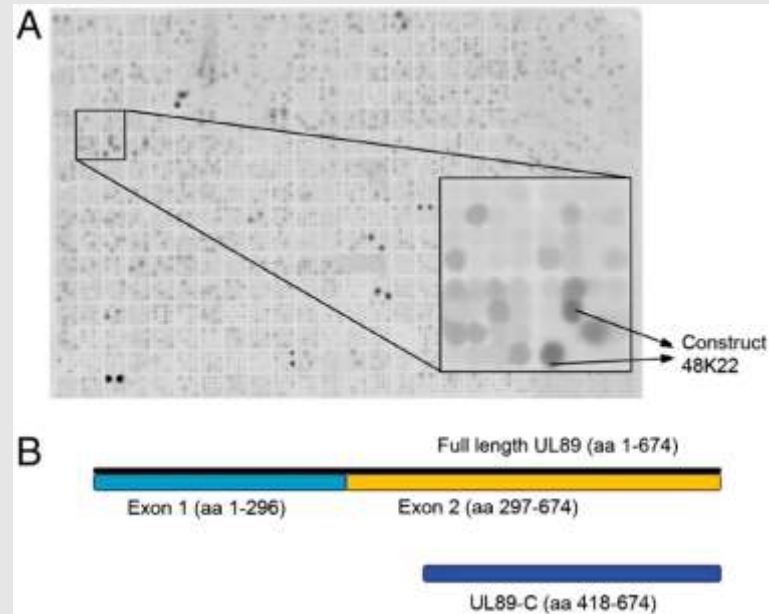
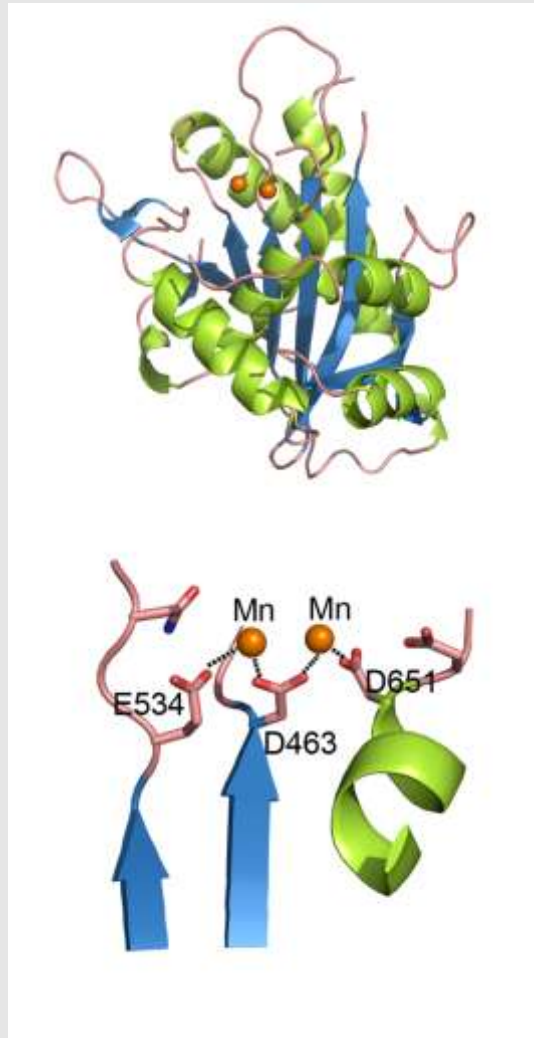


# Summary of Influenza Polymerase PB2





# HCMV terminase: a herpesviral drug target



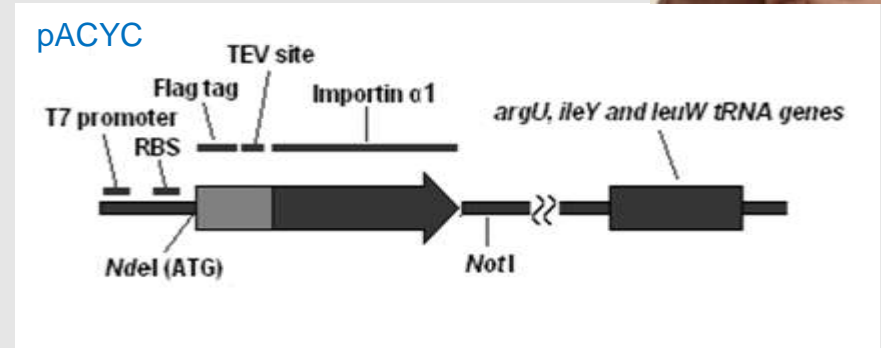
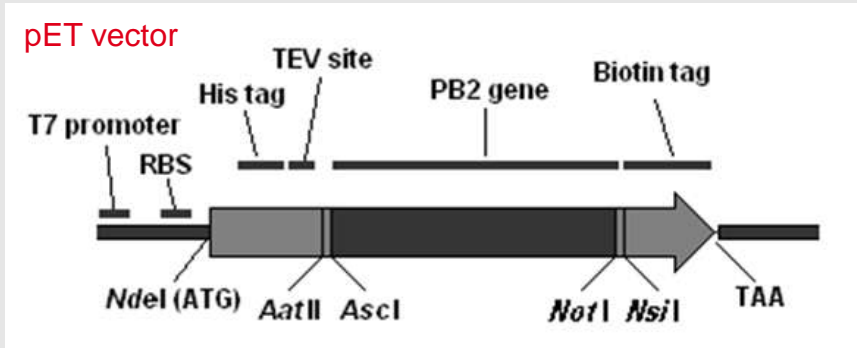
Coll Group, Barcelona  
3 yrs of failed expression expts  
ESPRIT: 28,000 constructs

# COMPLEX SCREENING

# CoESPRIT: Protein Complexes



Two compatible plasmids per cell:

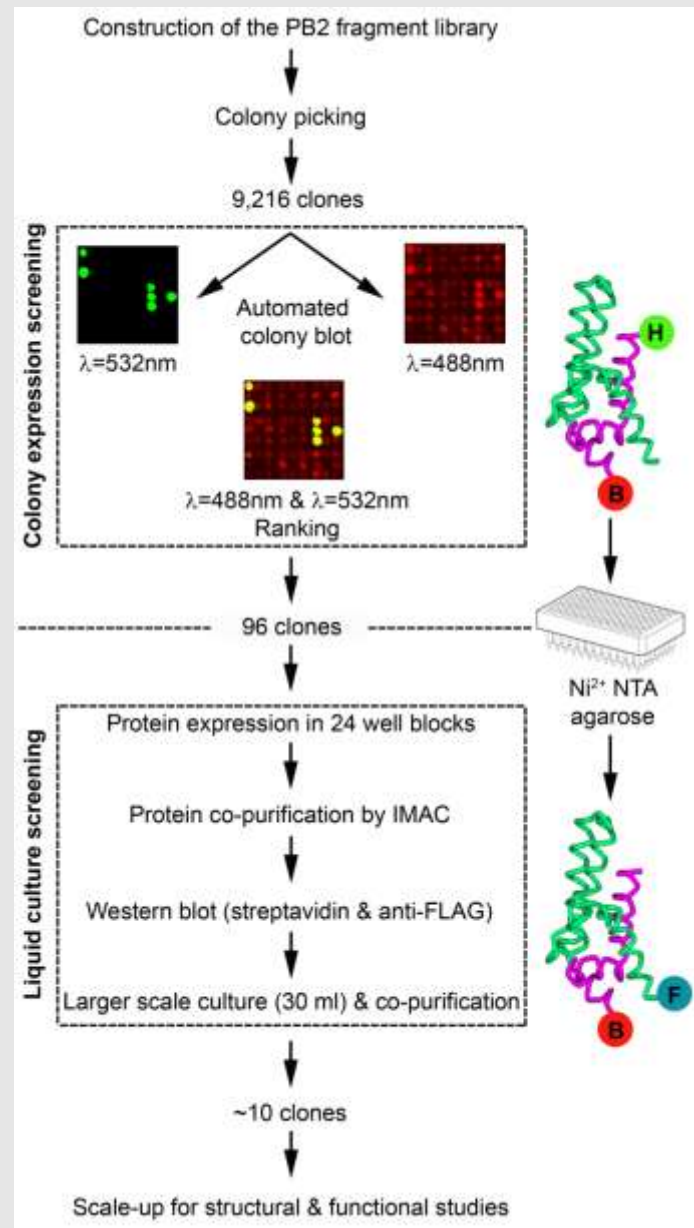


- “**Target**” plasmid 1
- Truncation library
- 6xHis *Purification* tag
- “**Bait**” plasmid 2
- Fixed partner
- Flag *Detection* tag

“*E. coli*-2-hybrid” expression screen

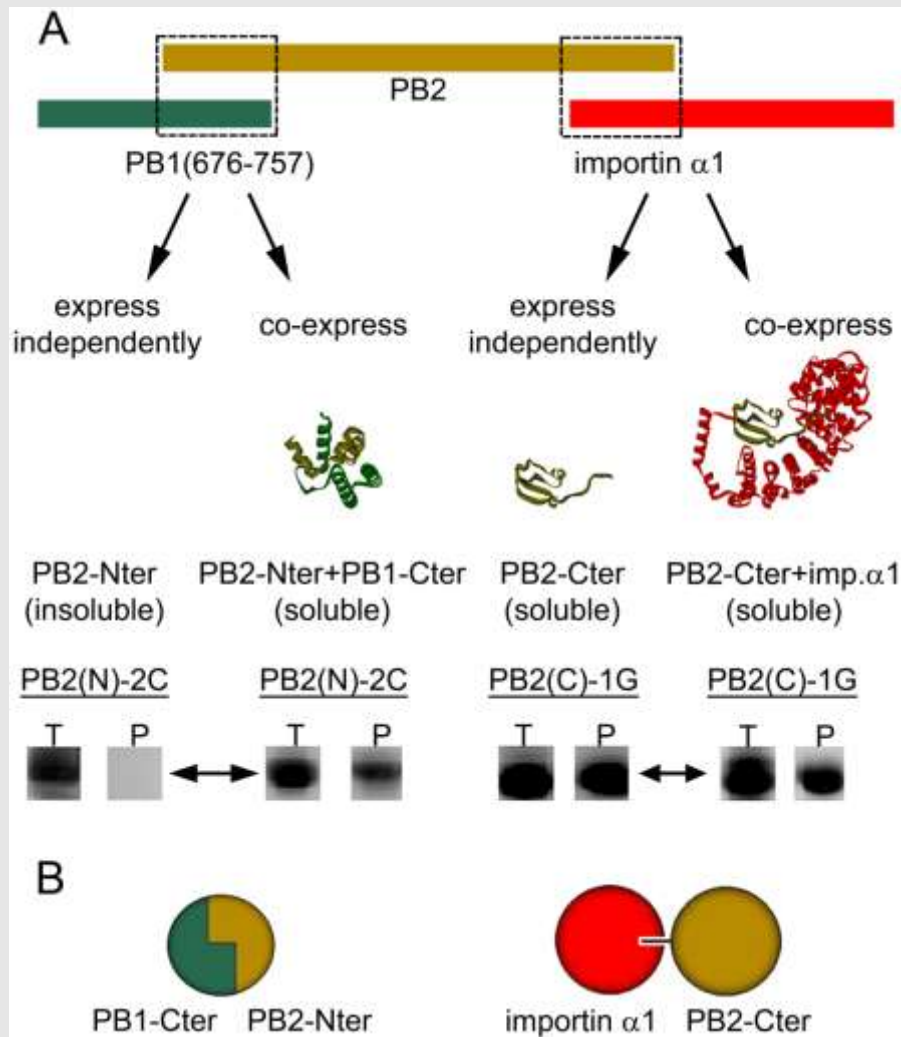
# CoESPRIT Screen

- 1) Colony screen
  - Library protein
  - His & biotin tags
- 2) Purification screen
  - Library – biotin
  - Bait – flag tag
  - Complex : double signal





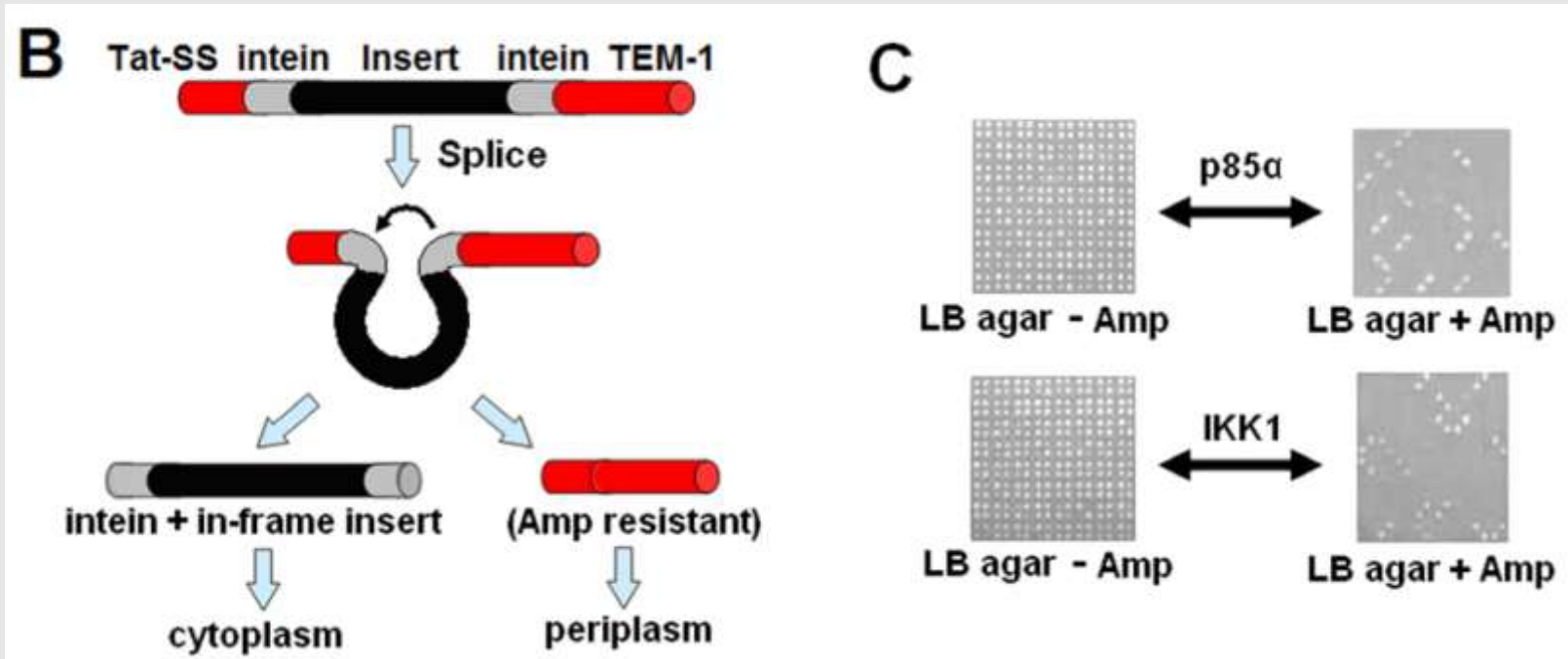
# Pre-folded & co-folded subunits



- Re-express hits without bait
- Stabilisation due to complex formation

# THE READING FRAME PROBLEM

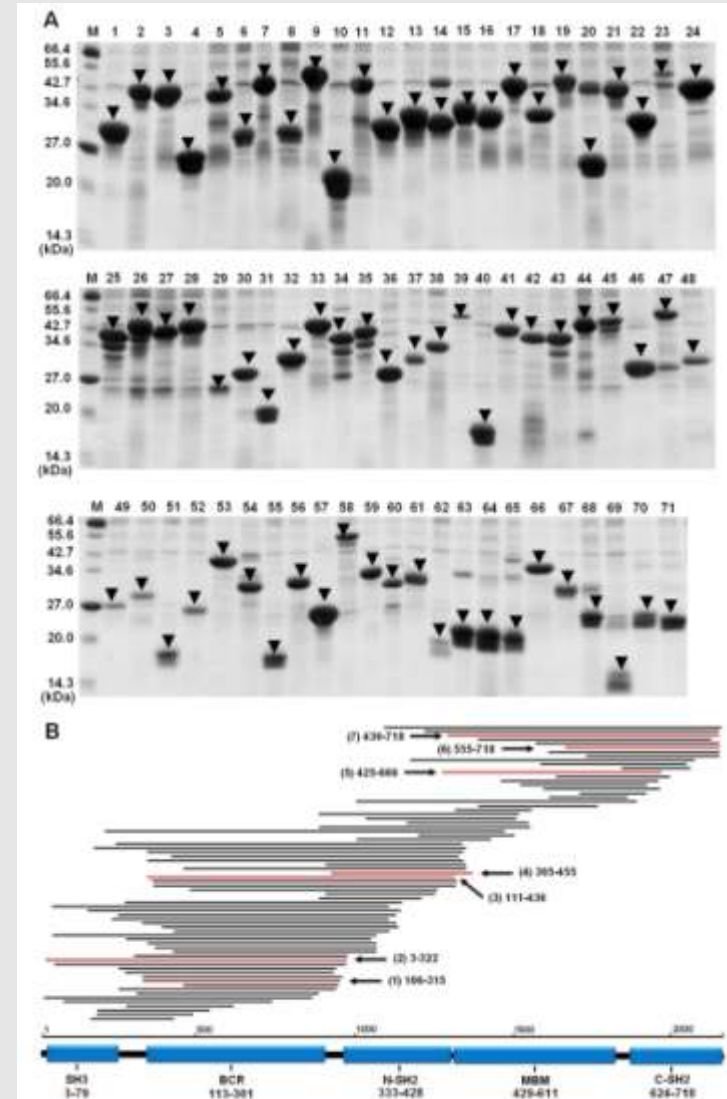
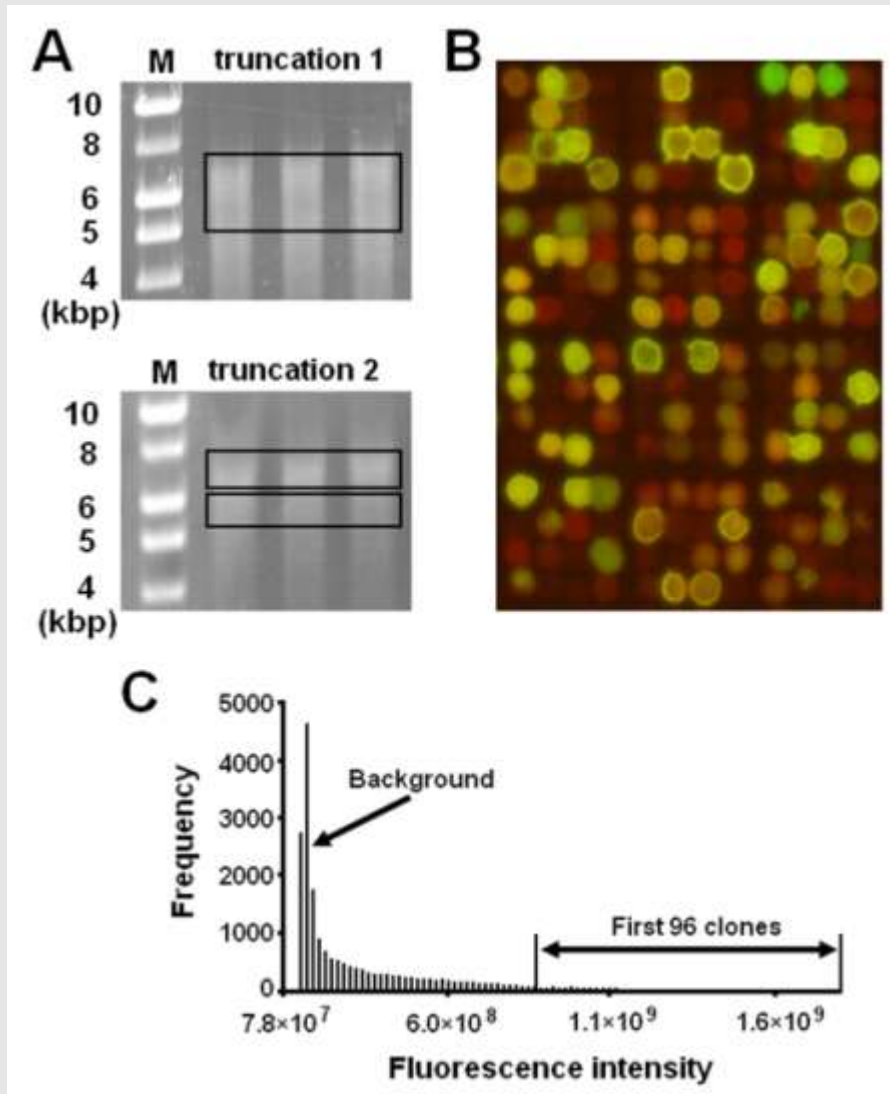
# The Reading Frame Problem



- Bidirectional libraries 1/9 in frame
- Genetic selection to eliminate out-of-frame constructs
- “ORF Selector ESPRIT”



# ORF Selector ESPRIT: p85 $\alpha$



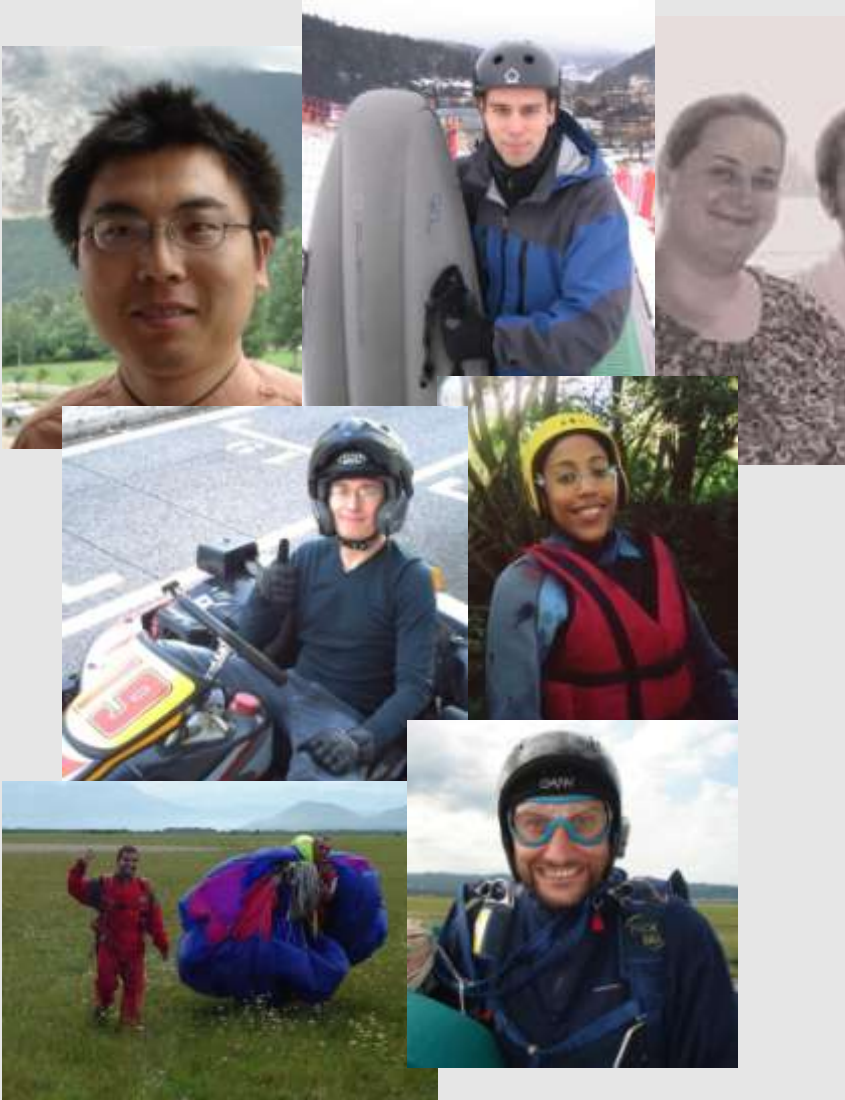
# ORF selector ESPRIT implications

- Tougher targets with rarer solutions
- Negative results become more definitive
- Library squared becomes feasible
- Eukaryotic hosts become feasible
  - ~200 constructs
  - Plate-based HEK293 cell transfections (Ariescu 2006)

# Summary

- Single gene ESPRIT well-established
  - Poorly annotated genes
  - Domain optimisation for expression/crystallisation
- CoESPRIT addresses binary complexes
- Complexes extended to IUPs
  - New insights into hub protein interactions
- ORF selector opens up new avenues
  - Tougher targets

# Acknowledgements



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EU 3D Repertoire  
EU FLUPOL  
EU SOUTH  
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Martin Blackledge & lab



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# Access to ESPRIT



[www.p-cube.eu](http://www.p-cube.eu)

- EU FP7 P-CUBE
  - 5 *Transnational* Projects funded per year by EU
    - Project review board
    - 3 week visit to EMBL Grenoble for screening (costs refunded)