Proteomics Course

LECTURE-29
Cell-free synthesis based protein microarrays

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Lecture outline

- Cell-free expression based microarrays
  - Protein *in situ* array (PISA)
  - Nucleic Acid Programmable Protein Array (NAPPA)
  - Multiple Spotting Technique (MIST)
  - DNA Array to Protein Array (DAPA)
  - HaloTag Array

Cell-free protein synthesis

Protein microarrays based on cell-free expression

Requirements of cell-free expression systems to generate protein microarrays

- Able to utilize wide variety of DNA templates
  - PCR products or plasmids
- Process should be simple, quick and cost-effective
- Avoid storage effects
- Simultaneous production of thousands of proteins in single reaction
- Methods to detect & analyze bound protein simple

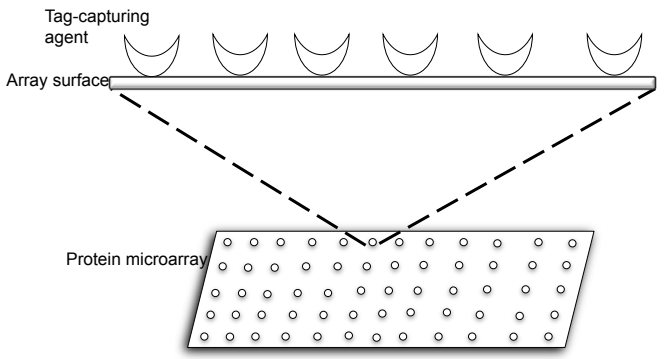
Protein *in situ* array (PISA)

He and Taussig. J Immunol Methods 2003, 274, 265

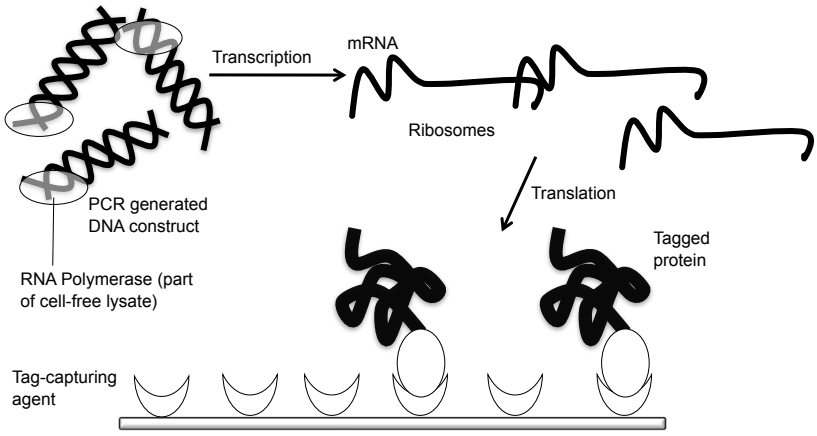
Protein *in situ* array (PISA)

- DNA construct produced by PCR
  - T7 promoter, sequences for translation initiation (Shine-Dalgarno or Kozak), an N- or C-terminal tag for immobilization, suitable termination sequences
- Used hexahistidine (His6) binding sequences and microtiter plate coated with Ni-NTA
- Protein expression with *E. coli* S30 or RRL
- After translation, protein bound specifically on surface through tag sequence

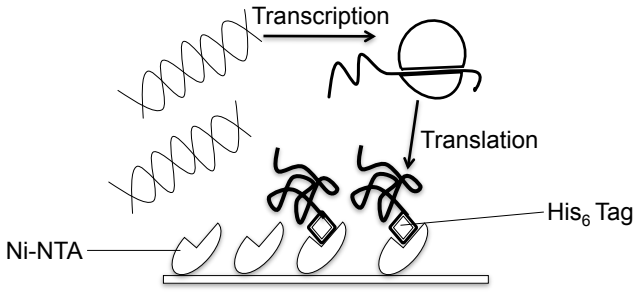
Protein *in situ* array (PISA)



Protein *in situ* array (PISA)



Protein *in situ* array (PISA)



PCR DNA encoding N- or C-terminal tag sequence expressed; bound protein gets specifically captured by tag-capturing agent

Protein *in situ* Arrays: *Animation*

PISA: Merits and demerits

- Merits
  - Protein purification not required
  - Rapid, single step process
  - Specific protein attachment
  - Soluble proteins formed
- Demerits
  - Possible loss of function during immobilization
  - Relatively high volume of cell-free lysate required

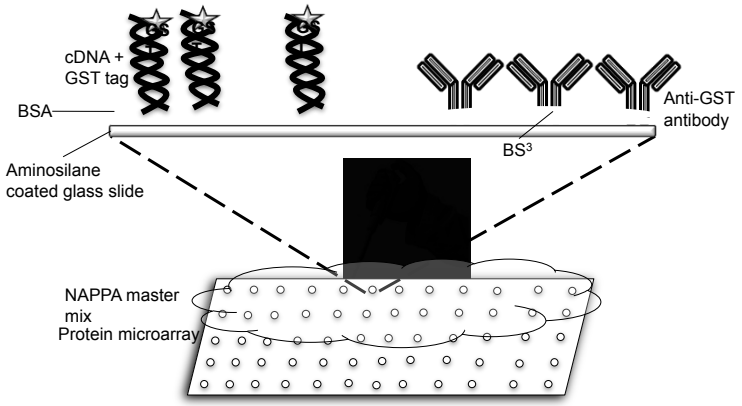
Nucleic Acid Programmable Protein Array (NAPPA)

Ramachandran et al. Science 2004, 305, 86
Ramachandran et al. Nat Methods 2008, 5, 535

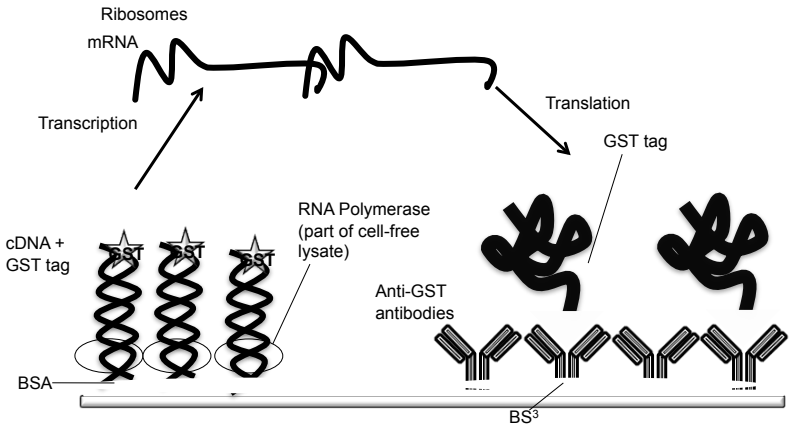
Nucleic Acid Programmable Protein Array (NAPPA)

- Plasmid encoding target proteins fused with an affinity tag are affixed to surface
- Array is activated by the addition of a cell free expression system
- Target proteins are expressed and immobilized in situ, and detected using a universal anti-tag antibody

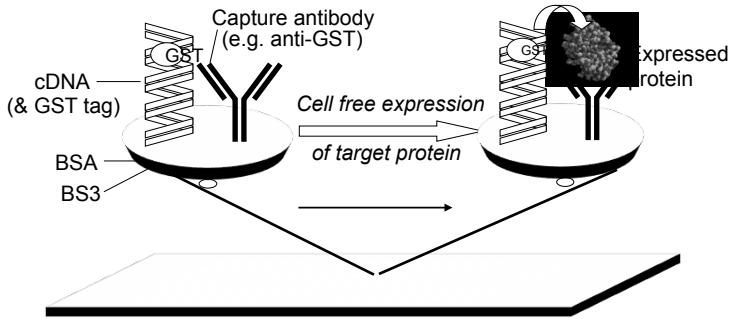
Nucleic Acid Programmable Protein Array (NAPPA)



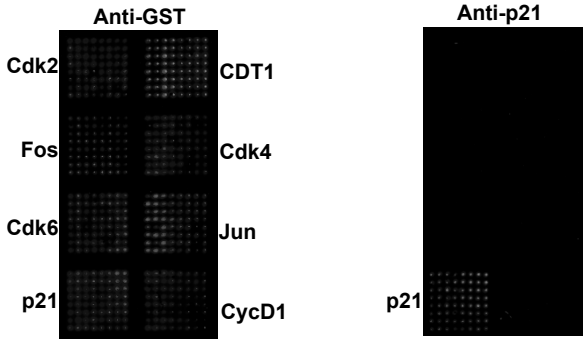
Nucleic Acid Programmable Protein Array (NAPPA)



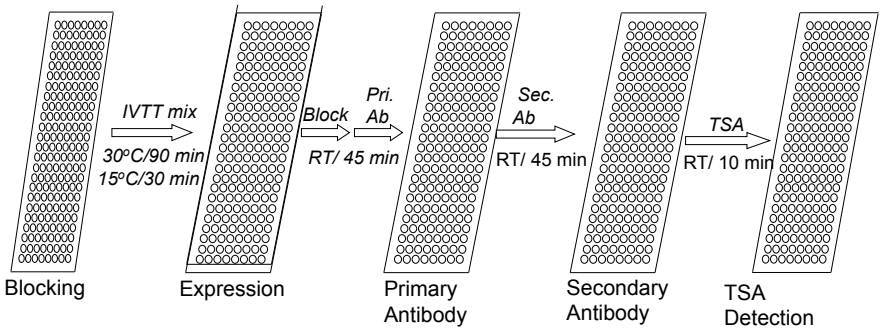
NAPPA protein arrays



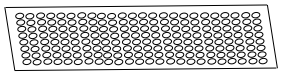
NAPPA: Detection of protein expression



NAPPA protein display: expression, capture & detection



NAPPA: Protein expression



- In high-density NAPPA arrays > 95% of proteins express and capture well
- > 10,000 proteins tested
- Multiple organisms tested
- Membrane proteins express well

NAPPA protein arrays

Transcription factors	Kinases	Membrane proteins
96% (136/141)	97% (38/39)	93% (239/258)

No protein class or size biases
Very tight range of protein levels

Size < 50 kDa	Size 50-100 kDa	Size < 100 kDa
98% (617/631)	92% (253/275)	88% (30/34)

Ramachandran et al. Nat Methods 2008, 5, 535

NAPPA: Merits and demerits

- Merits
  - No need to express and purify protein separately
  - Expression in mammalian milieu (natural folding)
  - Proteins produced just-in-time for assay
  - Shelf life not an issue
  - Access to all cloned cDNAs
  - Express & capture more than protein spotting arrays
  - Retains functionality of traditional protein arrays
  - Arrays stable on bench until activated

NAPPA: Merits and demerits

- Demerits
  - Cloning procedure required
  - Pure protein array not produced
  - Peptide tags may lead to sterical effects blocking important binding domains
  - Functionality of proteins?

Nucleic Acid Programmable Protein Array (NAPPA): *Animation*

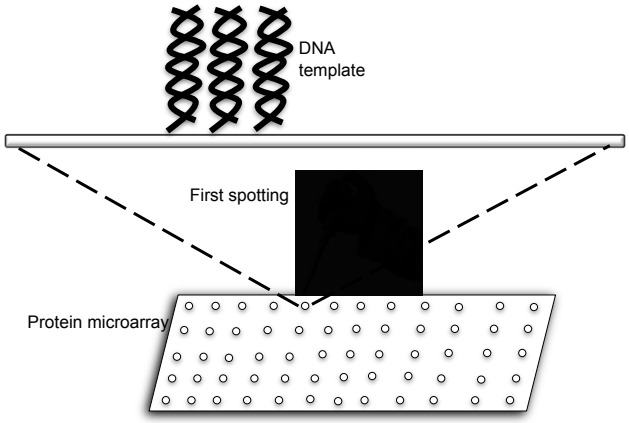
Multiple Spotting Technique (MIST)

Angenendt et al. Mol Cell Proteomics 2006, 5, 1658

Multiple Spotting Technique (MIST)

- 1st spotting step - addition of DNA template onto solid support
- 2nd spotting step - cell-free expression mixture transferred directly on top of first spot
- Proteins immobilized on activated array surface after translation by means of a tag-capturing agent or non-specific ionic interactions

Multiple Spotting Technique (MIST)



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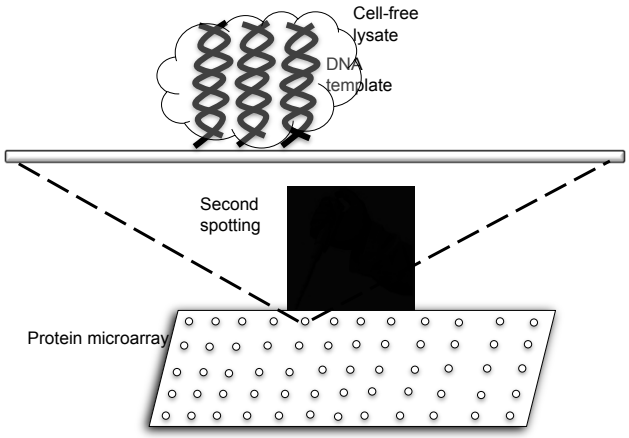

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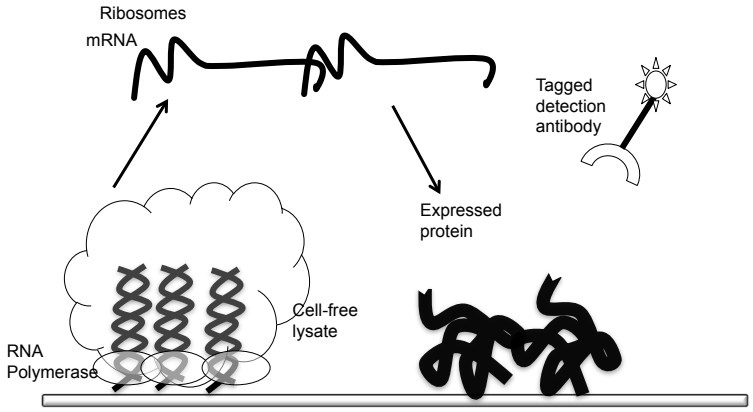
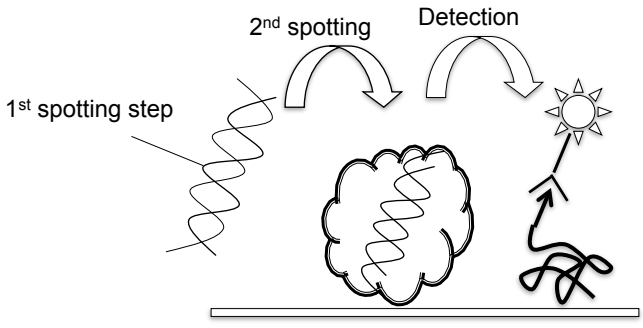


Multiple Spotting Technique (MIST)



Multiple Spotting Technique (MIST): *Animation*

MIST: Merits and demerits

- Merits
  - Unpurified DNA products used as template
  - Very high density protein arrays generated
- Demerits
  - Loss of signal intensity with prolonged incubation time
  - Non-specific protein binding
  - Time consuming process

DNA Array to Protein Array (DAPA)

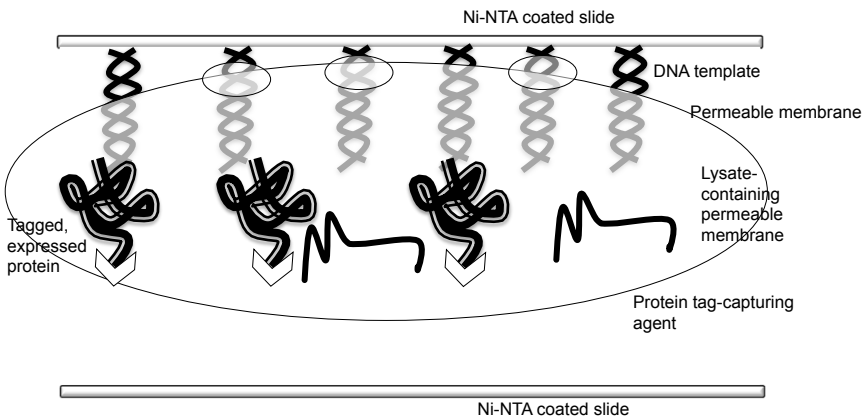
DAPA

- PCR amplified DNA fragments encoding tagged protein immobilized onto a Ni-NTA coated slide and assembled face-to-face with another Ni-NTA slide bearing protein tag-capturing agent
- Repeated use of same DNA template slide to print multiple protein arrays

DAPA

- Permeable membrane having cell-free lysate positioned in between the slides
- Protein synthesis took place from immobilized DNA spots
- Newly synthesized proteins penetrates membrane and bind to surface of capture slide

DAPA



DNA Array to Protein Array: *Animation*

DAPA: Merits and demerits

- Demerits
  - Reusable DNA template array
  - Pure protein array generated
  - DNA template array can be stored for long durations
- Demerits
  - Broadening of spots due to diffusion
  - Not ascertained if multimeric proteins assemble effectively
  - Time consuming process

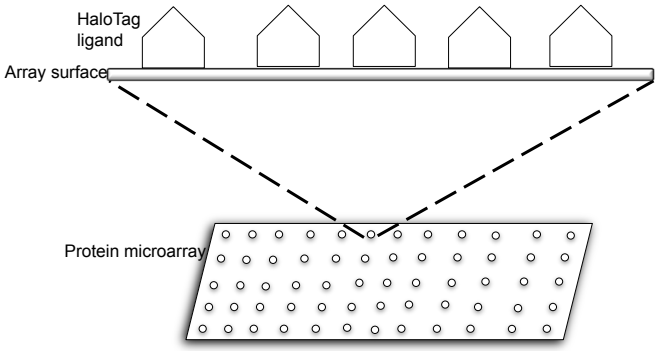
HaloTag Arrays

Nath et al., J. Proteome Res. 2008, 7, 4475

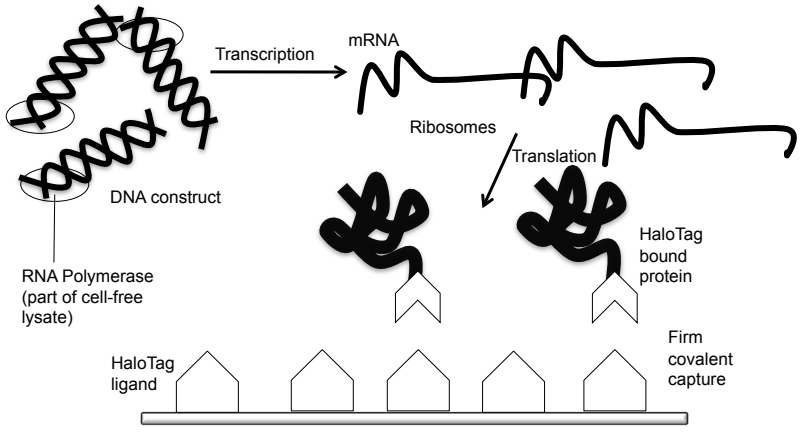
HaloTag Arrays

- HaloTag - a 33 kDa engineered derivative of bacterial hydrolase, used to tag desired protein
- Proteins fused with HaloTag expressed using WGE/RRL and covalently captured on a PEG-coated slide, activated with HaloTag ligand
- Enables oriented capture of proteins
  - ensuring no loss of function

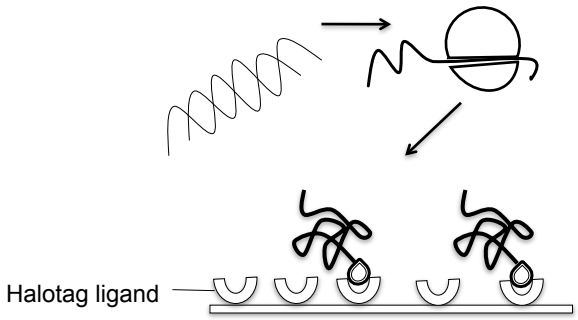
HaloTag Arrays



HaloTag Arrays



HaloTag Arrays



HaloTag Arrays: *Animation*

HaloTag Arrays: Merits and demerits

- Merits
  - Strong covalent bond between protein and ligand
  - No material loss during washing
  - Oriented capture of protein
  - No non-specific adsorption
  - Easy quantification
  - No need for a microarrayer printer
- Demerits
  - Possible loss of function on binding to Halotag
  - HT application will require optimization of printing

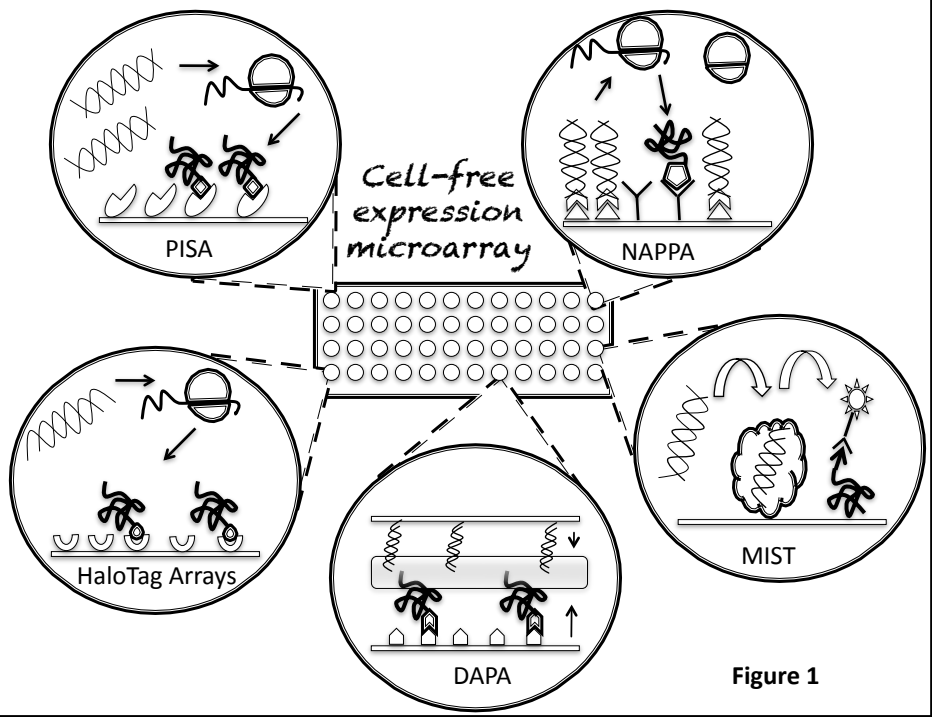


Figure 1

Summary

- Cell-free microarrays principle, merits and demerits
  - Protein in situ array (PISA)
  - Nucleic Acid Programmable Protein Array (NAPPA)
  - Multiple Spotting Technique (MIST)
  - DNA Array to Protein Array (DAPA)
  - HaloTag Array

References

- Chandra, H. & Srivastava, S. Cell-free synthesis-based protein microarrays and their applications. Proteomics 2010, 10, 1-14.
- He, M., Stoevesandt, O., Taussig, M. J., In situ synthesis of protein arrays. Curr. Opin. Biotechnol. 2008, 19, 4–9.
- Jackson, A. M., Boutell, J., Cooley, N., He, M., Review: cell-free protein synthesis for proteomics. Brief Funct. Genom.Proteomic 2004, 2, 308–319.
- He, M., Taussig, M. J., Single step generation of protein arrays from DNA by cell-free expression and in situ immobilisation (PISA method). Nucleic Acids Res. 2001, 29, e73.
- Ramachandran, N., Hainsworth, E., Bhullar, B., Eisenstein,S. et al., Self-assembling protein mircoarrays. Science 2004,305, 86–90.
- Ramachandran, N., Raphael, J. V., Hainsworth, E., Demirkan,G. et al., Next-generation high-density self-assembling functional protein arrays. Nat. Methods 2008, 5, 535–538.

References

- Angenendt, P., Kreutzberger, J., Glokler, J., Hoheisel, J. D., Generation of high density protein microarrays by cell-free in situ expression of unpurified PCR products. Mol. Cell.Proteomics 2006, 5, 1658–1666.
- He, M., Stoevesandt, O., Palmer, E. A., Khan, F. et al., Printing protein arrays from DNA arrays. Nat. Methods 2008, 5, 175–177.
- Nath, N., Hurst, R., Hook, B., Meisenheimer, P. et al., Improving protein array performance: Focus on washing and storage conditions. J. Proteome Res. 2008, 7, 4475–4482.
- Katzen, F., Chang, G., Kudlicki, W. The past, present and future of cell-free protein synthesis. Trends Biotechnol. 2005, 23 (3), 150-156.
- Amita Nand, Anju Gautam, Javier Batista Pérez, Alejandro Merino, Jinsong Zhu. Emerging technology of in situ cell free expression protein microarrays. Protein & Cell. February 2012, Volume 3, Issue 2, pp 84-88.

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