

Proteomics Course

LECTURE-30

Generating Protein Microarrays: *Focus on Nucleic Acid Programmable Protein Array*

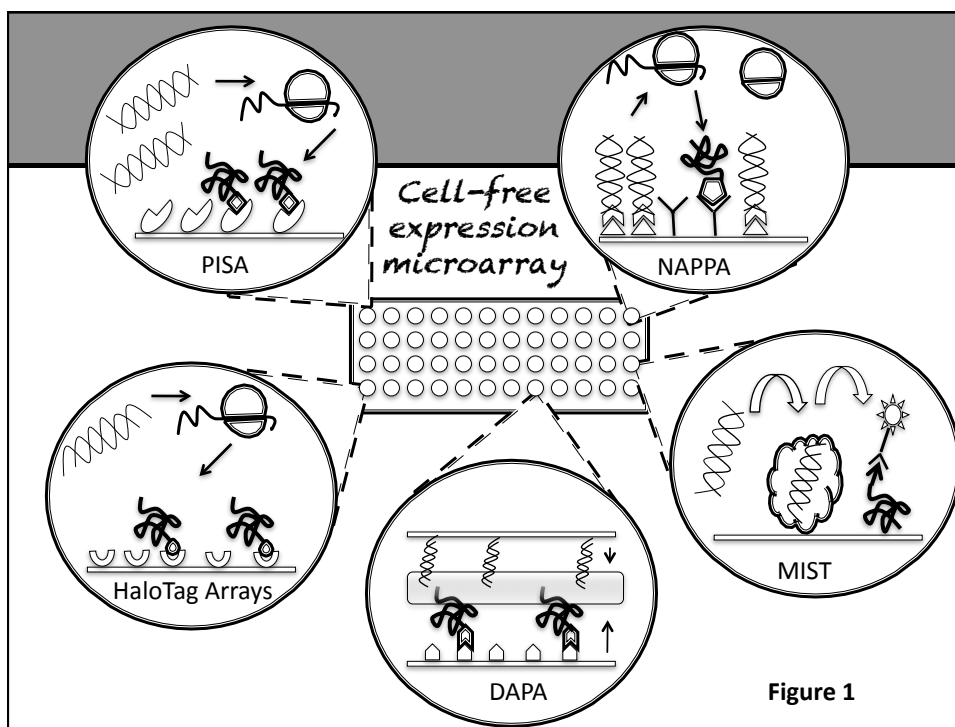


Dr. Sanjeeva Srivastava
IIT Bombay

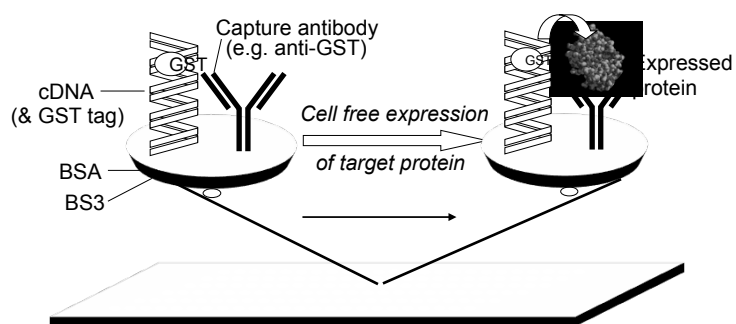


Lecture outline

- Nucleic Acid Programmable Protein Array
- Workflow



Nucleic Acid Programmable Protein Array (NAPPA)



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

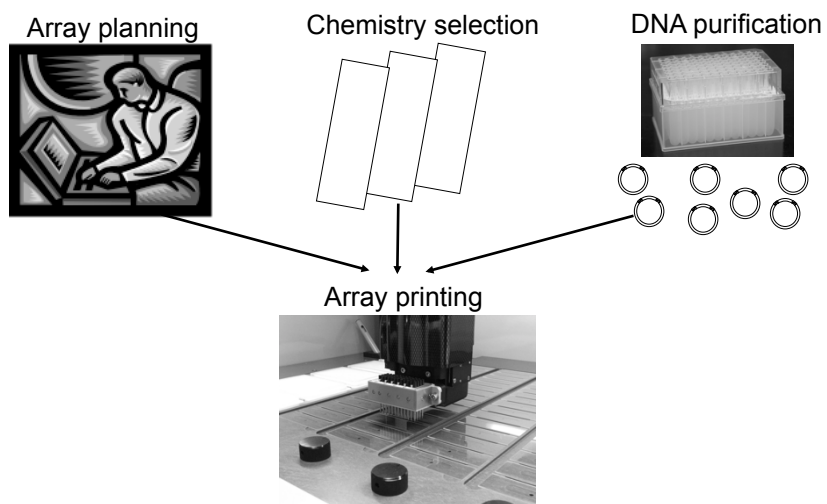
Assembling protein array: requirements

- A repository of expression-ready clones in flexible cloning system that enables easy sub-cloning between various expression constructs
- A pipeline for quickly purifying DNA constructs and arraying them
- Robust printing chemistry
- HT capability at every step

NAPPA protein array: work-flow

- Array design and experimental planning
- Selection of surface and printing chemistries
- Culture expression construct clones
- Purification and concentration of DNA
- Re-suspension of expression construct DNA in array spotting buffer
- Array printing
- Quality control check

Work-flow: an overview



Array design

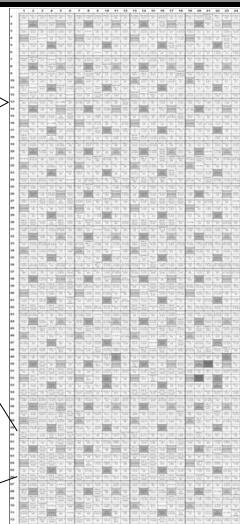
Array design

- Design experiment type & appropriate target protein test set
- Fit NAPPA chemistry utilized to assay being executed
- Decide array density & mapping
- Select robotic parameters for array execution

Experimental planning and Array design

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|--------|--------|---------|-------|---------|--------|--------|---------|--------|--------|----------|-----------|
| A | BCL2 | RAB31 | IGFBP3 | ETS1 | IGF1 | ALDOA | PCNA | FIGF10 | GAGE4 | HST3H3 | N-EENNA1 | Mage3 |
| B | CHEK2 | CDKN1B | CDK2 | MMP1 | AKT3 | CSF3 | LDHA | PHB | IFITM1 | MAGEA6 | C-EENNA1 | Mage5 |
| C | NCOA4 | BAK1 | CDK4 | PPARG | PDGFB | ARH1 | CCNI | EDG3 | S100A9 | NDUFA6 | p53 | PDEF |
| D | S100A4 | EF4E | TACSTD2 | AKT1 | TFE1 | SFN | APOD | GJA1 | GAGEB1 | IL18 | p21 | MYC |
| E | SNCG | BCL2L1 | JUN | RARB | NUMB | STC2 | MAGEA3 | TACSTD1 | CCND1 | FGF7 | CYCD1 | LDHB |
| F | CDKN2A | BAG1 | RAO51 | MUC1 | ARRC | CALU | MAPK13 | MAP3B | TPD52 | CytA1 | PCNA | S100A7 |
| G | BK | IGFBP7 | FEN1 | CD44 | ARH8 | IGFBP6 | OSM | RPL34 | RBP1 | CytB1 | MLIAP | Prngranth |
| H | HRAS | CCND2 | FOS | FLAUR | HST2DAA | MAP2K3 | FGF16 | GAGE2 | IL15 | CytB2 | SURVIVIN | |

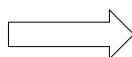
| | | | | | |
|-------------------------|----------------------------|----------------------------|---------------------------------|---------------------------------|-------------------------|
| PRELP 4-B5 | RAB27 A 4-D5 | RABL2 B 4-F5 | DNAJB 1 4-H5 | SCYE1 4-B11 | PPP1C B 4-D11 |
| RPL35 A 4-F11 | GST 3 | DKFZP 564B14 7 4-H11 | NA 8- B5 No Grow | Non Spots | MTMR9 8-D5 |
| PRTD- NY 3 8- F5 | TG737 8-H5 | SPAG8 8-B11 | CHC1L 8-D11 | DKFZP 434E23 18 8- F11 | NA 8- H11 No Grow |
| Non Spots | DKFZP 564B14 7 4-H11 | RPL35 A 4-F11 | SCYE1 4-B11 | RABL2 B 4-F5 | PRELP 4-B5 |
| RAB27 A 4-D5 | DNAJB 1 4-H5 | PPP1C B 4-D11 | GST 3 | NA 8- B5 No Grow | MTMR9 8-D5 |
| NA 8- H11 No Grow | CHC1L 8-D11 | TG737 8-H5 | DKFZP 434E23 18 8- F11 | SPAG8 8-B11 | PRTD- NY 3 8- F5 |



Control features on array

6 x 6 spots
per block

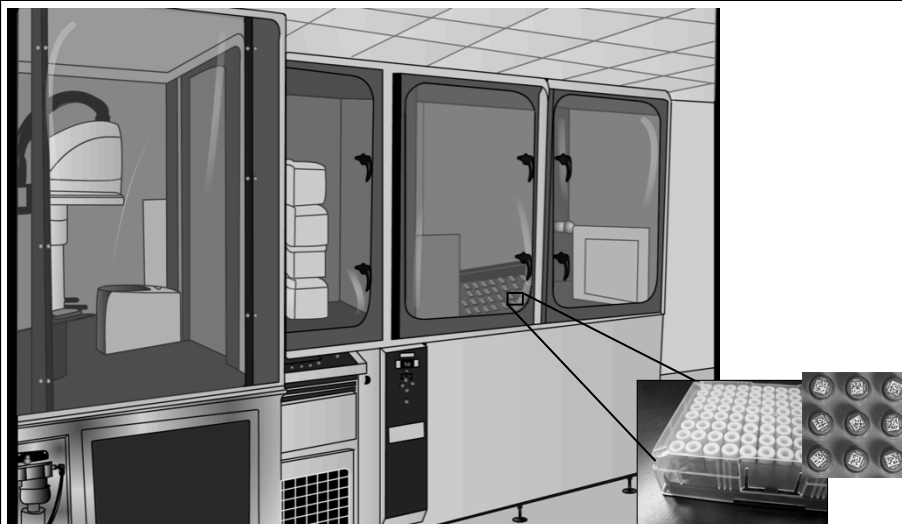
| | | | | | |
|---|---|---|---|---|---|
| ○ | ○ | ○ | ○ | ○ | ○ |
| ○ | ○ | ○ | ○ | ○ | ○ |
| ○ | ○ | ○ | ○ | ○ | ○ |
| ○ | ○ | ○ | ○ | ○ | ○ |
| ○ | ○ | ○ | ○ | ○ | ○ |
| ○ | ○ | ○ | ○ | ○ | ○ |



| | | | | | |
|----|----|----|----|----|----|
| 1 | 2 | 3 | 4 | 5 | 6 |
| 7 | 8 | 9 | 10 | 11 | 12 |
| 13 | 14 | 15 | 16 | 17 | 18 |
| 11 | 9 | 7 | 5 | 3 | 1 |
| 2 | 4 | 6 | 8 | 10 | 12 |
| 18 | 16 | 14 | 17 | 15 | 13 |

Cloning and Plasmid Preparation

Recombinational cloning: cDNA clone repositories



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Cloning and DNA extraction

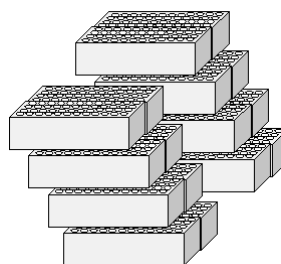
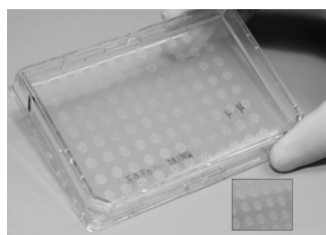
- Bioinformatics selection of target cDNAs
- Primer design and PCR amplification
- Gel purification of PCR amplicons
- Cloning into entry construct
- Selection and culturing of isolated transformants

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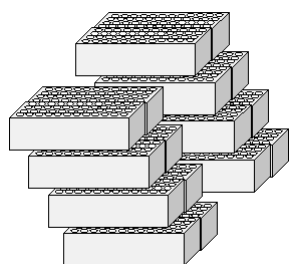
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Culture expression clones



Purification of DNA expression construct

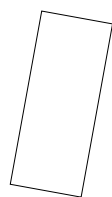


Purification of DNA expression construct

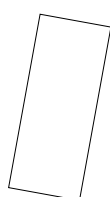


Selection of surface chemistry: Aminosilane coating

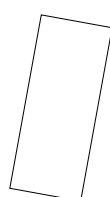
Selection of surface chemistry



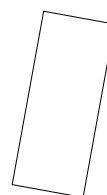
Glass



Gold



Nitrocellulose



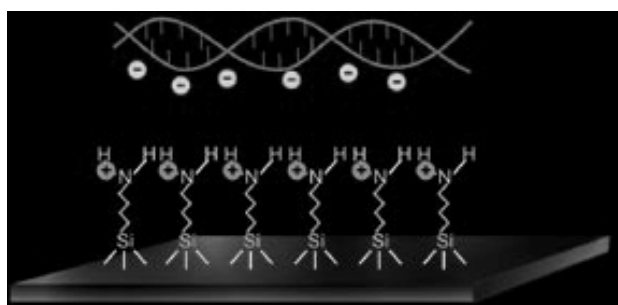
Hydrogel

Silane based chemistry

- Silicon-based chemistry
- DNA can be attached covalently to slide by baking or UV irradiation
- Bake arrayed slides at 85°C
- UV cross-linking also provides good results

Silane based chemistry

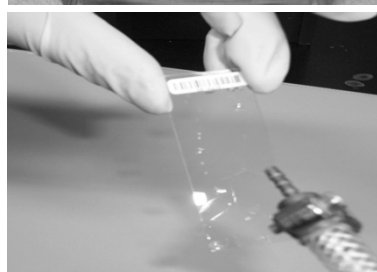
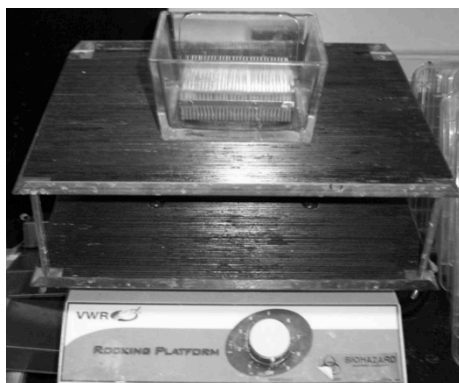
- Aminosilane coated slides contain high concentration of primary amino groups
- Amino group (+ charge)
- DNA backbone (- charge)



Aminosilane coating

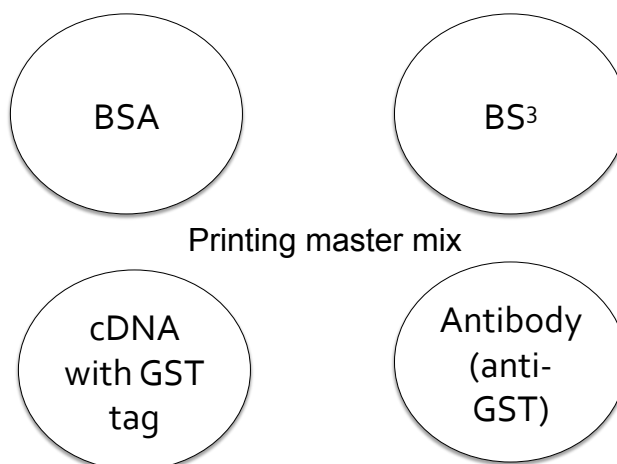
- Glass slides
- Acetone 99.9%
- Aminosilane (2% aminosilane solution in acetone)
- Metal 30-slide rack
- Rocking shaker

Aminosilane coating



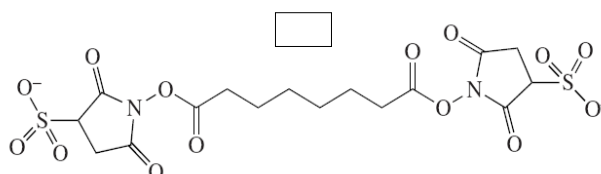
Printing arrays

Printing mix: role of each component



Role of BS3

- BS3 cross-linker - water soluble, non cleavable, membrane impermeable
- Amino-reactive groups of N-hydroxysulfosuccinimide (Sulfo-NHS), separated by spacer of 8C atoms
- Each protein contains a terminal amino group



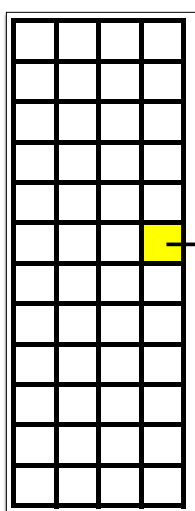
Control features on array

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Control features



| | | | | | |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 |

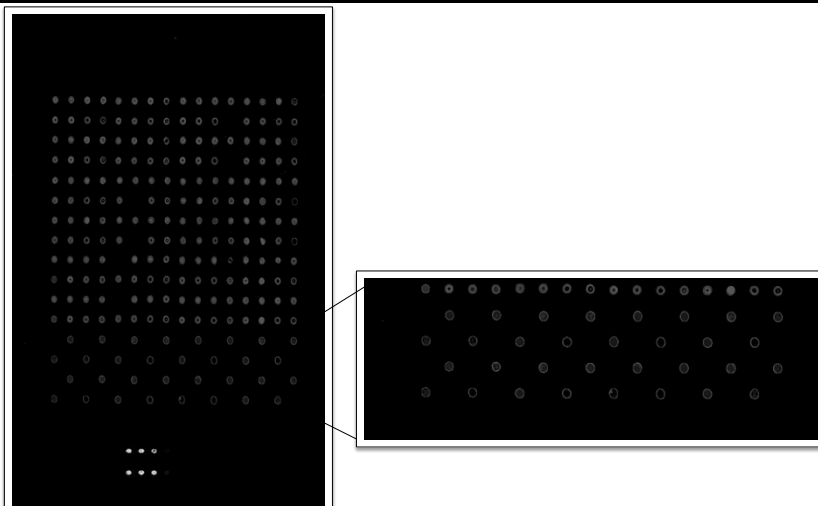
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|----|----|----|----|----|----|
| 1 | 2 | 3 | 4 | 5 | 6 |
| 7 | 8 | 9 | 10 | 11 | 12 |
| 13 | 14 | 15 | 16 | 17 | 18 |
| 11 | 9 | 7 | 5 | 3 | 1 |
| 2 | 4 | 6 | 8 | 10 | 12 |
| 18 | 16 | 14 | 17 | 15 | 13 |

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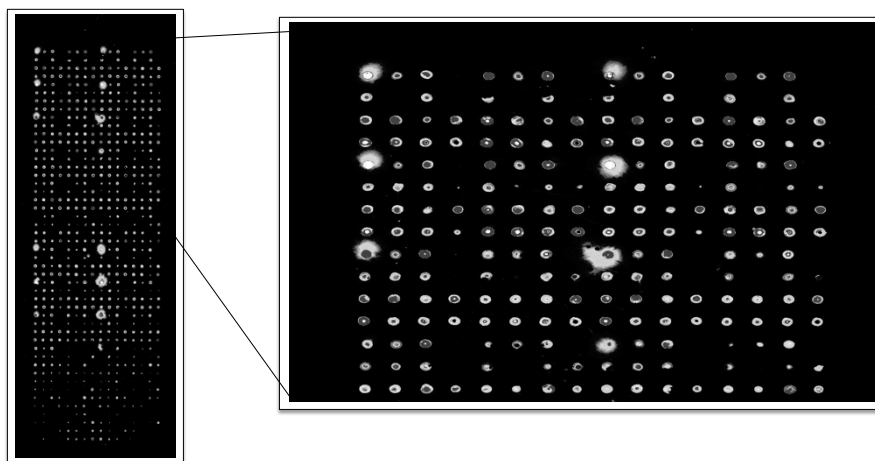
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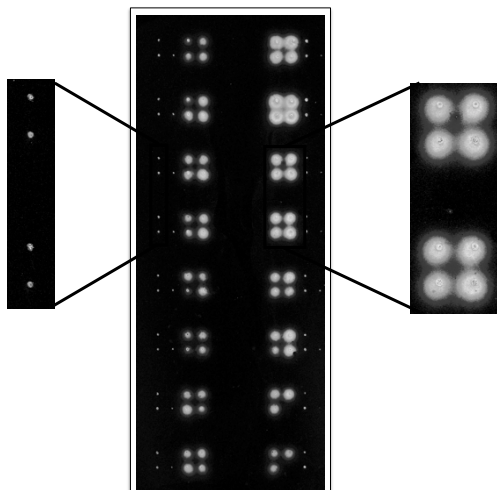
Control features: testing background



Assessing protein expression and detection



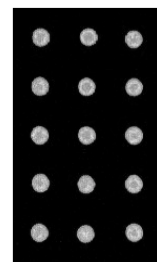
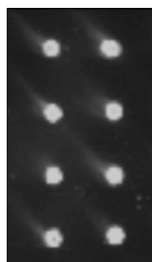
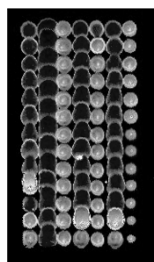
Control features



Qualities for good protein arrays



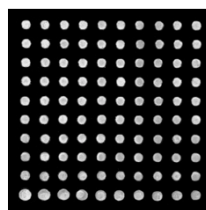
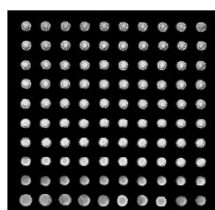
Qualities for good protein arrays



- Spot size, shape & morphology

- Spot-to-spot consistency

Qualities for good protein arrays

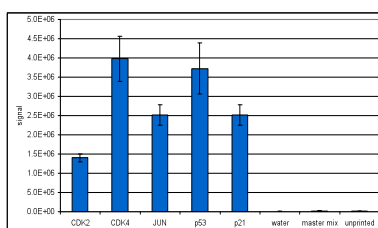
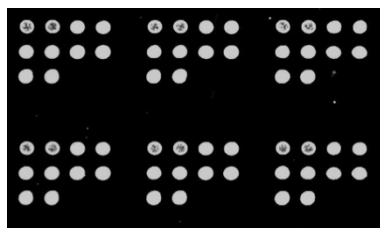


Printing quality arrays

- Precise liquid handling techniques
- Strict sample tracking throughout entire process
- Careful quality control of materials and reagents
- Maintain detailed description in notebooks
- Fidelity to protocols

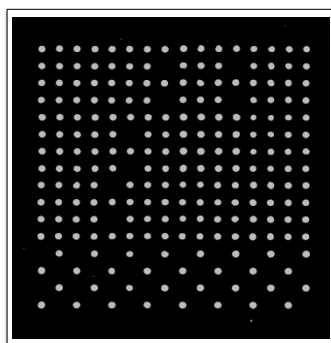
Testing NAPPA Printing Quality

QC check: DNA staining

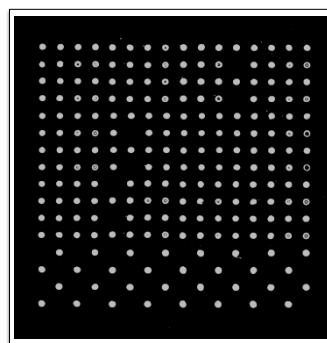


| | | | | | | | | | | | |
|-------|-------|------|------|-------|-------|------|------|-------|-------|------|------|
| CDK2 | CDK2 | CDK4 | CDK4 | CDK2 | CDK2 | CDK4 | CDK4 | CDK2 | CDK2 | CDK4 | CDK4 |
| JUN | JUN | p53 | p53 | JUN | JUN | p53 | p53 | JUN | JUN | p53 | p53 |
| p21 | p21 | MM | MM | p21 | p21 | MM | MM | p21 | p21 | MM | MM |
| water | water | MM | MM | water | water | MM | MM | water | water | MM | MM |
| CDK2 | CDK2 | CDK4 | CDK4 | CDK2 | CDK2 | CDK4 | CDK4 | CDK2 | CDK2 | CDK4 | CDK4 |
| JUN | JUN | p53 | p53 | JUN | JUN | p53 | p53 | JUN | JUN | p53 | p53 |
| p21 | p21 | MM | MM | p21 | p21 | MM | MM | p21 | p21 | MM | MM |
| water | water | MM | MM | water | water | MM | MM | water | water | MM | MM |

QC check: DNA staining



Printing batch 1



Printing batch 2

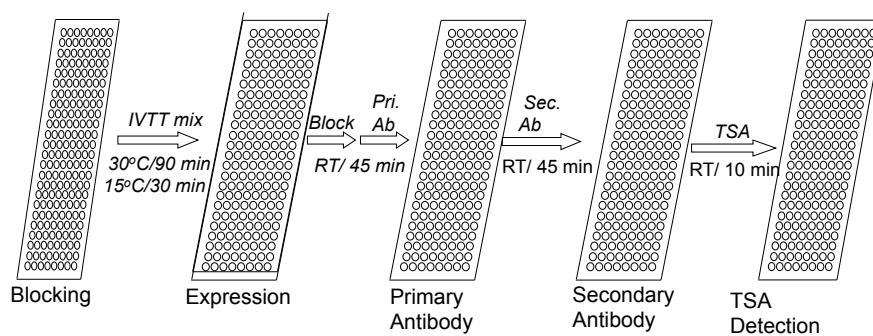
Testing Protein Expression

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NAPPA protein display: expression, capture & detection



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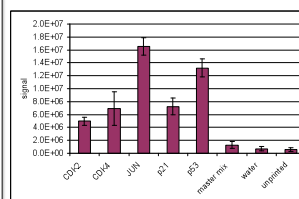
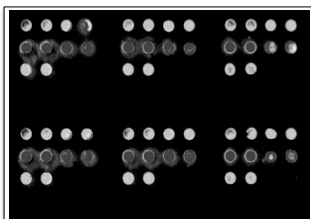
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QC check: protein expression

Anti-GST antibody

| | | | |
|-------|-------|------|------|
| CDK2 | CDK2 | CDK4 | CDK4 |
| JUN | JUN | p53 | p53 |
| p21 | p21 | MM | MM |
| water | water | MM | MM |



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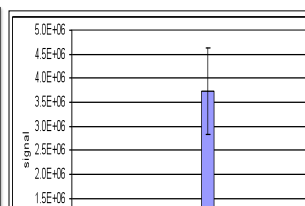
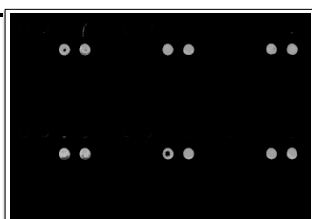
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QC check: protein expression

Anti-p53 antibody

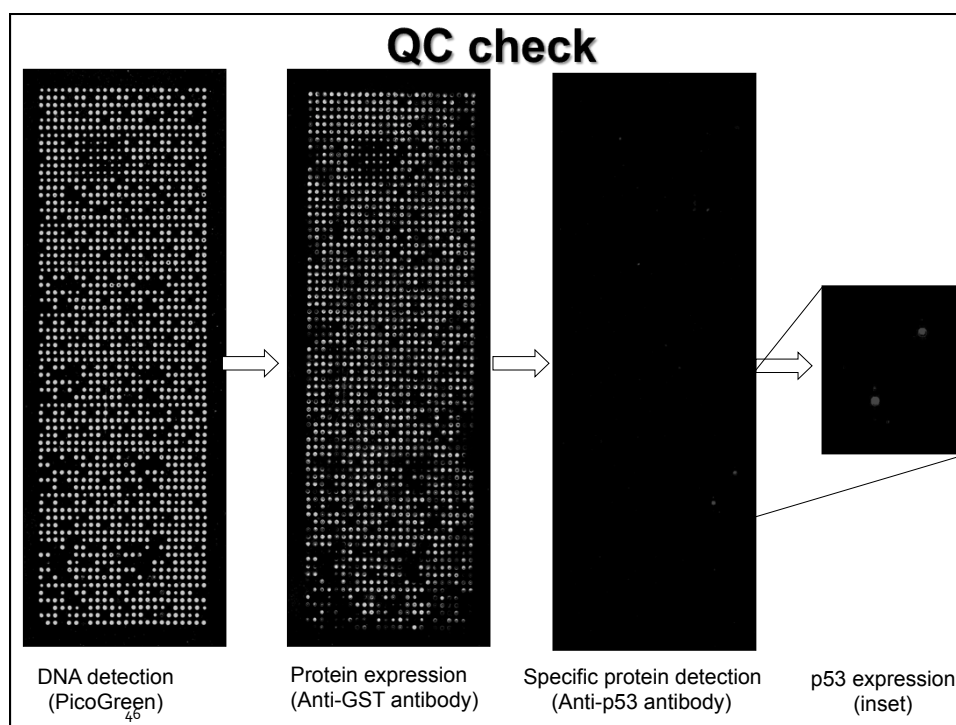
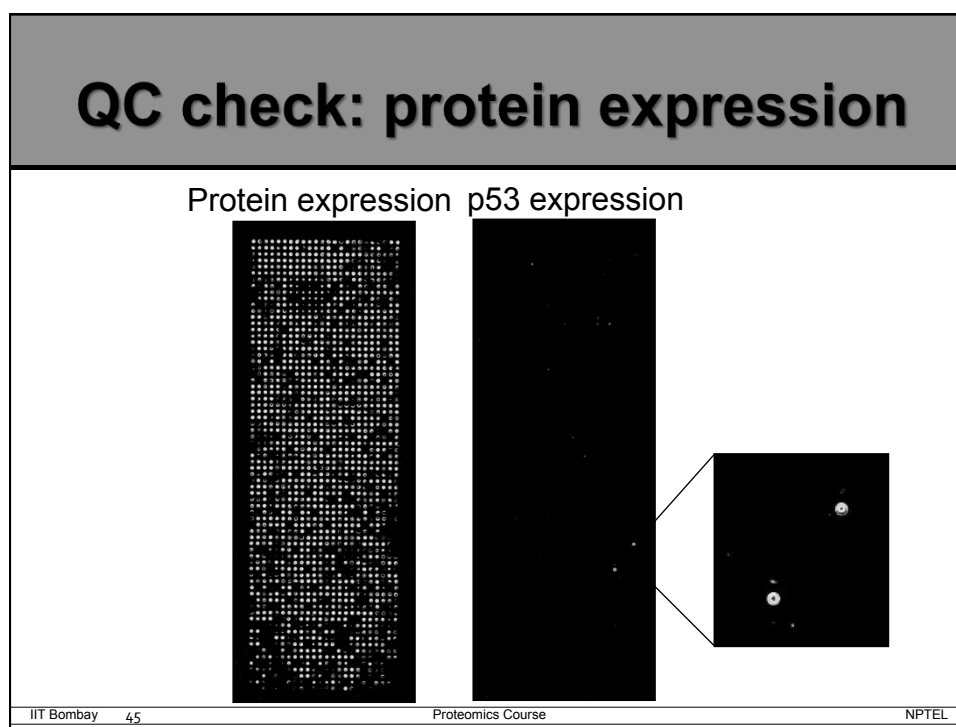
| | | | |
|-------|-------|------|------|
| CDK2 | CDK2 | CDK4 | CDK4 |
| JUN | JUN | p53 | p53 |
| p21 | p21 | MM | MM |
| water | water | MM | MM |



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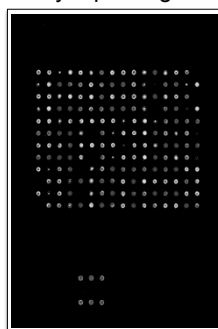
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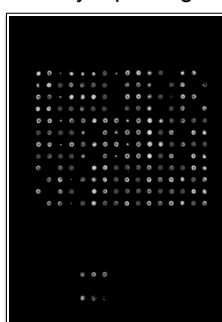


QC check: batch variations

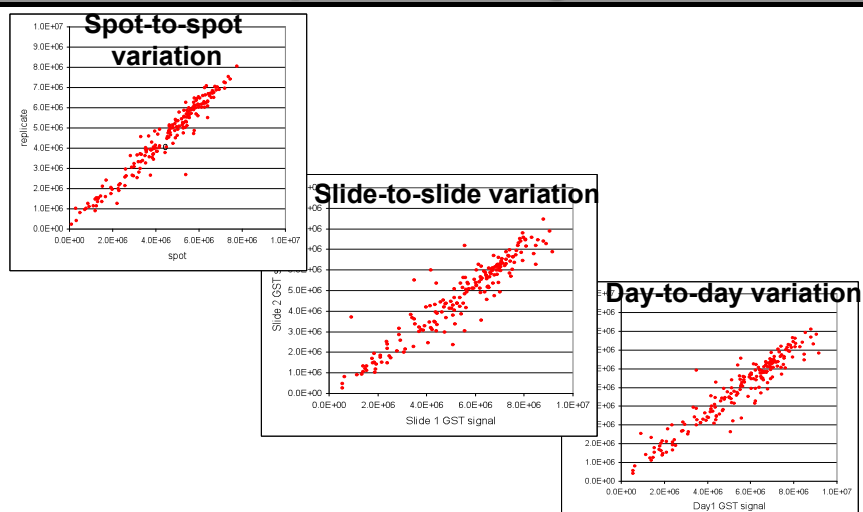
Day 1 printing



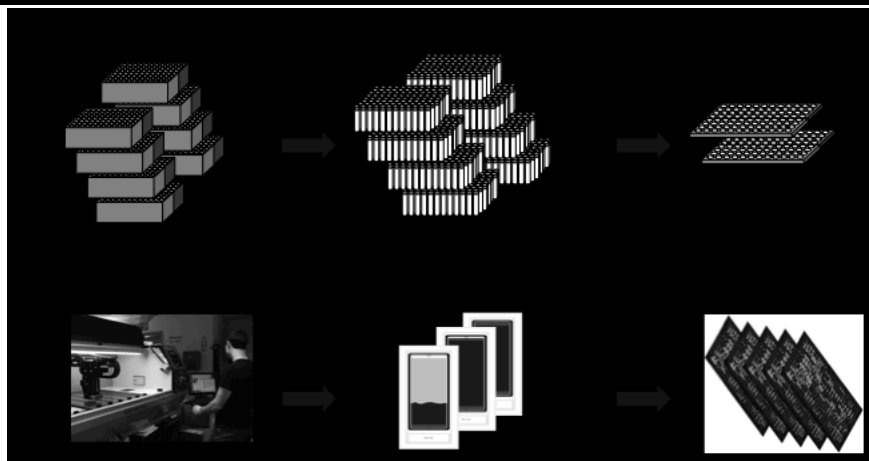
Day 2 printing



QC check: Reproducibility in slide processing



Making NAPPA arrays: Summary



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References

- Ramachandran, N., Hainsworth, E., Bhullar, B., Eisenstein, S. et al., Self-assembling protein microarrays. *Science* 2004, 305, 86–90.
- Ramachandran N, Raphael JV, Hainsworth E, Demirkan G, Fuentes MG, Rolfs A, Hu Y, LaBaer J. Next-generation high-density self-assembling functional protein arrays. *Nat Methods*. 2008 Jun;5(6):535-8.
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- Harini Chandra, Panga Jaipal Reddy and Sanjeeva Srivastava. 2011. Expert Review of Proteomics. Protein microarrays and novel detection platforms. February 2011, Vol. 8, No. 1, Pages 61-79 10.1586/epr.10.99
- Joshua LaBaer , Niroshan Ramachandran. 2005. Protein microarrays as tools for functional proteomics. *Current Opinion in Chemical Biology*. Volume 9, Issue 1, February 2005, Pages 14–19.

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