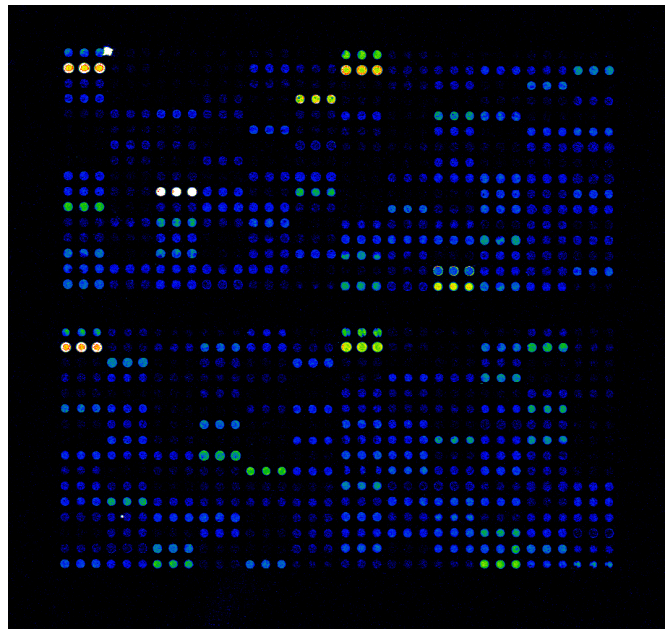
Microarray Printing, Design and Controls

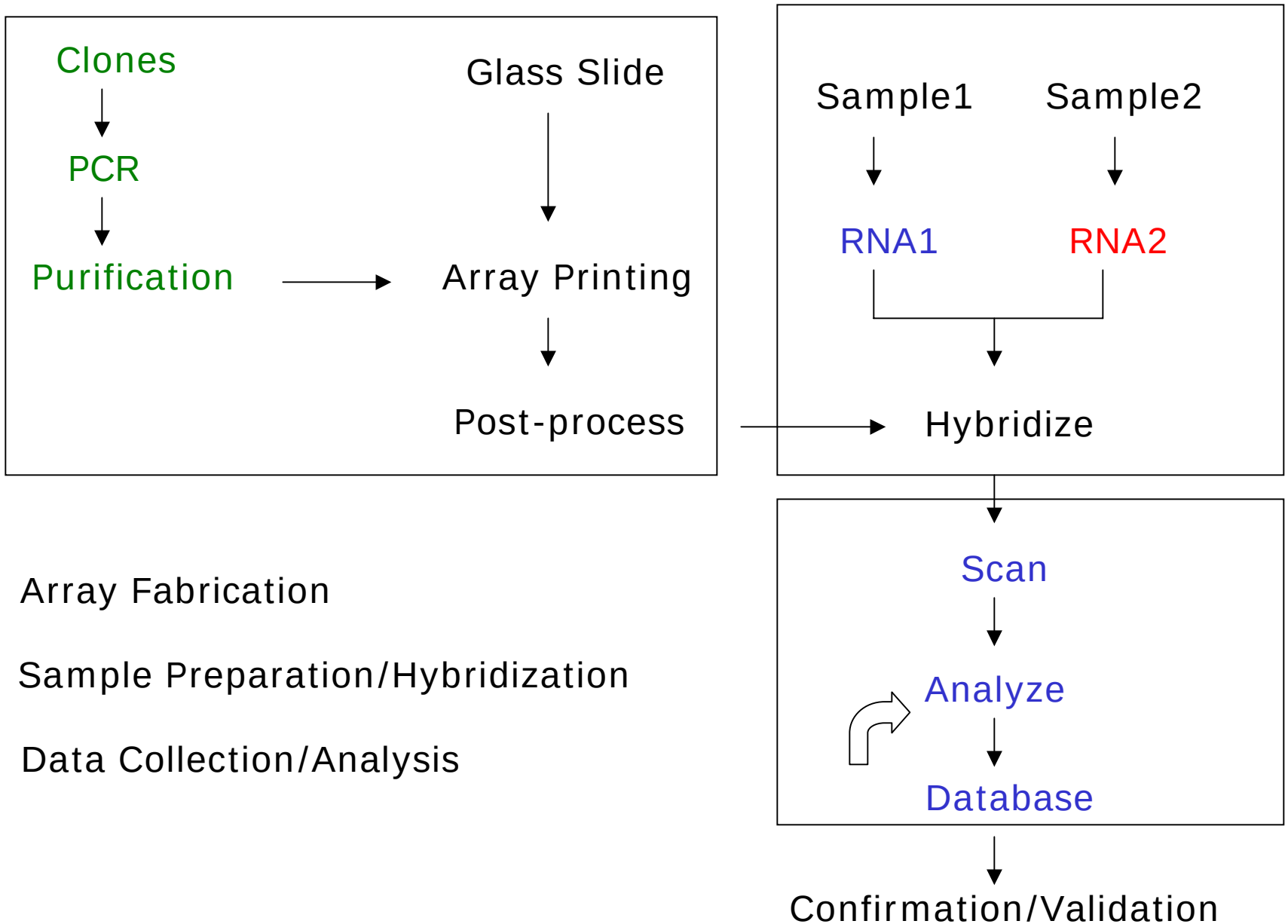


Debbie Laudencia-Chingcuanco

WHEAT MICROARRAY WORKSHOP

AUGUST 12-16, 2002

Microarray Process

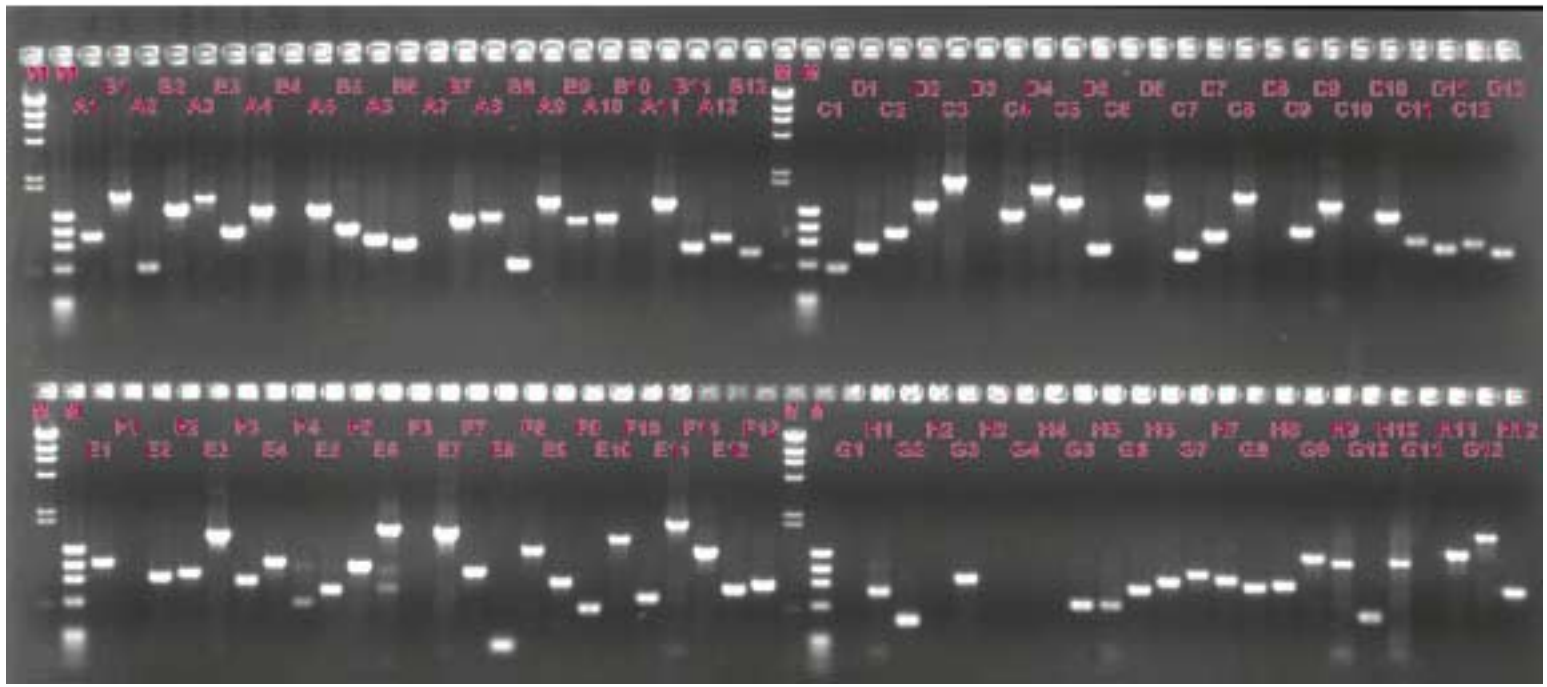


Array Fabrication

Sample Preparation/Hybridization

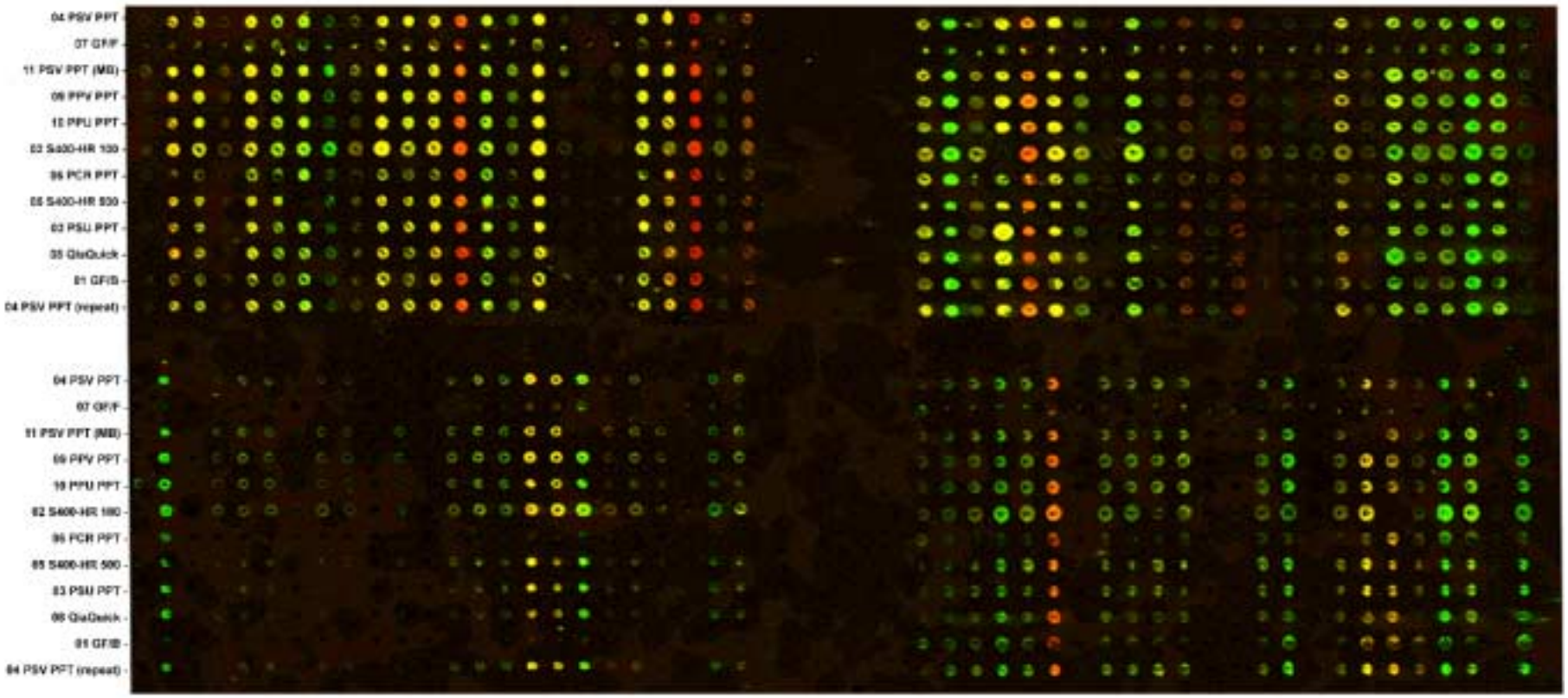
Data Collection/Analysis

TEMPLATE PREPARATION



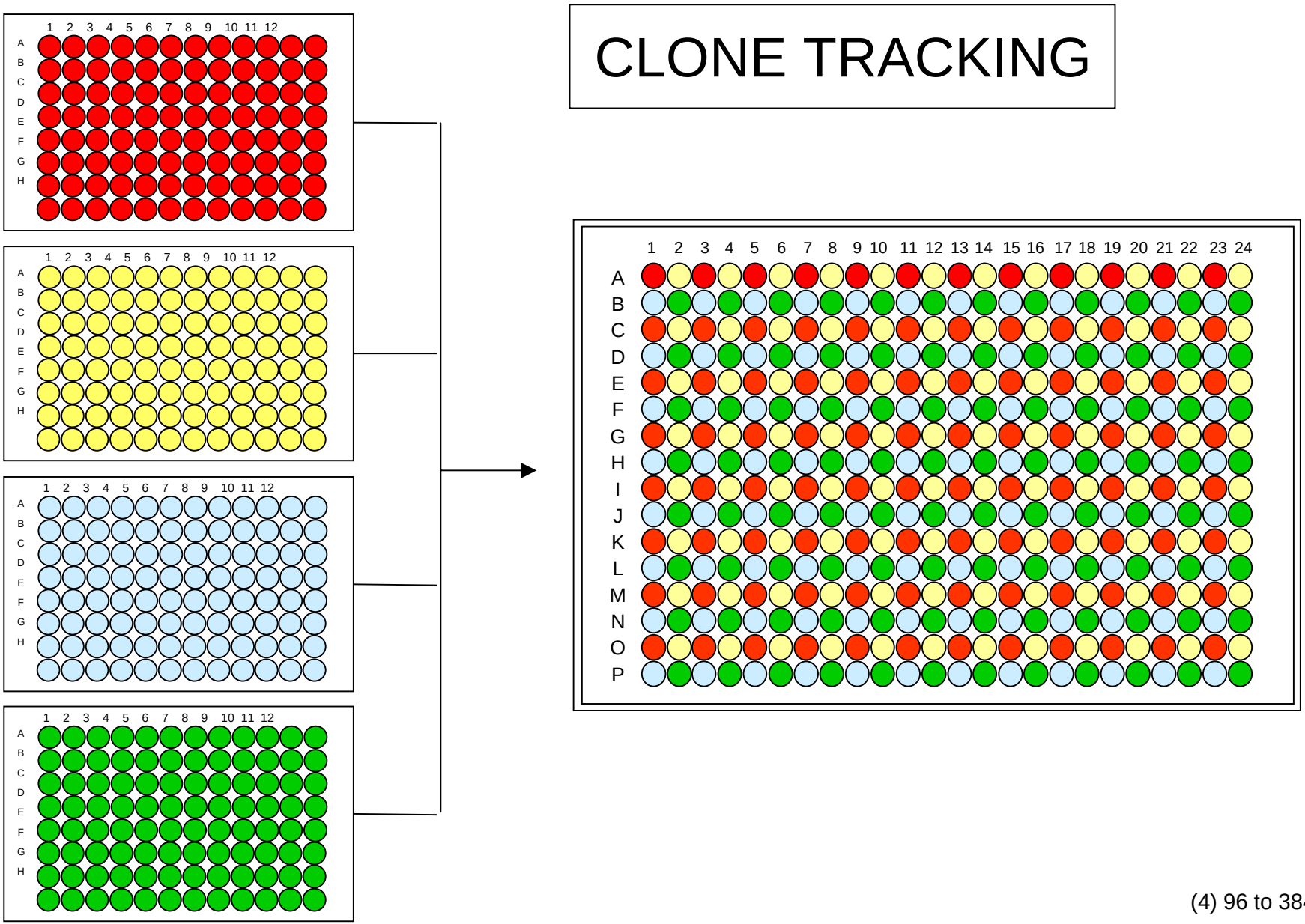
Single band/clone
Yield > 2 ug/PCR reaction
Size of insert

Comparison of PCR Product Purification Methods
03/06/1998



Pat Brown's lab?

CLONE TRACKING



(4) 96 to 384

ARRAY SPOTTER

OmniGrid from GeneMachines

+/- 2.5 micron accuracy

100 slides capacity

74 plates capacity

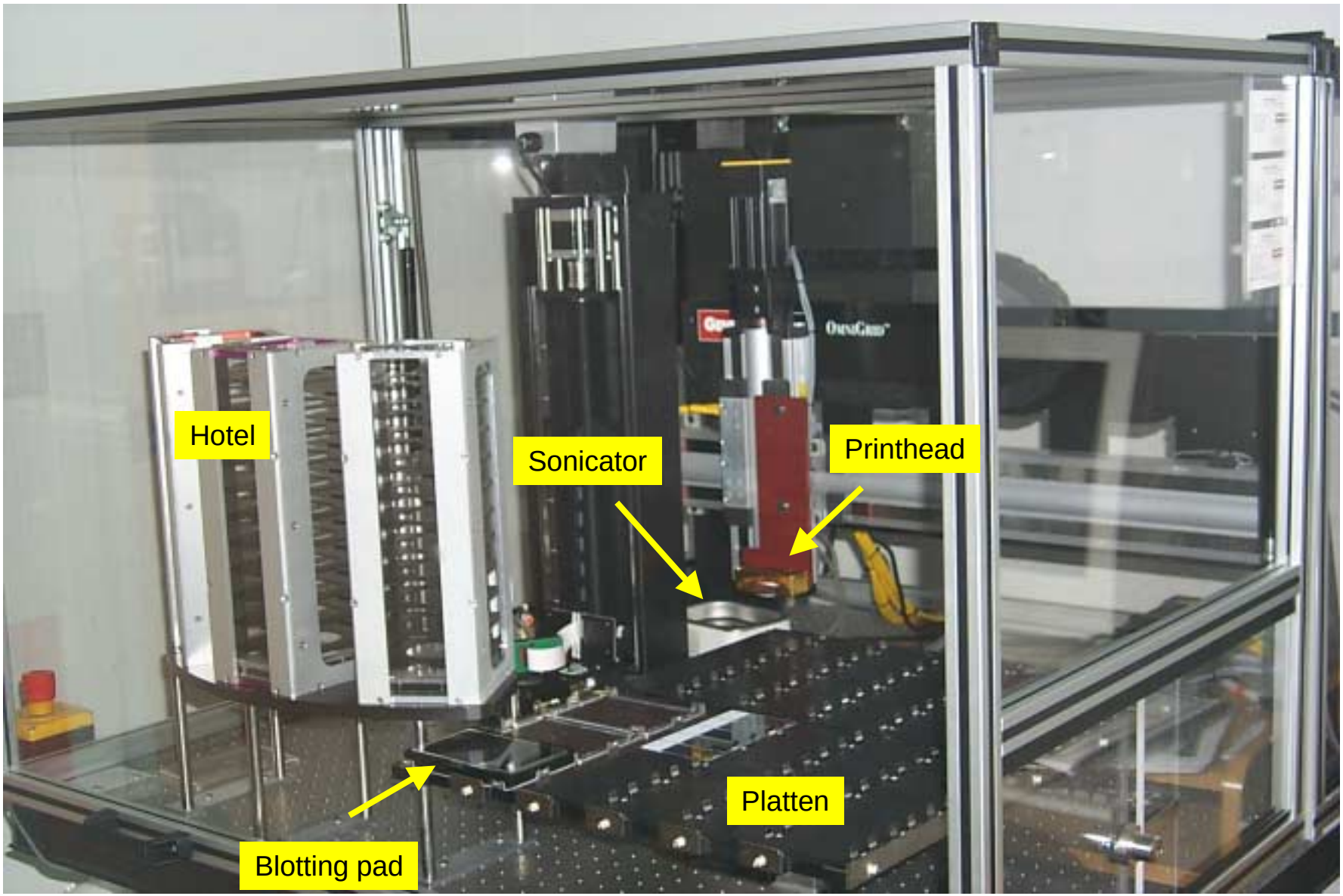
Multiple pin types/brands

Up to 48 pins

Blotting Capability

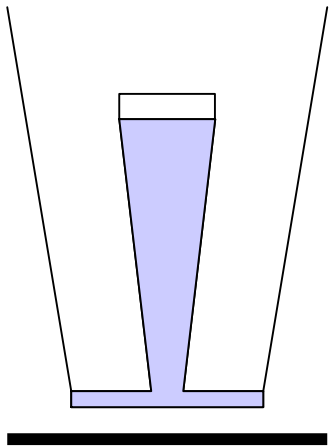
Sample Tracking

Humidity control/HEPA

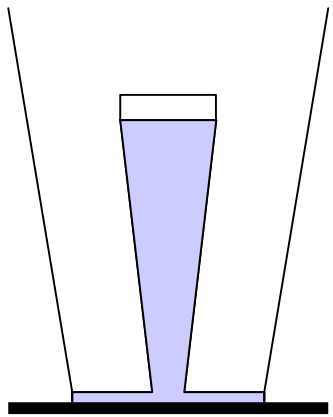


Array Spotter II

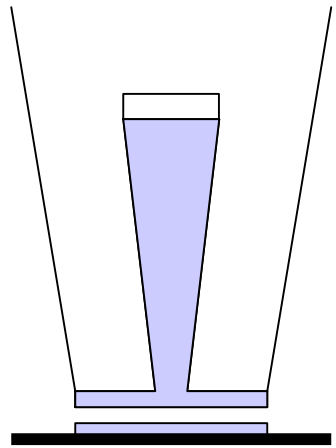
FACTORS AFFECTING SPOT MORPHOLOGY



Slide surface chemistry
Printing solution



Sample DNA viscosity
Pin tip quality



Dwell time
Speed of pin removal

Chamber humidity and temperature

Print Pin Wash Optimization

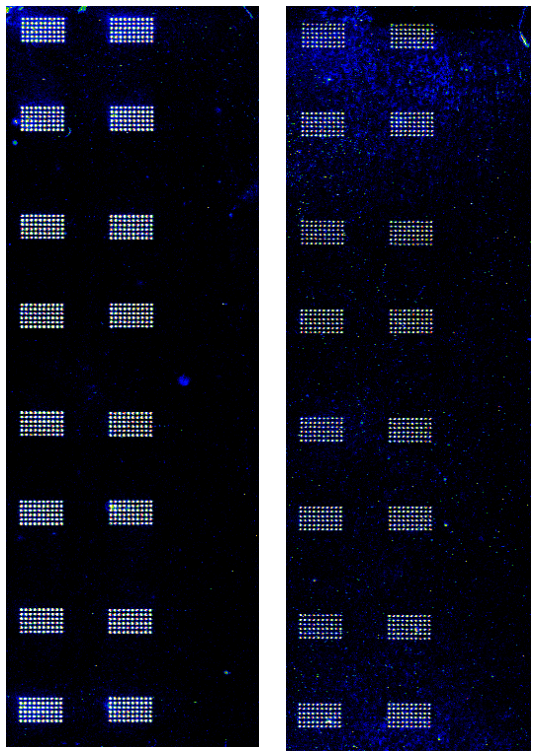
Sonicate, 2000ms
Loop, X times
Wash #1, 2000ms
Dry, 2000ms
Endloop

8x

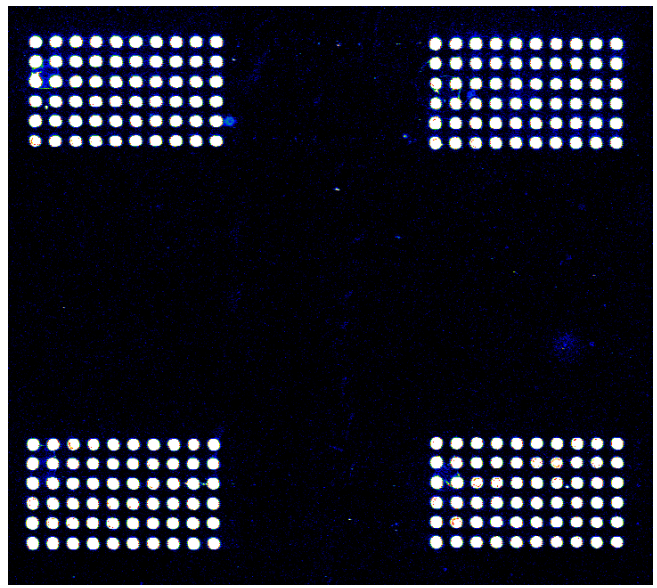
6x

6x

4x



SAMPLE	BUFFER	SAMPLE

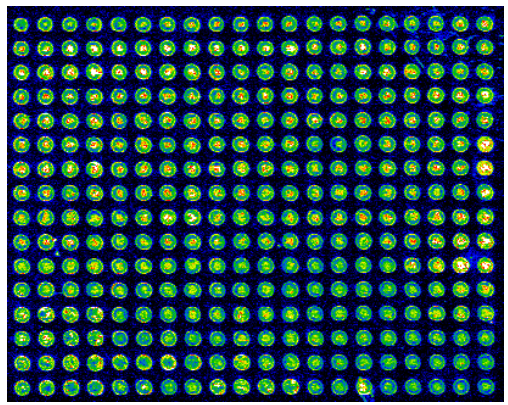


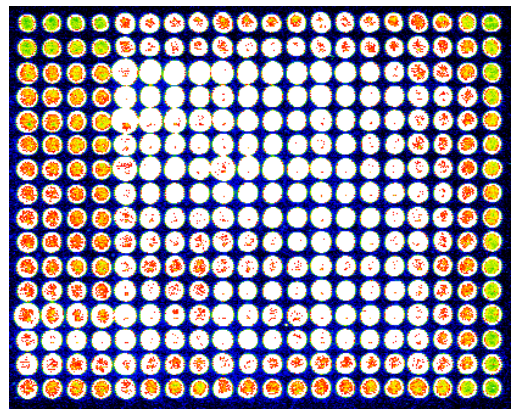
SAMPLE = 500 ug/ml Salmon Sperm DNA in 2XSSC

PRINTING SOLUTION vs SLIDE CHEMISTRY

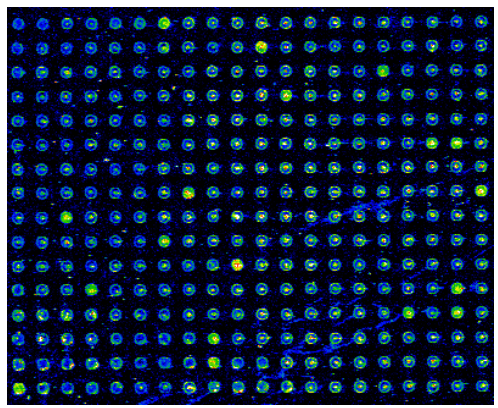
2XSSC 50%DMSO

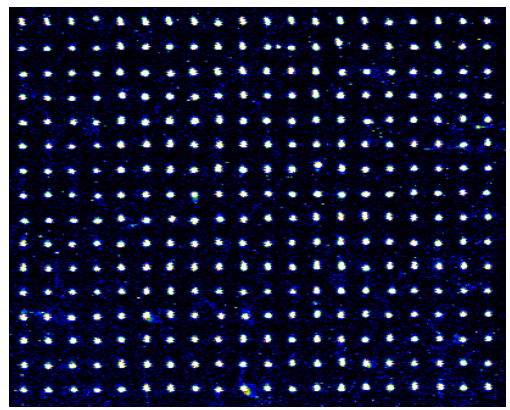
Aminosilane
(Corning GAPS II)





SuperAldehyde
(Telechem)



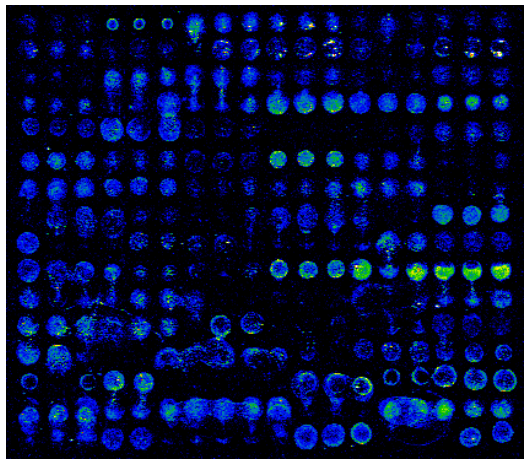


Signal Intensity:

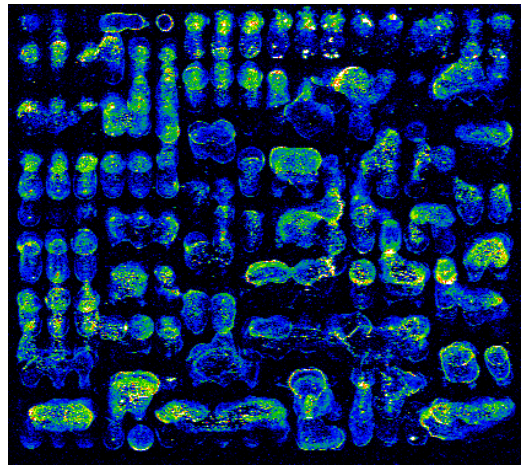
White > red > green > blue

SS Concentration:
125 ug /10 ul

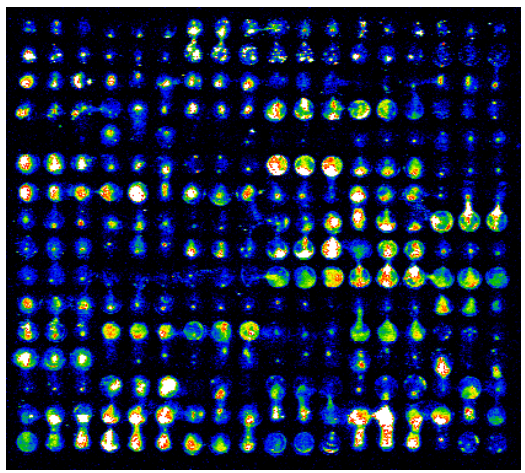
TestArray spot morphology (50% DMSO)



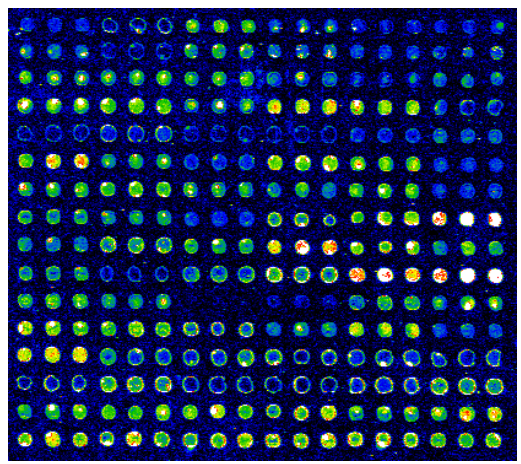
SuperAldehyde
Telechem



SuperAmine
Telechem



AminoSilane
Corning GAPS

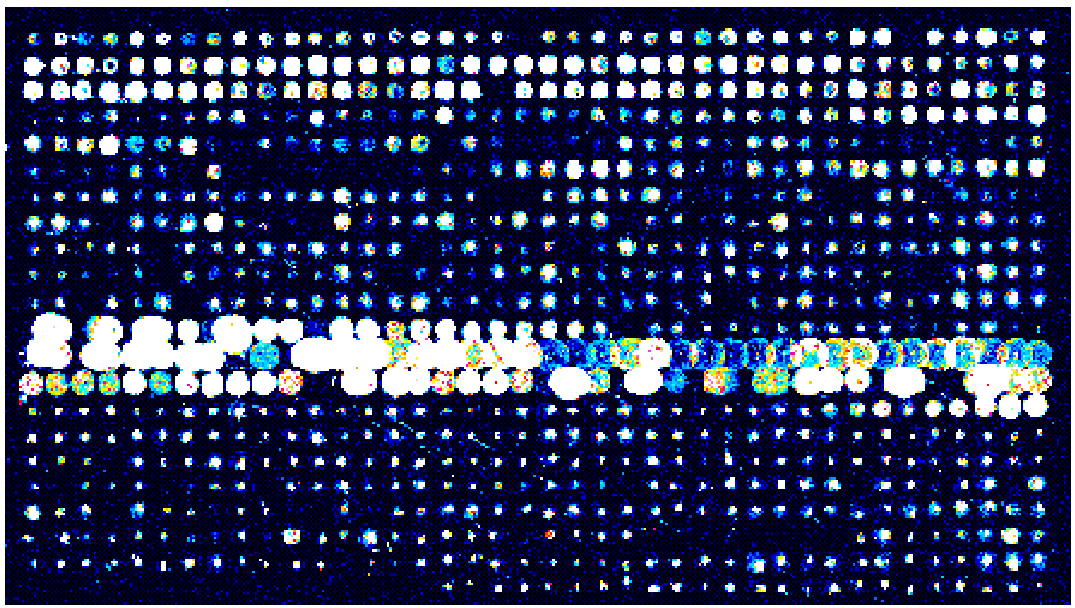


AminoSilane
US Bio-one

Print soln vs
slide II

SPOT MORPHOLOGY

EFFECT OF RELATIVE HUMIDITY



72°F (22.2°C)
40-45 % Relative Humidity

62°F (16.7°C)
40-45 % Relative Humidity

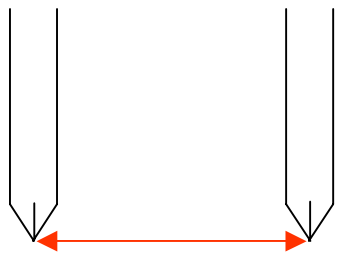
80°F (26.7°C)
80-85 % Relative Humidity

62°F (16.7°C)
40-45 % Relative Humidity

PRINT SPACING

Pin Spacing

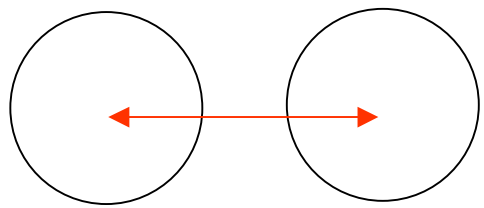
384-well plate: 4.5mm
96-well plate: 9mm



Spot Spacing

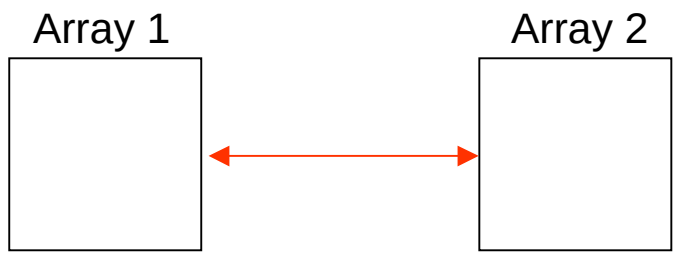
Center-to-center

Pin	List Diameter	Seen Diameter
SMP3	90-100µm	120-140µm
SMP7	200-220µm	430-450µm

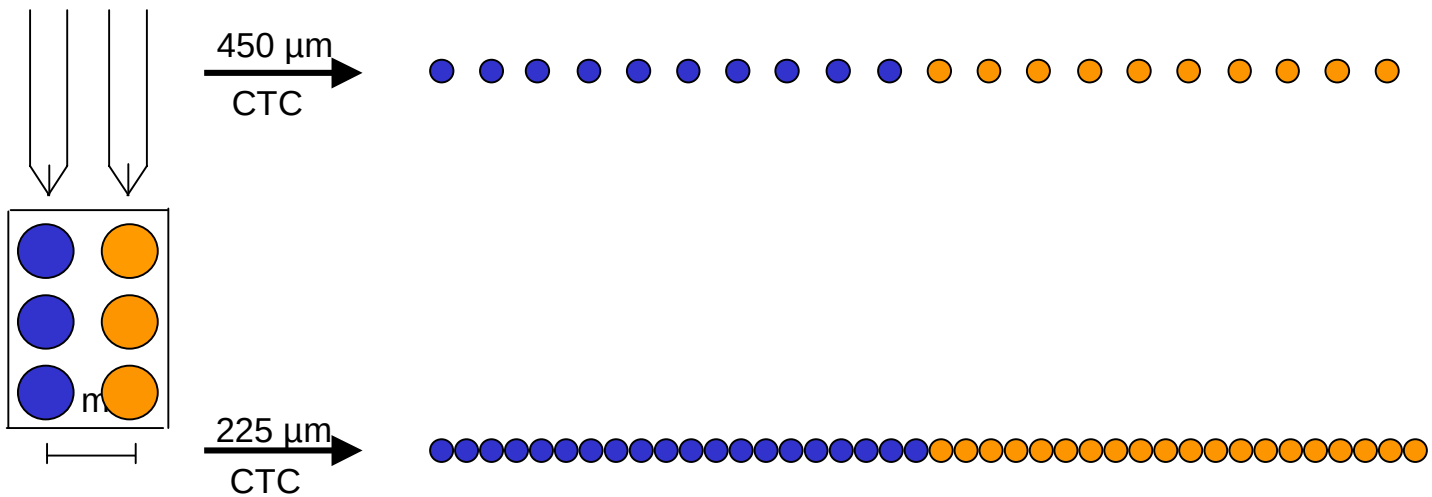


Array Spacing

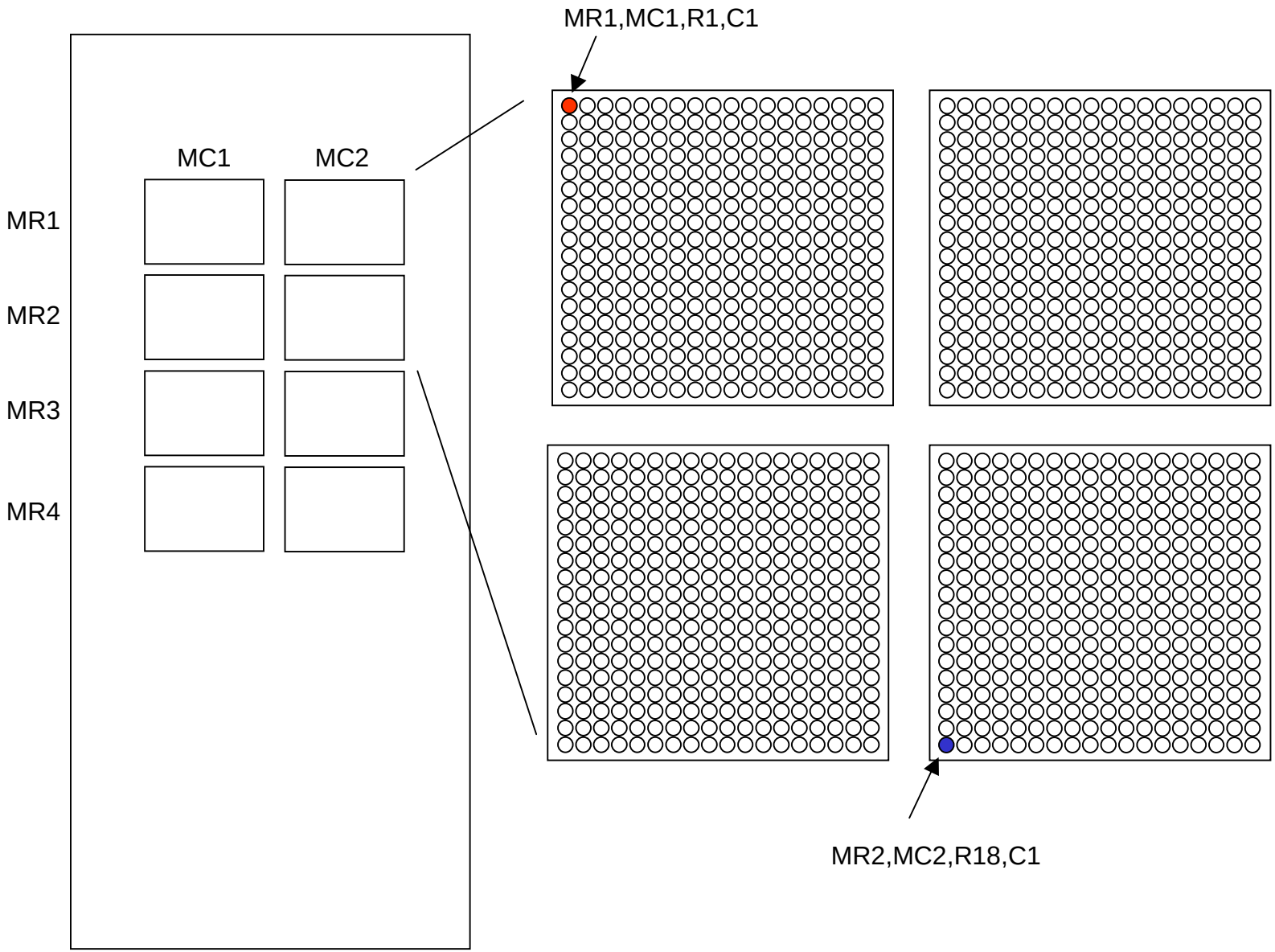
At least the same as spot spacing



ADAPTED FROM A TORREY MESA SLIDE

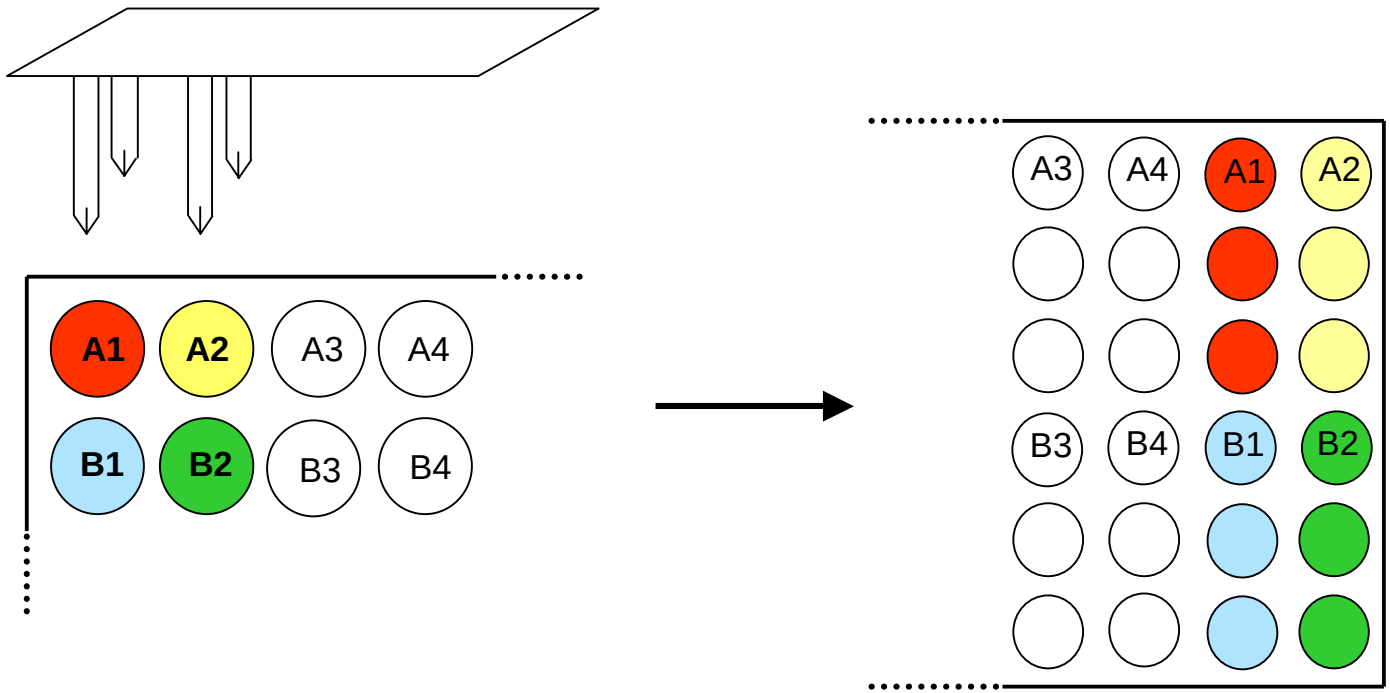


10 vs 20 spots



Array Address

CLONE TRACKING



Printing Plate

Slide

GENELIST

ROW	COL	GENBANK NO.	EST CLONE ID	LOCATOR	BLASTN	BLASTN BEST HIT	SCORE	E-VAL
1	1	Control1	Actin					
1	2	Control1	Actin					
1	3	Control2	Tubulin					
1	4	Control2	Tubulin					
1	5	Control3	50% DMSO					
1	6	Control3	50% DMSO					
1	7	Control4	50% DMSO					
1	8	Control4	50% DMSO					
1	9	Control5	SP1					
1	10	Control5	SP1					
1	11	Control6	SP3					
1	12	Control6	SP3					
1	13	Control7	Salmon Sperm DNA					
1	14	Control7	Salmon Sperm DNA					
1	15	BE399237	WHE0032.E03F990514	9032_ D08_ H16	WHTGLIABG	Wheat (T.aestivum) alpha-Wbeta-c	402	e-110
1	16	BE399675	WHE0033.E12F990516	9035_ C11_ F21	HVTRYPINH	H.vulgare mRNA for trypsin inhibi	383	e-104
1	17	BE399200	WHE0031.H03F990514	9033_ A05_ A09	AC004142	Homo sapiens BAC clone CTB-11	44.1	0.084
1	18	BE399612	WHE0038.G04F990516	9035_ F10_ L19	AC005608	Homo sapiens chromosome 5, PA	42.1	0.19
1	19	BE399282	WHE0034.A04F990516	9033_ B05_ C09	TAU76558	Triticum aestivum alpha-tubulin mR	529	e-148
1	20	BE399852	WHE0041.A08F990703	9035_ H04_ P07	CELY92H12A	Caenorhabditis elegans cosmid Y	44.1	0.043
1	21	BE399290	WHE0034.B02F990516	9033_ C10_ E19	AC008857	Homo sapiens chromosome 5 clon	40.1	0.99
1	22	BE399866	WHE0041.B10F990703	9036_ B03_ D06	HVU43505	Hordeum vulgare profilin (Hvpro1)	464	e-128
1	23	BE399364	WHE0035.C07F990516	9033_ E10_ I19	AL355365	Human DNA sequence from clone	42.1	0.28
1	24	BE399965	WHE0044.C10F990602	9033_ G05_ M03	AC084729	Papio hamadryas clone RP41-12N	40.1	0.35
2	1	Control1	Actin					
2	2	Control1	Actin					
2	3	Control2	Tubulin					
2	4	Control2	Tubulin					
2	5	Control3	50% DMSO					
2	6	Control3	50% DMSO					
2	7	Control4	50% DMSO					
2	8	Control4	50% DMSO					
2	9	Control5	SP1					
2	10	Control5	SP1					
2	11	Control6	SP3					
2	12	Control6	SP3					
2	13	Control7	Salmon Sperm DNA	9045_A07		ABllqb|AF286320.1|AF286320 Triticum aestivum gran		
2	14	Control7	Salmon Sperm DNA					
2	15	BE488825	WHE1078_G03_N06ZS	9045_A08		ABllqb|AF017362.1|AF017362 Oryza sativa aldolase mR		
2	16	BE424340	WHE0083_D10_G19ZS	9023_ F04_ L07	RICGA3	Rice (YK426) mRNA, complete c	313	4.00E-83
2	17	BE423462	WHE0064_D09_G18ZS	9045_A09		ABllqb|AF280606.1|AF280606 Triticum aestivum omec		
2	18	BE424540	WHE0086_F07_L14ZS	9024_ A10_ B20	AMY010454	Alopecurus myosuroides mRNA f	367	4.00E-93
2	19	BE638053	WHE0995-0998_E01	9045_A10		ABlldbj|D89802.1|D89802 Oryza sativa mRNA for clor		
2	20	BE424936	WHE0088_A03_A06ZS	9024_ D03_ H06	RICRPL3A	Rice mRNA for ribosomal protein	470	e-130
2	21	BE439591	WHE0962_F09_L18ZS	9045_A11		ABllqb|U09450.1|OSU09450 Oryza sativa enolase mRN		
2	22	BE424948	WHE0088_C02_E04ZS	9024_ F04_ L08	AFU80041	Avena fatua Af10-protein mRNA,	117	6.00E-24

CLONE TRACKING

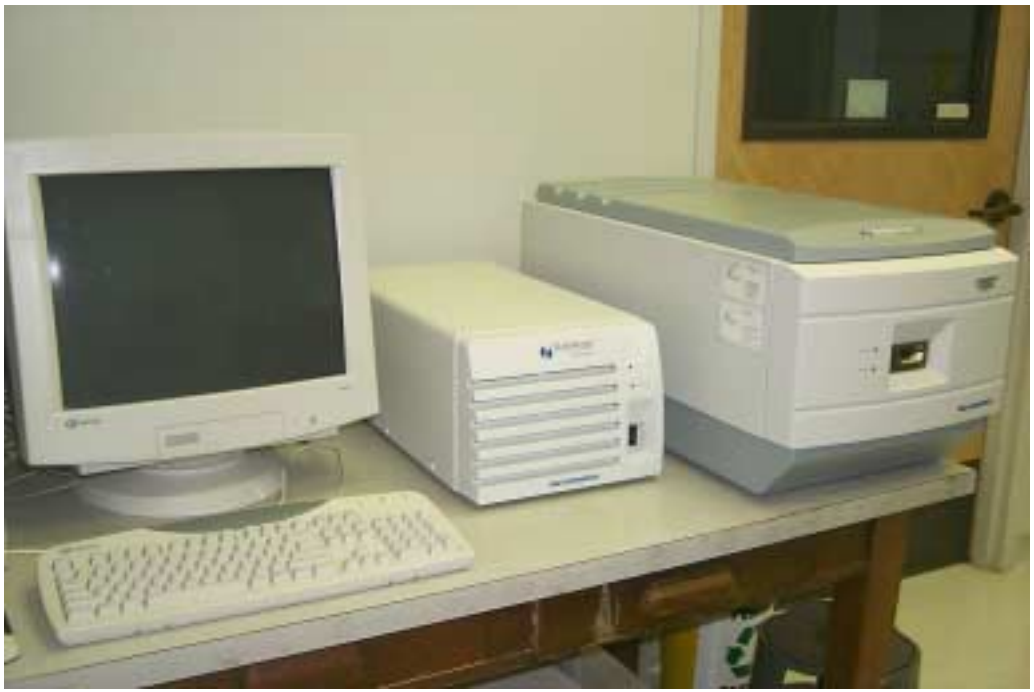
GAL FILE

(Genes on Array List)

	1	2	3	4	5	6	7
1	1	1	1	1	Control1		
2	1	1	1	2	Control1		
3	1	1	1	3	Control1		
4	1	1	1	4	Control2		
5	1	1	1	5	Control2		
6	1	1	1	6	Control2		
7	1	1	1	7	Control3		
8	1	1	1	8	Control3		
9	1	1	1	9	Control3		
10	1	1	1	10	Control4		
11	1	1	1	11	Control4		
12	1	1	1	12	Control4		
13	1	1	1	13	Control5		
14	1	1	1	14	Control5		
15	1	1	1	15	Control5		
16	1	1	1	16	Control6		
17	1	1	1	17	Control6		
18	1	1	1	18	Control6		
19	1	1	2	1	Control7		
20	1	1	2	2	Control7		
21	1	1	2	3	Control7		
22	1	1	2	4	BE399675		
23	1	1	2	5	BE399675		
24	1	1	2	6	BE399675		
25	1	1	2	7	BE399612		
26	1	1	2	8	BE399612		
27	1	1	2	9	BE399612		
28	1	1	2	10	BE399852		
29	1	1	2	11	BE399852		
30	1	1	2	12	BE399852		
31	1	1	2	13	BE399866		
32	1	1	2	14	BE399866		
33	1	1	2	15	BE399866		
34	1	1	2	16	BE399965		
35	1	1	2	17	BE399965		
36	1	1	2	18	BE399965		
37	1	1	3	1	BE399468		

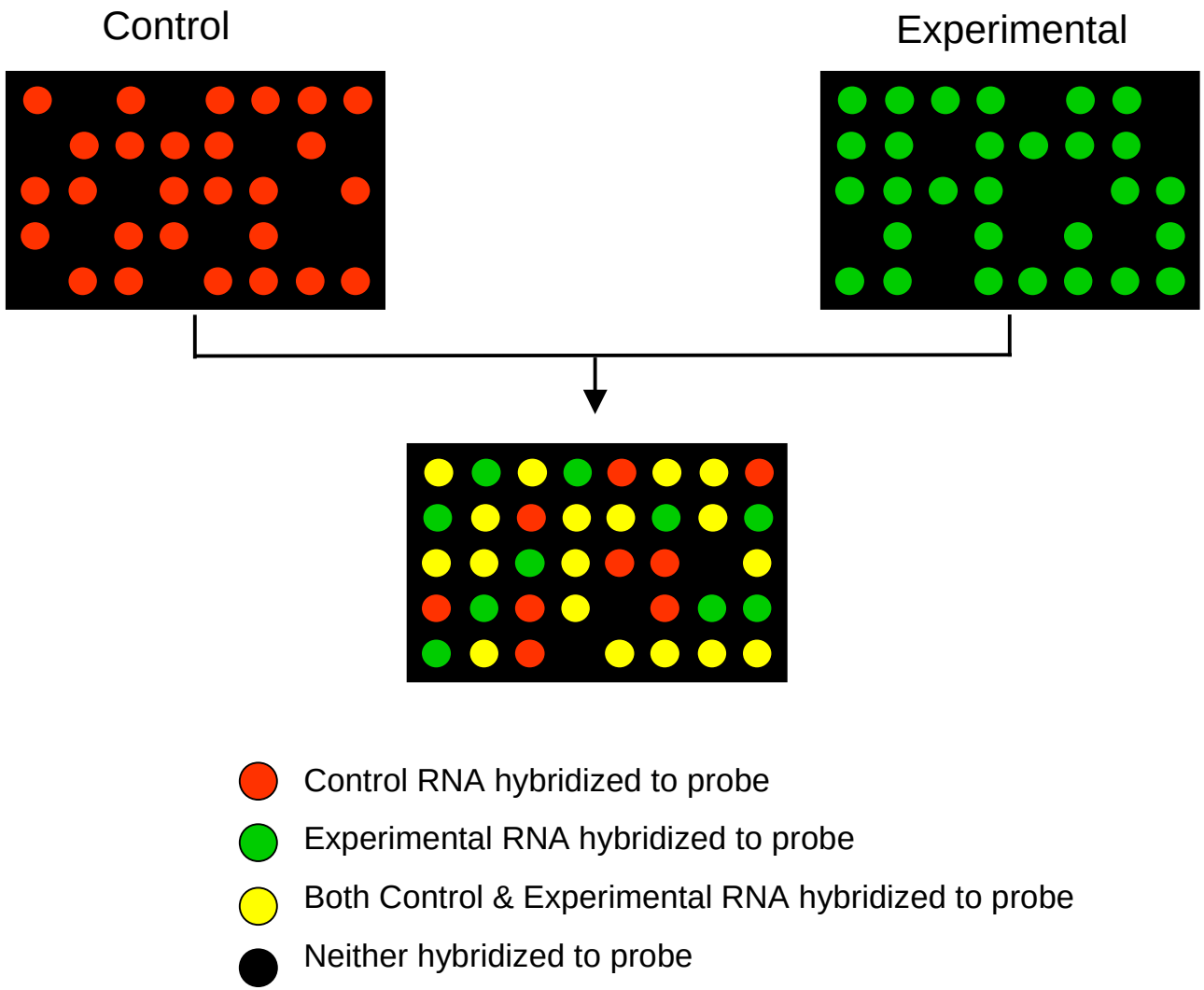
GAL

MICROARRAY READER



ScanArray 5000XL from PerkinElmer (GSI Lumonics)

5-50 um/pixel resolution
Sequential scanning
~5 minutes/scan
5 Lasers
Quantarray software


Control
Experimental
Control RNA hybridized to probe
Experimental RNA hybridized to probe
Both Control & Experimental RNA hybridized to probe
Neither hybridized to probe

Power and PMT settings for Cy3 and Cy5 lasers

PMT \ POW	60	65	70	75	80	85	90
60	🔴	🔴	🔴	🟡	🟡	🟢	🟢
65	🔴	🟡	🟡	🟢	🟢	🟢	🟢
70	🟡	🟢	🟢	🟢	🟢	🟢	🟢
75	🟢	🟢	🟢	🟢	🟢	🟡	🟡
80	🟢	🟢	🟢	🟡	🟡	🔴	🔴
85	🟡	🟡	🟡	🔴	🔴	🔴	🔴
90	🔴	🔴	🔴	🔴	🔴	🔴	🔴

🟢 - optimal for linearity and saturation

🟡 - should be avoided if possible

🔴 - should not be used

U Toronto Array Facility?

Separate SIGNAL from NOISE

…to reliably detect a biologically significant difference in expression level.

CONTROL SET

Poly (dA)	Negative control, since most spotted DNA are from EST inserts with polyA
5'-end, middle, 3'end of a few genes	Relative signal from this control indicate the quality of labeled probe as reflected by length
Genomic DNA from	Signal indicates non-specific hybridization to
Salmon, T. aestivum	Low-entropy sequences present in some % of cDNA
E.coli, lambda and Plasmid DNA	Negative control test for vector contamination
t-RNA and rRNA	Signal indicates extent of labeling of non-poly A+ RNAs (especially useful when using total RNA for labeling)
Replicate positive controls i.e actin	Spaced throughout the array, especially corners, to orient the grid and to check overall consistency of array element deposition
Transgene markers	Check for transgenic identity
Spiking Controls	Test sensitivity, dynamic range and for normalization
Housekeeping genes	Act as positive controls, maybe useful for normalization

LIST OF CONTROLS

Constitutive Genes
Actin
Alpha-Tubulin1 (T. aestivum)
Alpha-Tubulin2
Alpha-Tubulin3 (H. vulgare)
Cytochrome P-450 monooxygenase
EF1-alpha
EF1-beta
EF1-gamma
EF2
Enolase
Gluthathione S-transferase I
Heat Shock protein (hsp70)
Histone 1
Histone 2
Histone 3
Histone 4
Lipid Transfer Protein
Mitochondrial F-1-ATPase subunit 2
Polyubiquitin
Ribosomal protein L3
Ribosomal protein L7
Ribosomal protein S4
Ribosomal protein S7
S-adenolsylmethionine decarboxylase
S-adenolsylmethionine synthetase
Ubiquitin

Tissue-specific Markers
Alpha/Beta gliadin
chlorophyll a/b binding complex
ferredoxin
Gamma gliadin
HMW glutenin
LMW glutenin
Omega gliadin
oxygen-evolving complex
PS I proteins
PS II protein
Rubisco small subunit

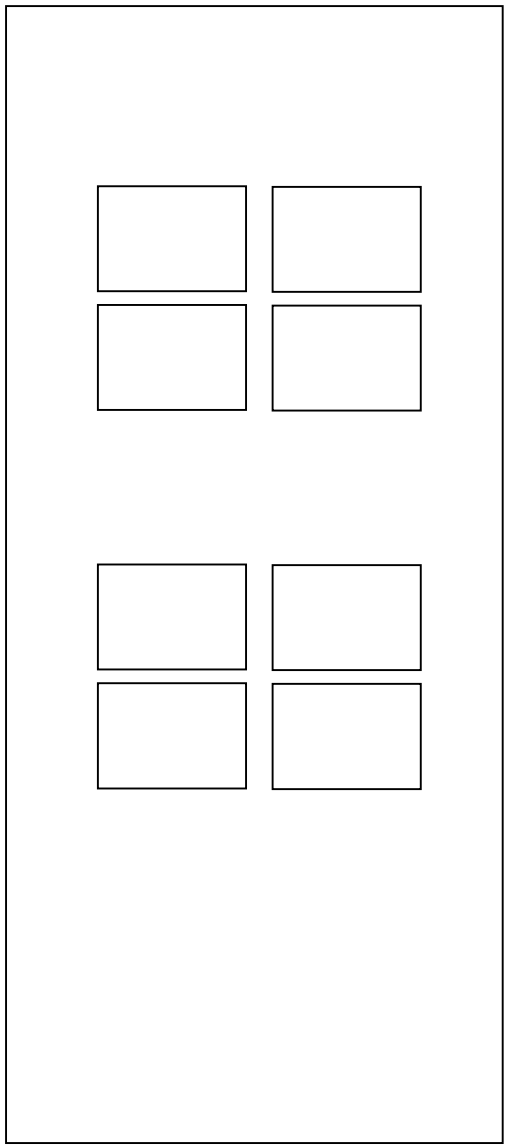
Contamination
Agrobacterium Genomic DNA
E. coli DNA
rRNA (25S)
rRNA (18S)

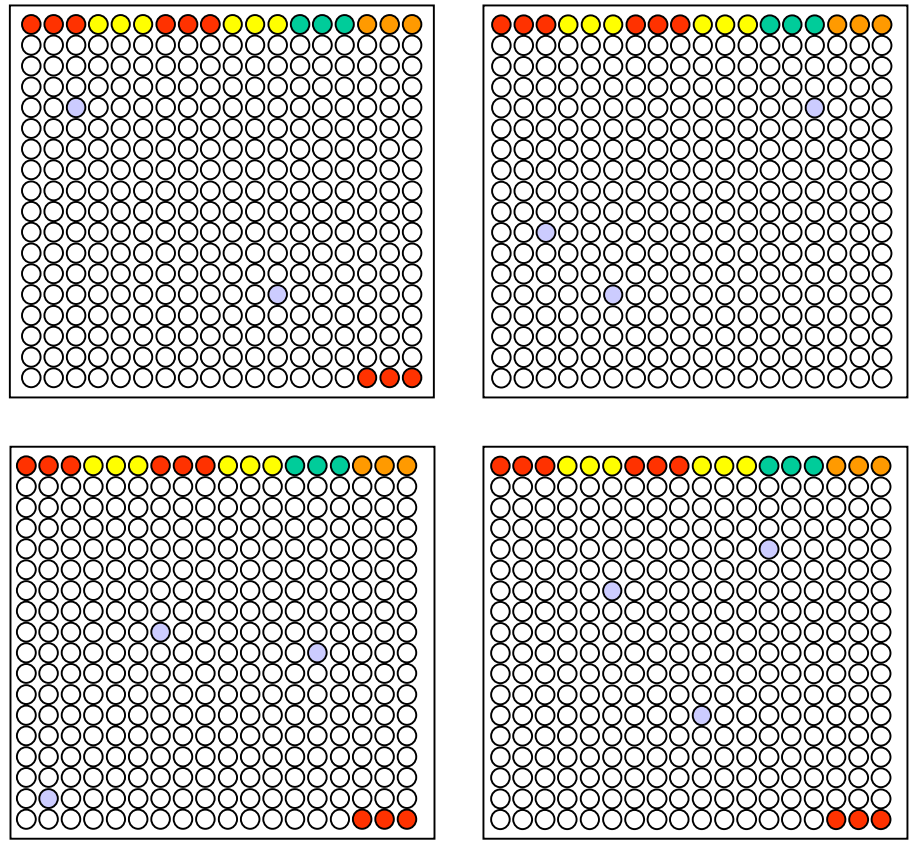
Negative Controls
Triticum aestivum genomic DNA
Salmon Sperm DNA
30 Campylobacter genes
printing solution
blanks

Spiking Controls
Sp1: B-cell receptor prot (IMAGE:1420858)
Sp2: Myosin Heavy Chain (IMAGE:1593605)
Sp3: Myosin Reg. Light Chain2 (IMAGE: 1592600)
Sp4: Insulin-like Growth Factor (IMAGE: 1576490)
Sp5:FLJ1091fis
Sp6:HSPC120
Sp7:Beta2 microglobulin
Sp8:Phosphoglycerate kinase (pgk1)
Sp9:Tyrosine Phosphatase (pac-1)
Sp10:G10 Homolog (edg-2)

Transgenes
BT: B. Thuringiensis cry1 AC
BAR: Phosphinothricin acetyltransferase
GFP: Green Fluorescent Protein
Globin: Globin
GUS: Beta-Glucuronidase (uidA)
HPH: Hygromycin B phosphotransferse
Luc: Luciferase
nptII: Kanamycin/Neomycin phosphotransferase

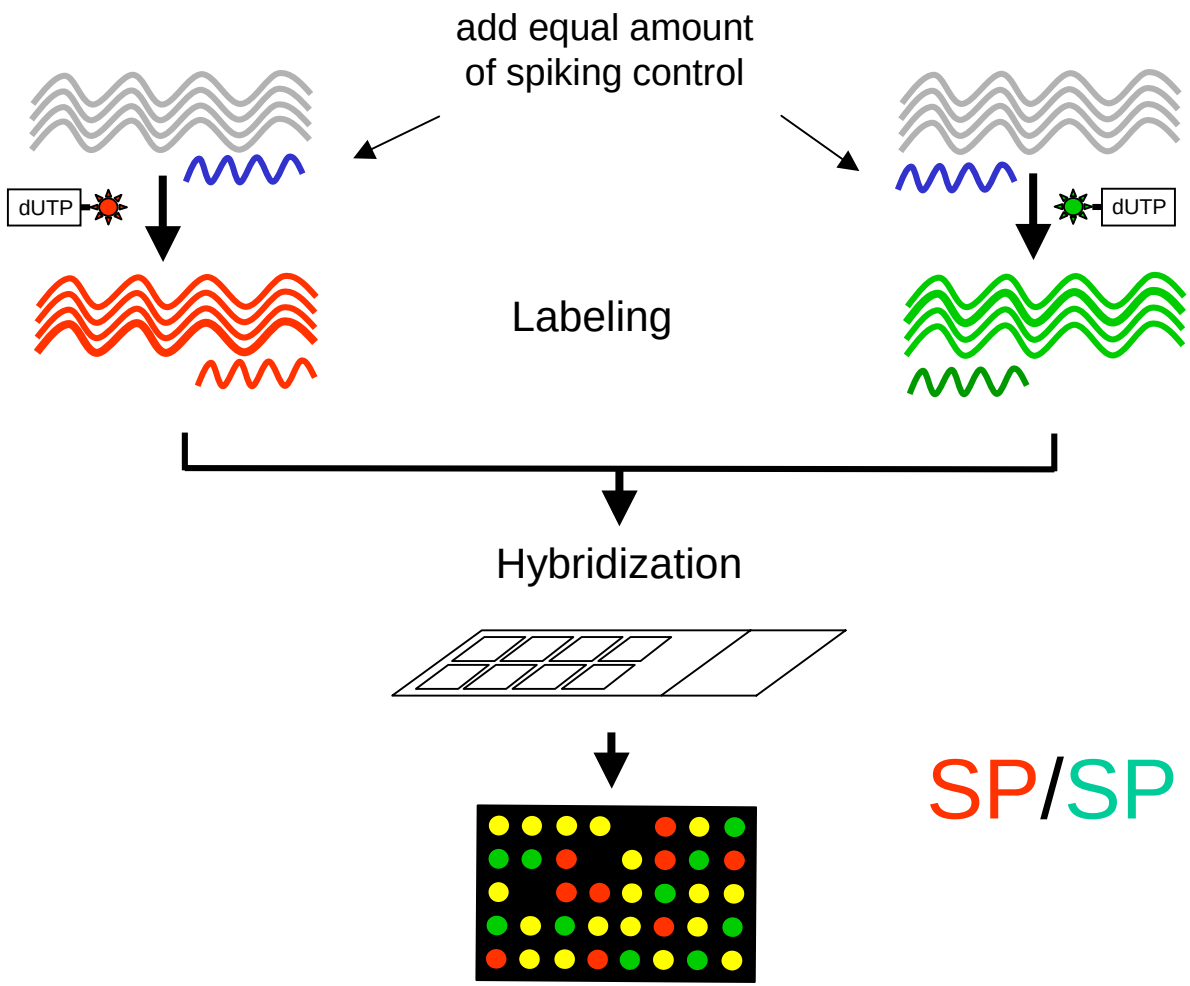
ARRAY DESIGN



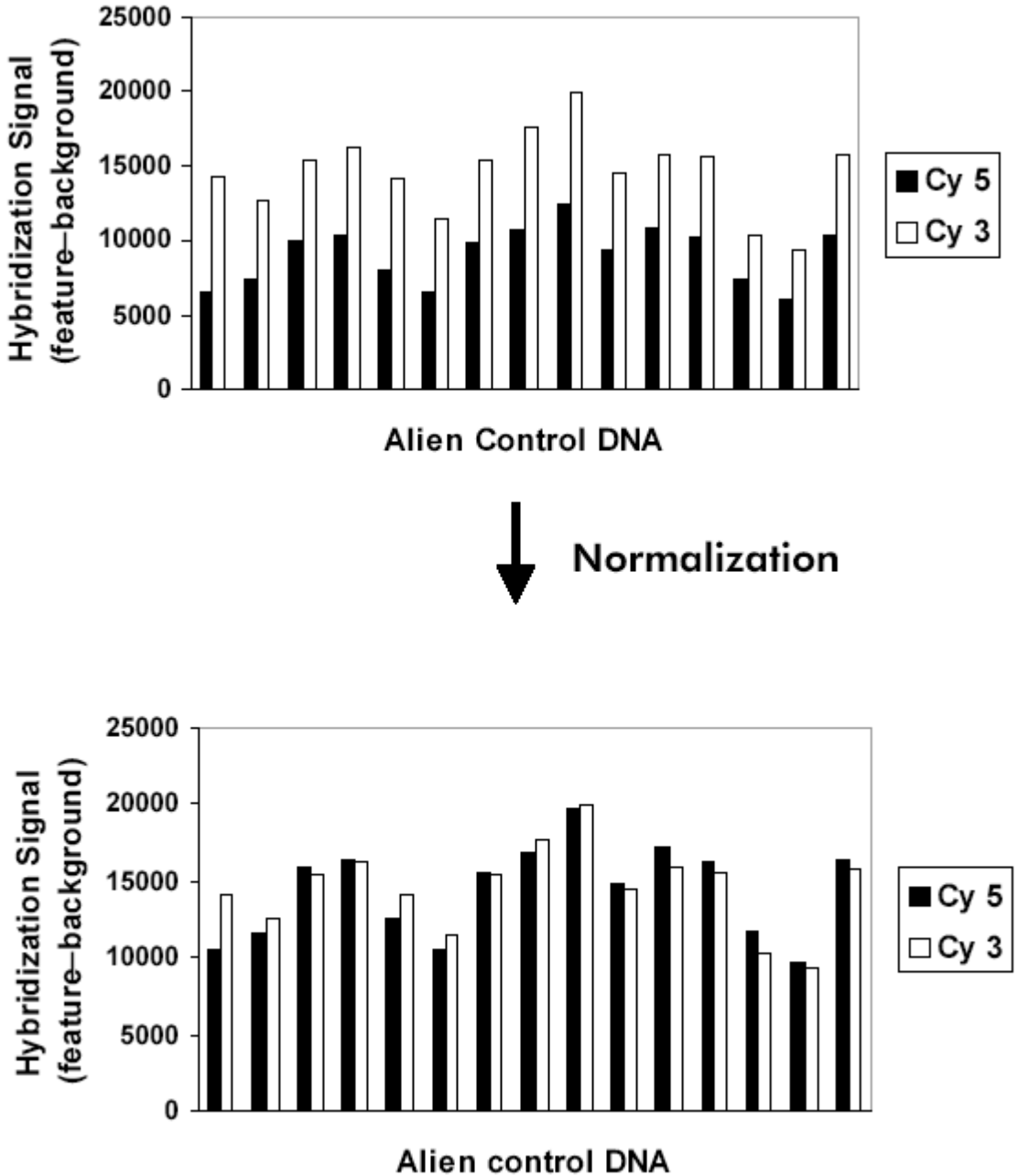


- Positive control
- Buffer/blank control
- Spiking control
- Negative controls
- Other controls

SPIKING CONTROL



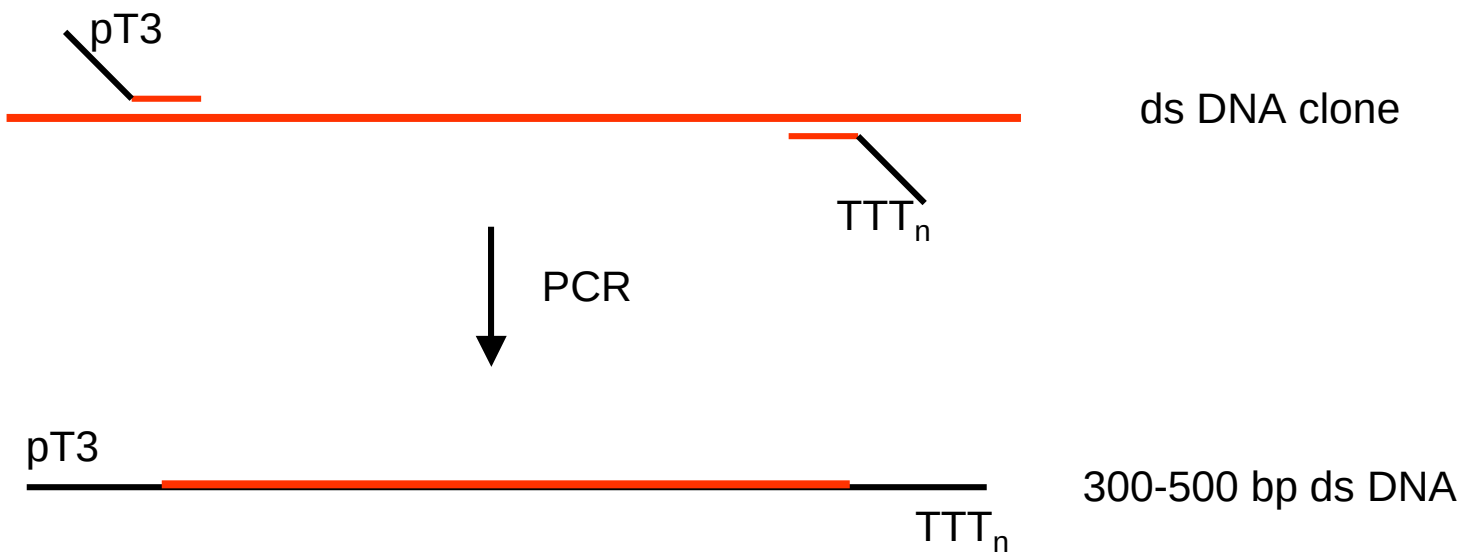
SP/SP = 1



Normalize with SC

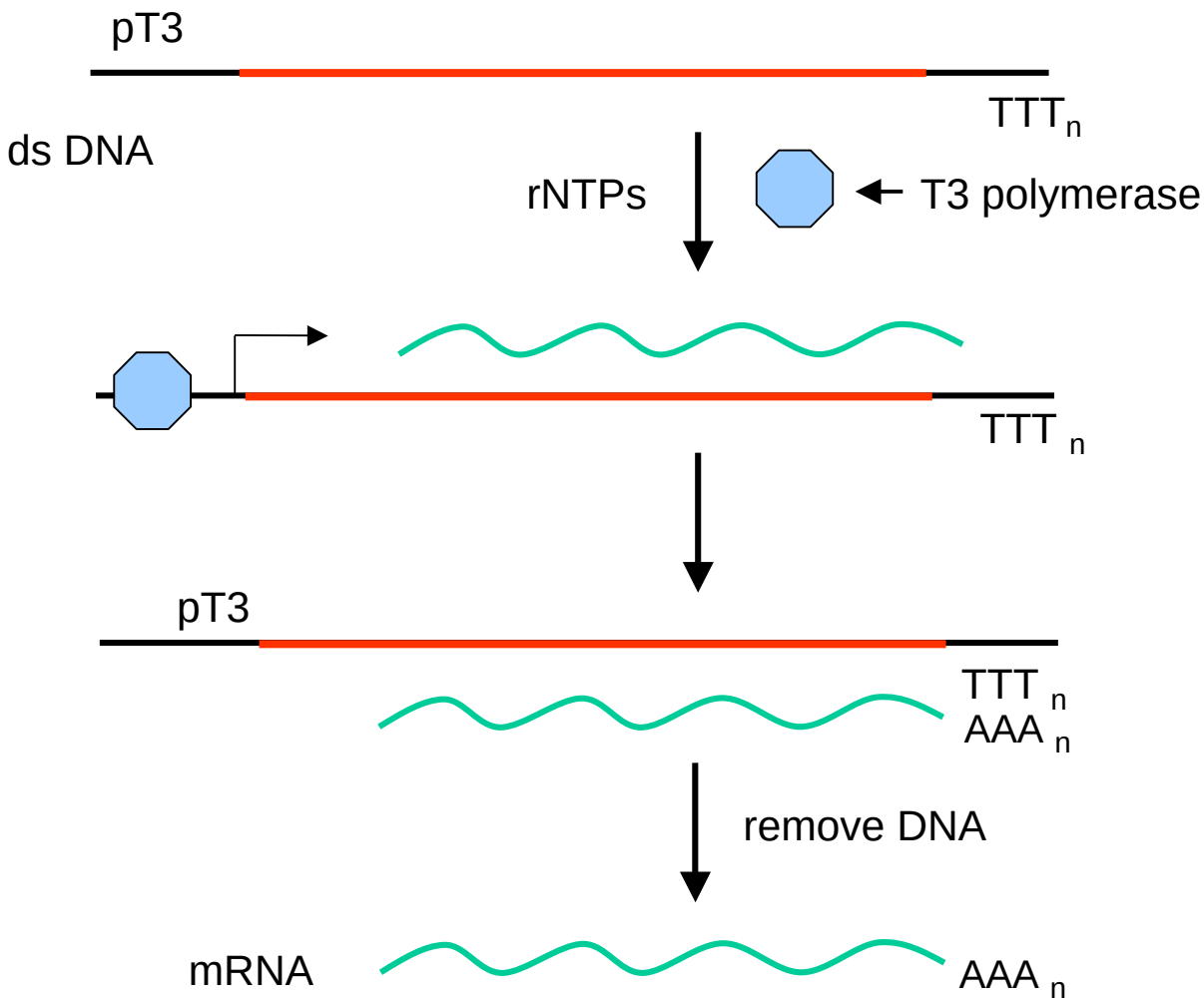
SPIKING CONTROL

I. Preparation of Template DNA



SPIKING CONTROL

II. *In vitro* Transcription



SPIKING CONTROL SET

Spiking Control with Custom Primers for *In Vitro* Transcription	GI	PCR band, vector primers	PCR band, custom primers
Sp01: B-cell Receptor Protein	AF126021	1324	342
Sp01-F: 5′–GCGCAATTAACCCTCACTAAAGGGCGCGAATCTGTGTTCACC–3′			
Sp01-R: 5′–TTTTTTTTTTTTTTTTTTTTTAATGGACGGCAACACTCG–3′			
Sp02: Myosin Heavy Chain	X13988	1180	543
Sp02-F: 5′–GCGCAATTAACCCTCACTAAAGGGAGGAGCAGCTGGCGATTGTGG–3′			
Sp02-R: 5′–TTTTTTTTTTTTTTTTTTTTTCTCTGTGTTCTTCTTCTGCTCTCC–3′			
Sp03: Myosin Light Chain 2	M21812	560	342
Sp03-F: 5′–GCGCAATTAACCCTCACTAAAGGGTCGGGGAGAAGCTCAAGGG–3′			
Sp03-R: 5′–TTTTTTTTTTTTTTTTTTTTTAGGTCGGGCCGAACAGAAG–3′			
Sp04: Insulin-like Growth Factor	X07868	1028	576
Sp04-F: 5′-GCGCAATTAACCCTCACTAAAGGGGCAAGCGCCCTGTGAGT-3′			
Sp04-R: 5′-TTTTTTTTTTTTTTTTTTTTTGGGATTGCAAGCGTTAC-3′			
Sp05: FLJ1091fis	AK001779	1562	519
Sp05-F:5′-GCGCAATTAACCCTCACTAAAGGGGTGAATCCTGAAACAAAGAGACCA-3′			
Sp05-R:5′-TTTTTTTTTTTTTTTTTTTTTGGCAGACCACAGACATGAAACAGT-3′			
Sp06: HSPC120	AF161469	1451	577
Sp06-F:5′-GCGCAATTAACCCTCACTAAAGGGTTACAGAAAGTTGGCGATAGG-3′			
Sp06-R:5′-TTTTTTTTTTTTTTTTTTTTTAACTCCCATTGCAGAACTTGA-3′			
Sp08: Phosphoglycerate Kinase (pgk1)	NM_000291	1228	504
Sp08-F:5′-GCGCAATTAACCCTCACTAAAGGGTGGAACACGGAGGATAAAG-3′			
Sp08-R:5′-TTTTTTTTTTTTTTTTTTTTTTGAGTAGTGAAACAGTGACAAAG-3′			
Sp09: Tyrosine Phosphatase (pac-1)	L11329	1230	571
Sp09-F:5′-GCGCAATTAACCCTCACTAAAGGGGGAGGCCGGGTGCTGGTG-3′			
Sp09-R:5′-TTTTTTTTTTTTTTTTTTTTTAAGGGCTTCAACATGGTGGTGGAC-3′			
Sp10: G10 Homolog (edg-2)	U11861	926	513
Sp10-F:5′-GCGCAATTAACCCTCACTAAAGGGAGGATGGCTGGGAGTTGATTGAG-3′			
Sp10-R:5′-TTTTTTTTTTTTTTTTTTTTTGAAGGGGGTGGCGTGACAGG-3′			

SP01: B-cell receptor protein

```
   1 aagttcgggt ccgtagtggg ctaaggggga gggtttcaaa gggagcgcac ttccgctgcc
  61 ctttctttcg ccagccttac gggcccgaac cctcgtgtga agggtgcagt acctaagccg
 121 gagcggggta gaggcgggcc ggcacccccct tctgacctcc agtgccgccg gcctcaagat
 181 cagacatggc ccagaacttg aaggacttgg cgggacggct gcccgccggg ccccggggca
 241 tgggcacggc cctgaagctg ttgctggggg ccggcgccgt ggcctacggt gtgcgcgaat
 301 ctgtgttcac cgtggaaggc gggcacagag ccatcttctt caatcggatc ggtggagtgc
 361 agcaggacac tatcctggcc gagggccttc acttcaggat cccttggttc cagtacccca
 421 ttatctatga cattcgggcc agacctcgaa aaatctcctc ccctacaggc tccaaagacc
 481 tacagatggt gaatatctcc ctgcgagtgt tgtctcgacc caatgctcag gagcttccta
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 601 aggtgctcaa gagtgtggtg gccaagttca atgcctcaca gctgatcacc cagcgggccc
 661 aggtatccct gttgatccgc cgggagctga cagagagggc caaggacttc agcctcatcc
 721 tggatgatgt ggccatcaca gagctgagct ttagccgaga gtacacagct gctgtagaag
 781 ccaaacaagt ggcccagcag gaggcccagc gggcccaatt cttggtagaa aaagcaaagc
 841 aggaacagcg gcagaaaatt gtgcaggccg agggtgaggc cgaggctgcc aagatgcttg
 901 gagaagcact gagcaagaac cctggctaca tcaaacttcg caagattcga gcagcccaga
 961 atatctccaa gacgatcgcc acatcacaga atcgtatcta tctcacagct gacaaccttg
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1201 cccacaccgg ttccctgaac ccctcttgga ttaaggaaga ctgaagacta gcccctttc
1261 tgggaaatta ctttcctcct ccctgtgtta actggggctg ttggggacag tgcgtgattt
1321 ctcagtgatt tcctacagtg ttgttccctc cctcaaggct gggaggagat aaacaccaac
1381 ccaggaattc tcaataaatt tttattactt aacctg
```

SP01-F 5'-CAA TTA ACC CTC ACT AAA GGG CGC GAA TCT GTG TTC ACC-3'

SP01-R 5'-TTT TTT TTT TTT TTT TTT TTT TAA TGG ACG GCA ACA CTC G–3'

SP03: Myosin Light Chain 2

```
  1 ctgactcctt gcttctttcc agccggtgcc gctgccttgc cccccggaga ctgaagacat
 61 ggcacccaag agggccaaga gaaggacagt agcagagggc ggaagctcca gcgtcttctc
121 catgttcgac cagactcaga tccaggagtt caaagaggcc ttcactgtga tcgaccagaa
181 ccgtgatggt attatagaca aggaggacct tcgggacacc ttcgcagcca tgggccgcct
241 caatgtgaag aatgaggagt tggatgccat gatgaaggaa gccagcggtc ccatcaactt
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421 ggaggagctg ctgaccacgc agtgtgaccg cttctcccag gaggagatca agaacatgtg
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541 cacgcacggc gacgccaagg accaggagta ggggcacccg cgggcctccg ctgcccgacg
601 cttctgttcg gcccgacctc caccccggct cccaataaaa tttaactgat ctttgtttct
661 ta
```
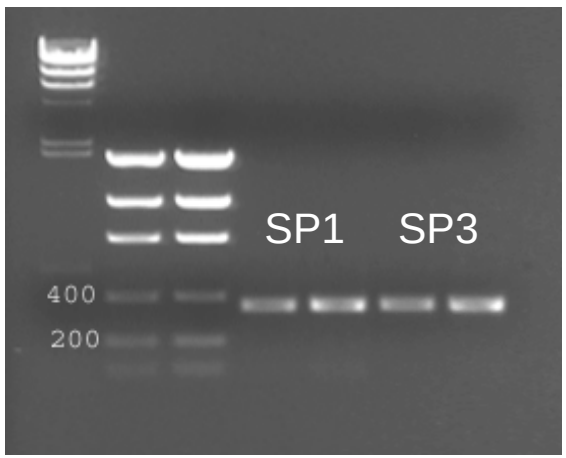

SP03-F 5'-CAA TTA ACC CTC ACT AAA GGG TCG GGG AGA AGC TCA AGG-3'

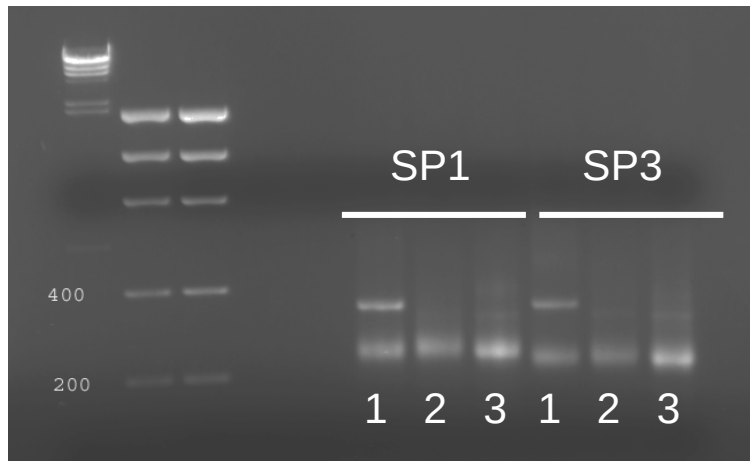
SP03-R 5'- TTT TTT TTT TTT TTT TTT TTT TAG GTC GGG CCG AAC AGA AG-3'

SPIKING CONTROL

Template preparation



In vitro transcription



1 Transcription product
2 After DNAse treatment
3 After purification

Today's lab:

AM

Prepare spiking controls

PM

QC Spiking control prep
Tour EST sequencing lab
Demo microarray printing
Demo use of Scanner
Practice use of Quantarray

In vitro Transcription

Nuclease-free water
5X Txn buffer, DTT,
RNAse Inhibitor, rNTP,
T3 Polymerase

+

Spiking Control
Template DNA
1-2.5 ug

↓

Incubate for 1 hour at 37-40 ^{o}C

↓

Remove template DNA
By DNAse treatment

↓

Purify RNA
(RNAeasy kit)

↓

Quantify