REPORT

Application of engine Protein Array Technology for the analysis of IgG autoantibody repertoires of five patients

engine | the biomarker company

Your Question. Our Service. Your Result.

BIOMARKER SCREENING with 15,000 antigens in one shot with serum samples. In a cooperation-project we have examined serum of 5 autoimmune patients with the engine UniPEx arrays (product No. 1008) .

We identified 7 overlapping potential antigens for serum IgG in all 5 patients.

Did we discover new biomarkers?! We are now investigating the association between autoimmune diseases and antibodies against the following antigens:

- MAFK 8
- HNRNPUL2
- MYH14
- CREBBP
- AATF
- SDE1
- DDX51

Properties of our UniPEx Array

- Clones from different protein expression libraries (lung, T-cells, colon and human fetal brain)

- Mainly clones that are "in frame" are present

- Different libraries in two different vectors and expressed in different *E.coli*-strains on one array

- Spotting in 4x4 pattern

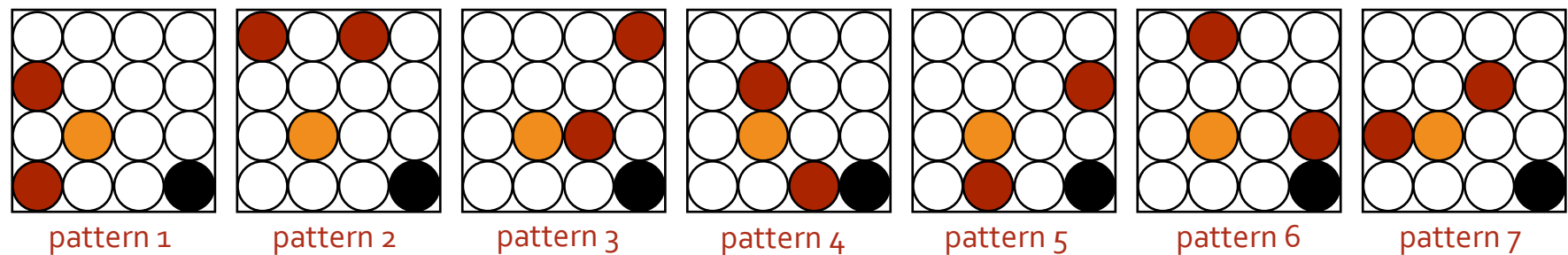- 15,300 clones representing 7,390 different human proteins

Creation of UniPEx Array
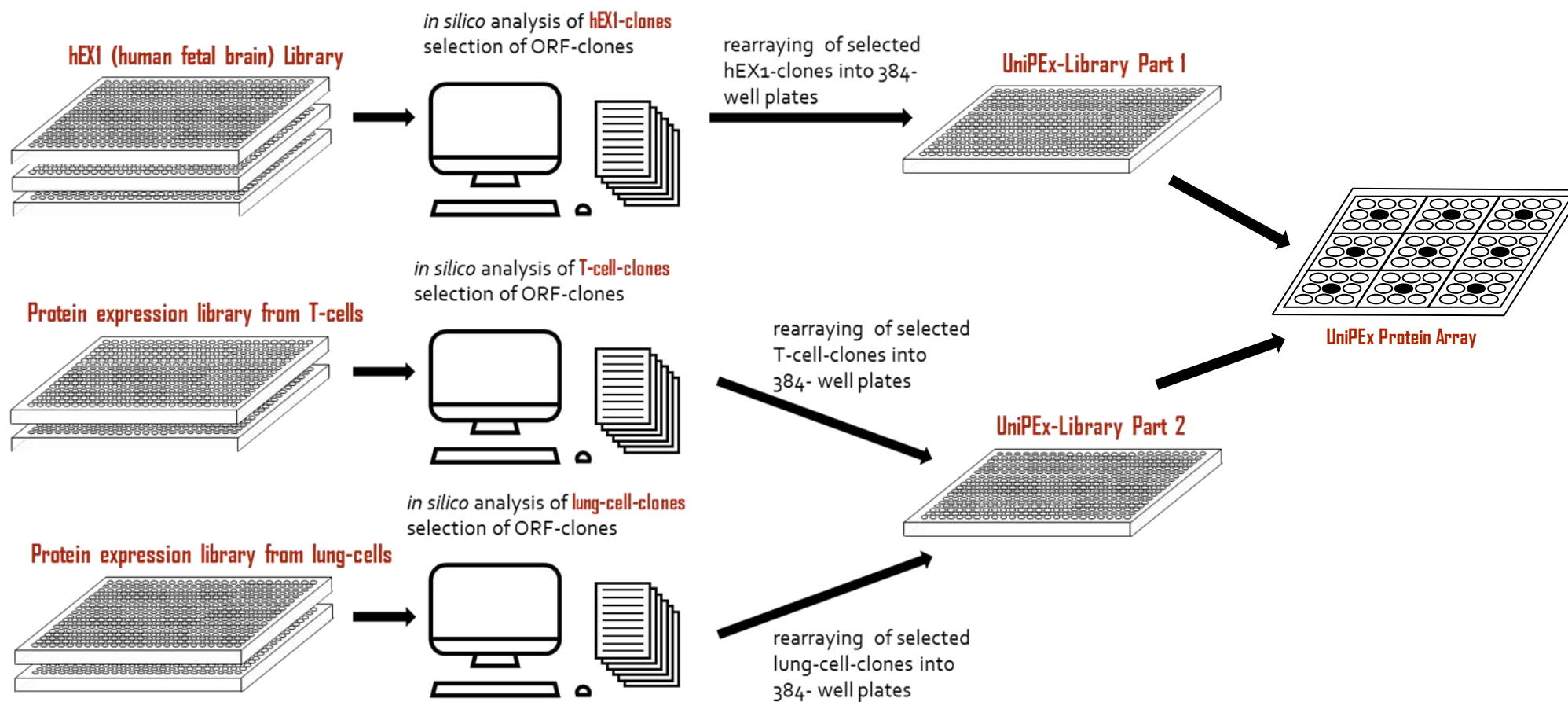
ArrayDesign_192x192
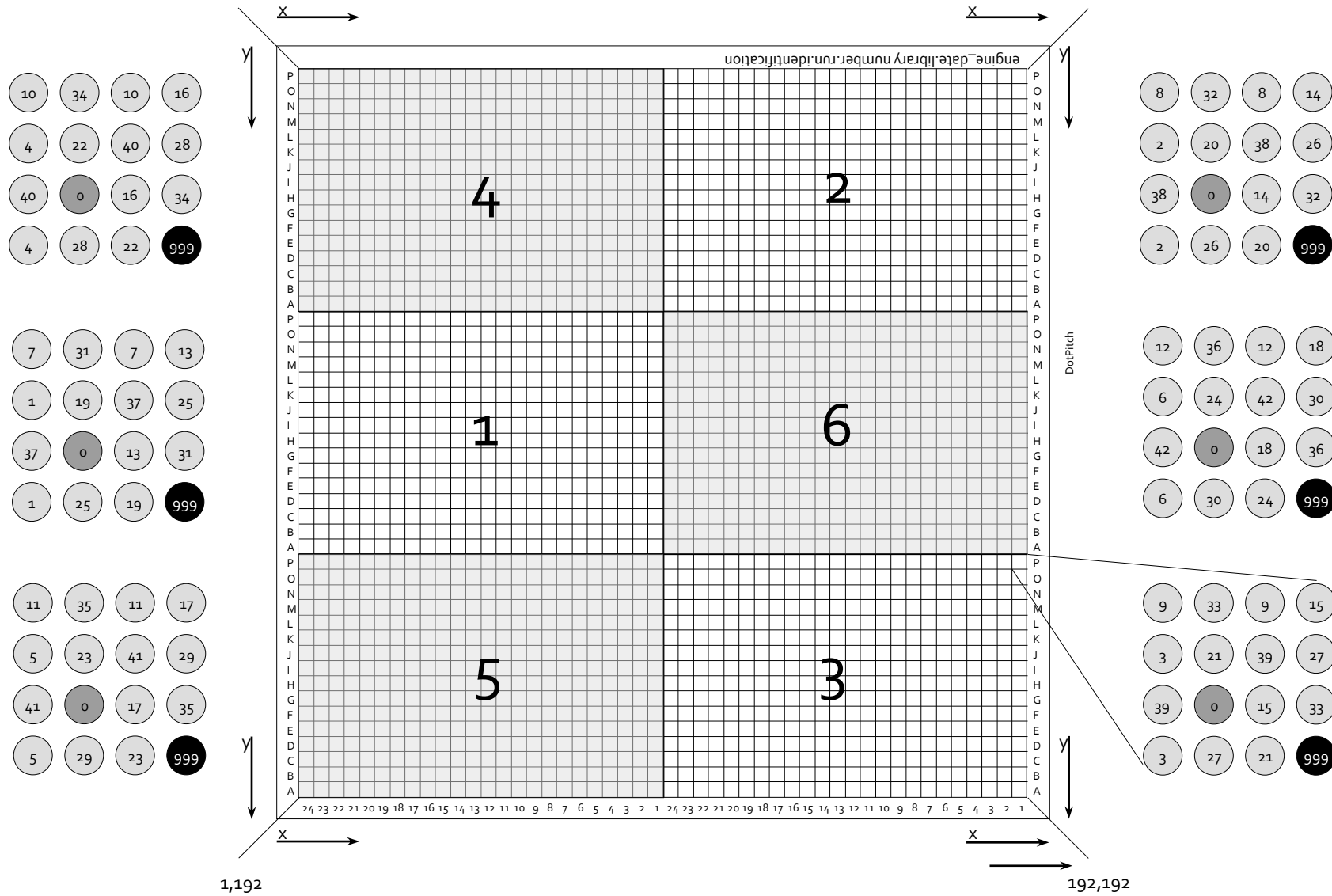
Scoring Pattern on engine Protein Arrays

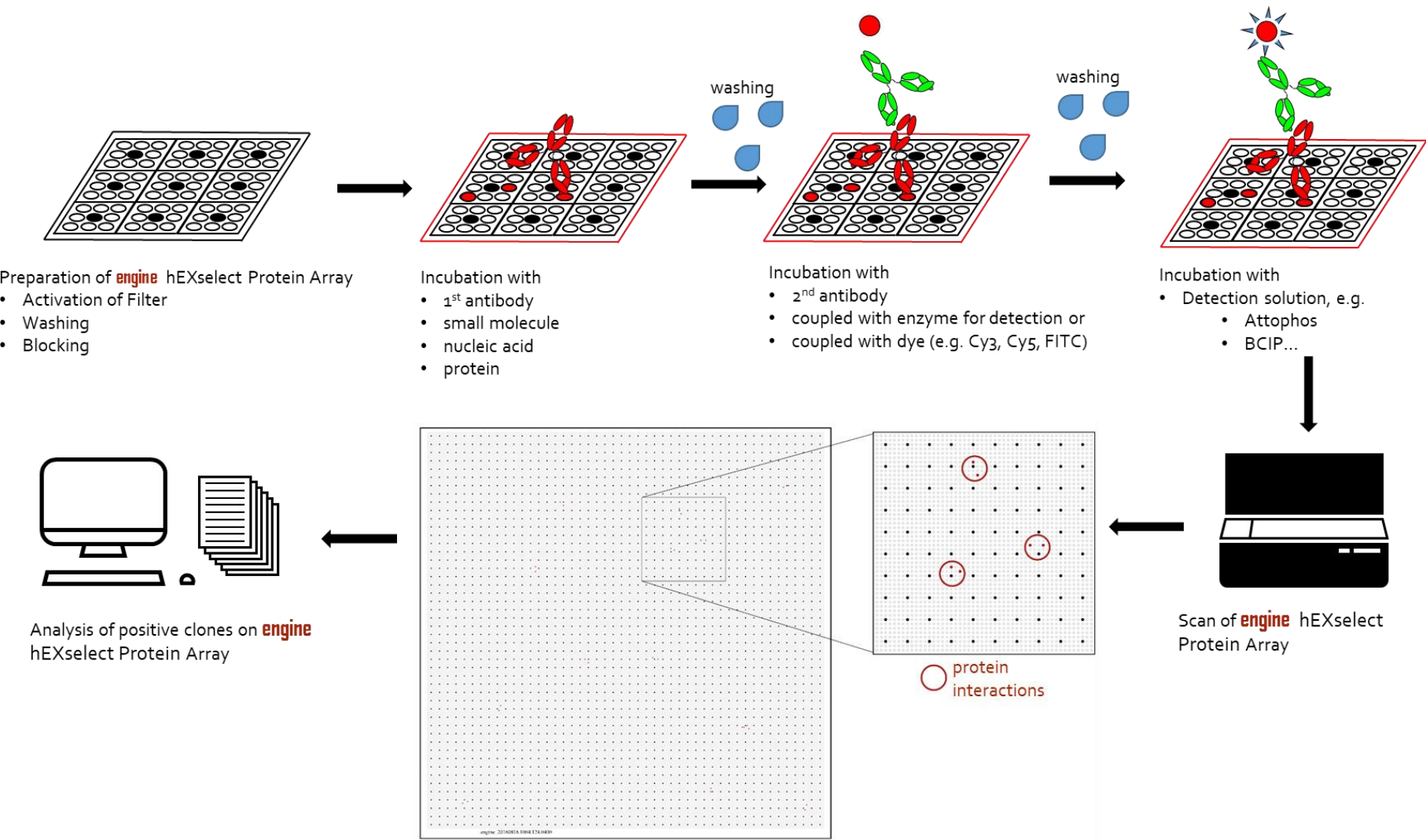pattern 1   pattern 2   pattern 3   pattern 4   pattern 5   pattern 6   pattern 7

- Spots **higlighted in red** show the corresponding pattern, in which clones were spotted
- A clone is only regarded as positive, if <u>both</u> spots can be detected

pattern 8 – guiding dot (black)
and empty position (orange)

engine Protein Arrays – as simple as a Western Blot

- Rehydration of array (PVDF membrane)
- Removal of E. coli debris with Kimwipe tissue
- Washing
- Blocking
- Incubation with sample (serum, antibody etc.)
- Washing
- Incubation with secondary antibody (AP-labeled)
- Detection with AttoPhos® substrate
- Software-based evaluation

Detection of positives hits at a glance

Preparation of **engine** hEXselect Protein Array
- Activation of Filter
- Washing
- Blocking

Incubation with
- 1st antibody
- small molecule
- nucleic acid
- protein

washing

Incubation with
- 2nd antibody
- coupled with enzyme for detection or
- coupled with dye (e.g. Cy3, Cy5, FITC)

washing

Incubation with
- Detection solution, e.g.
  - Attophos
  - BCIP...

Scan of **engine** hEXselect Protein Array

Analysis of positive clones on **engine** hEXselect Protein Array

protein interactions

Detection of positives hits at a glance

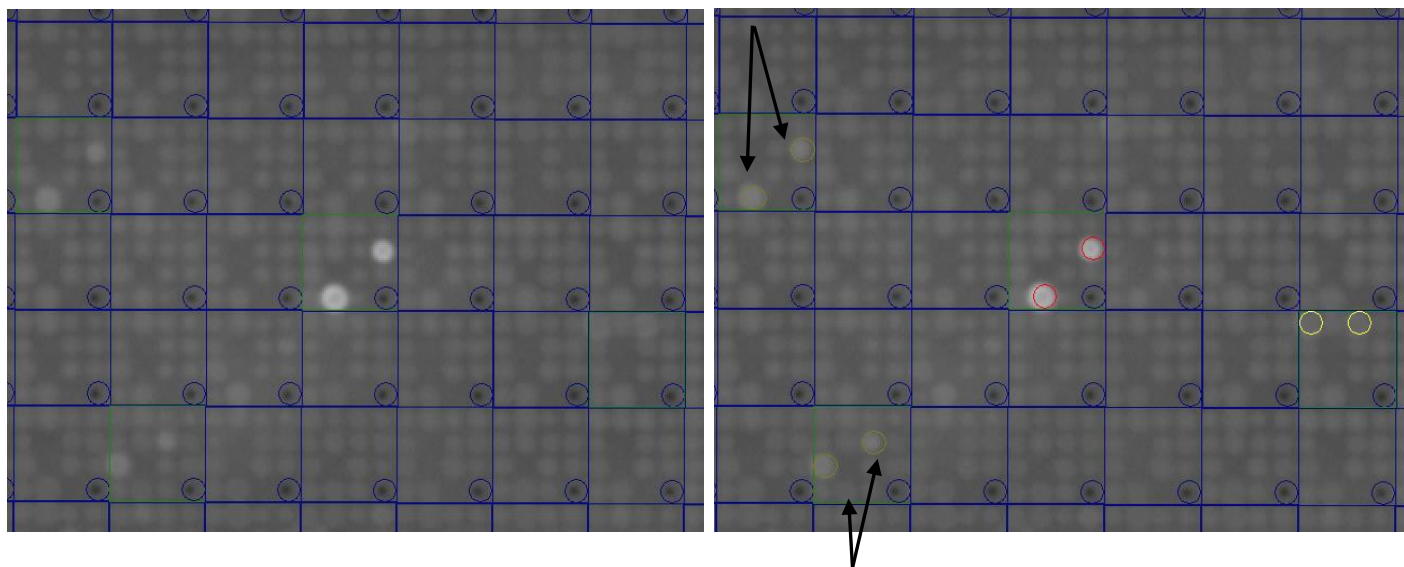## Example for Scoring:

Scoring (circle colour):

Red – 3 = strong

Brown – 2 = moderate

Yellow – 1 = low

As brown circles of score 2 are poorly visible, these are marked by arrows in addition

The same section of an Array was cropped and is shown without (left) and with (right) scoring of positive hits (bright spots), as displayed by the software.

- Hits clearly detectable at a zoom of 50% in the software, used for analysis were marked with a score of "3"

- Hits clearly detectable at a zoom of 200% in the software, used for analysis were marked with a score of "2"

- Hits visible, but not standing out prominently at zoom of 200 % in the software, used for analysis were marked with a score of "1"
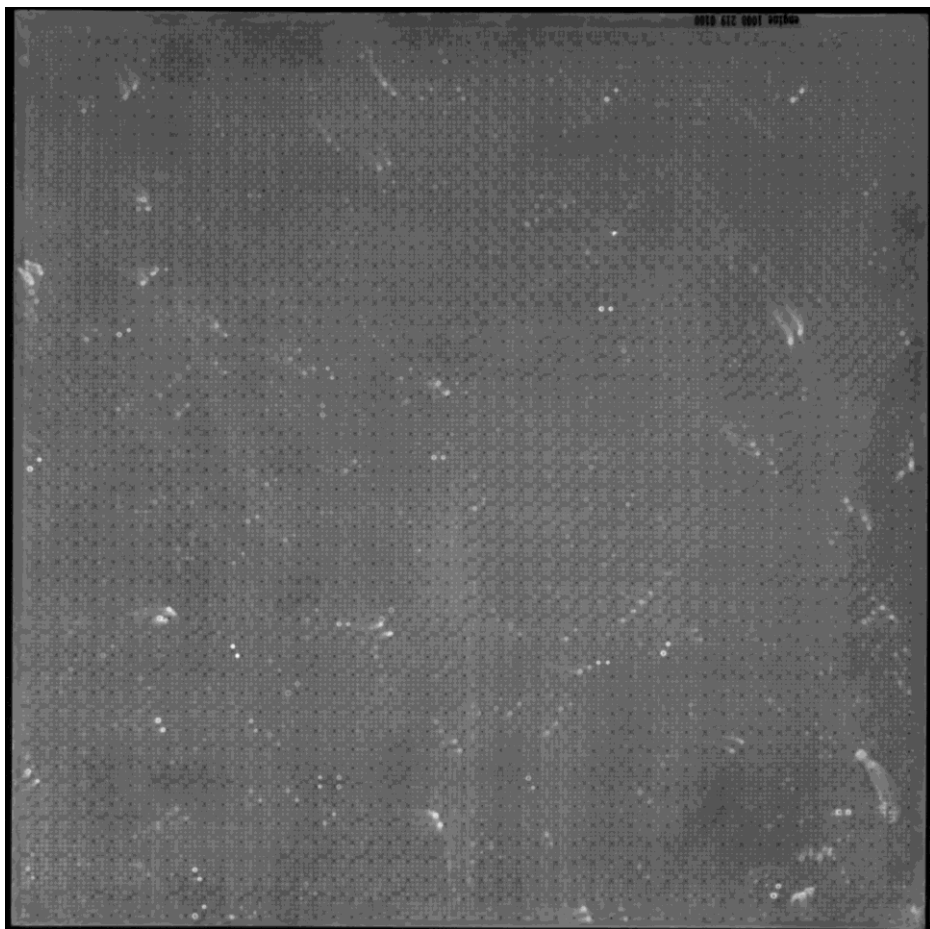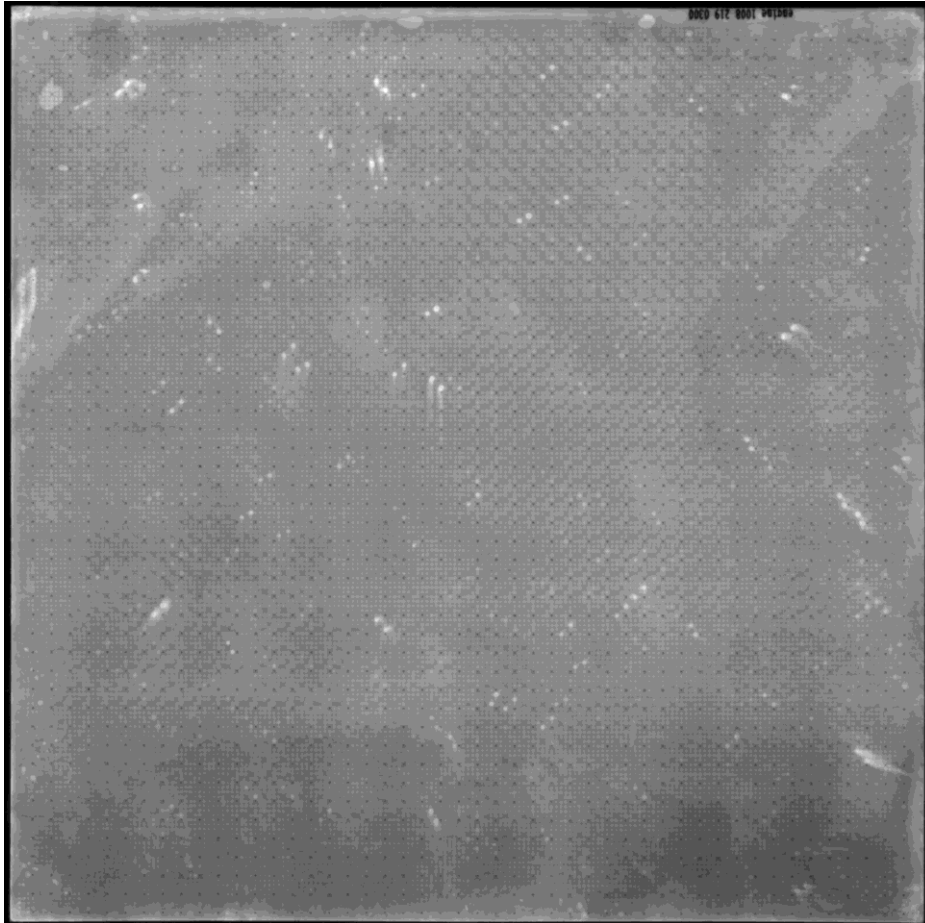
- Sample: Serum **Pat1** 1:1000

- Sec antibody: anti human IgG (Fc spec)-AP 1:5000
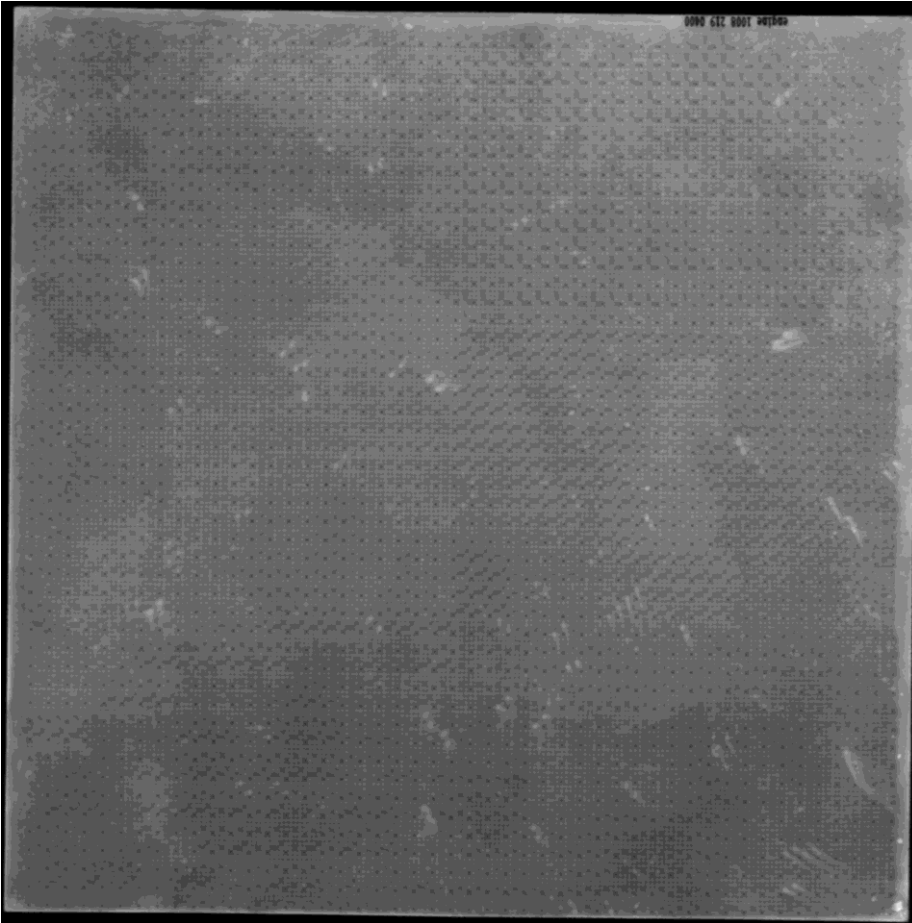
- Array: engine_1008_219_0100

- Positives total: 265

  - Intensity 1: 86

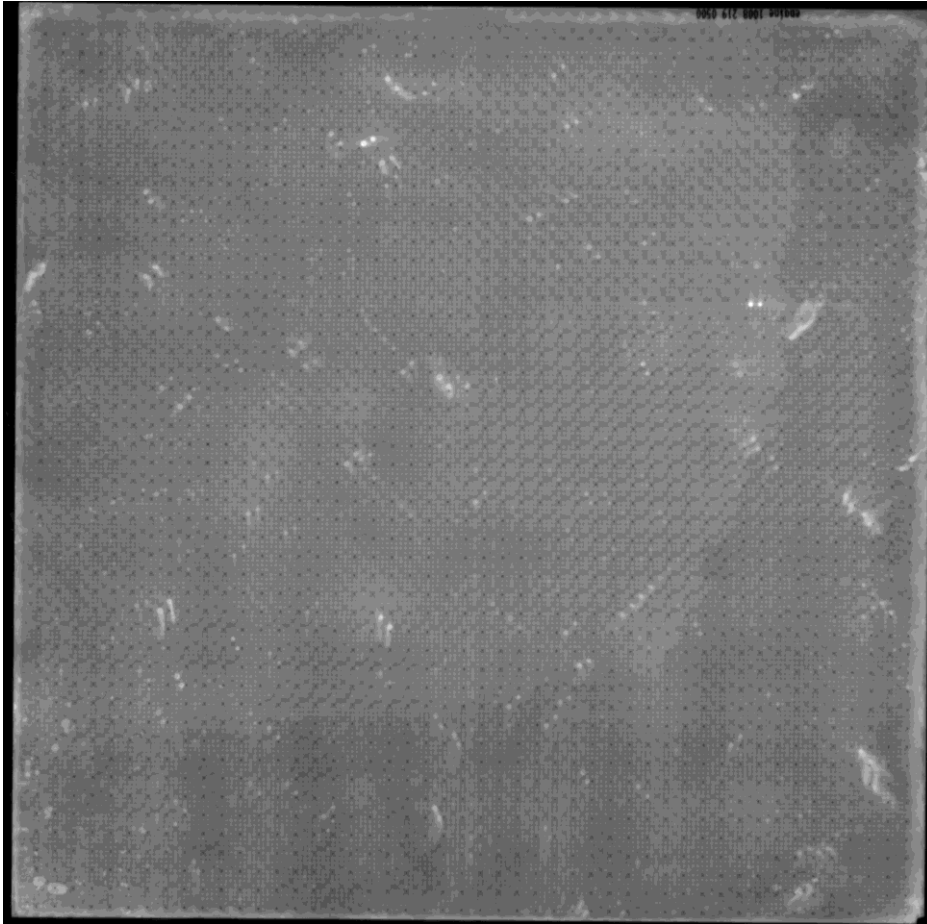 - Intensity 2: 140

  - Intensity 3: 39

Results – Sample 2

- Sample: Serum **Pat2** 1:1000
- Sec antibody: anti human IgG (Fc spec)-AP 1:5000
- Array: engine_1008_219_0300

- Positives total: 239
  - Intensity 1: 62
  - Intensity 2: 130
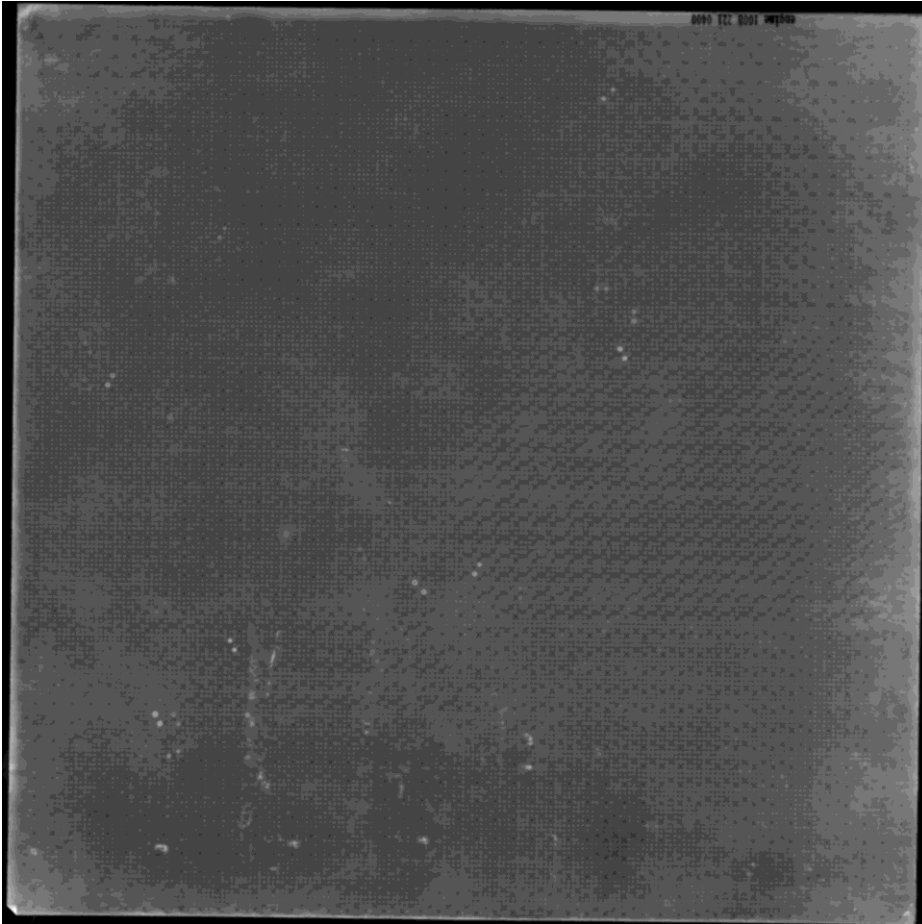 - Intensity 3: 47

Results – Sample 3

- Sample: Serum **Pat3** 1:1000
- Sec antibody: anti human IgG (Fc spec)-AP 1:5000

- Array: engine_1008_219_0400

- Positives total: 114
  - Intensity 1: 38
  - Intensity 2: 54
  - Intensity 3: 22

Results – Sample 4

- Sample: Serum **Pat4** 1:1000
- Sec antibody: anti human IgG (Fc spec)-AP 1:5000

- Array: engine_1008_219_0500

- Positives total: 242
  - Intensity 1: 75
  - Intensity 2: 115
  - Intensity 3: 52

Results – Sample 5

- Sample: Serum **Pat5** 1:1000
- Sec antibody: anti human IgG (Fc spec)-AP 1:5000
- Array: engine_1008_221_0400

- Positives total: 61
- Intensity 1: 41
- Intensity 2: 11
- Intensity 3: 9

Summary

For all samples, antibody binding to proteins on the membrane could be detected.

Distribution of hits and intensity of hits:

	total	visual score		
		1	2	3
Pat1	265	86	140	39
Pat2	239	62	130	47
Pat3	114	38	54	22
Pat4	242	75	115	52
Pat5	61	41	11	9

Overlap in positivity for all samples IgG

Total hits IgG

single	181
double	65
triple	69
quadruple	88
quintuple	10

Genes identified by all 5 patients excluding secondary antibody binding

Gene	Frame	Patient					Anti IgG	# positive
		1	2	3	4	5		
MAFK	inframe	3	3	1	3	1	0	5
HNRNPUL2	inframe	3	3	3	3	1	0	5
MYH14	inframe	3	2	2	3	1	0	5
CREBBP	inframe	2	3	2	2	1	0	5
AATF	inframe	3	3	3	3	1	0	5
CSDE1	inframe	3	2	3	3	1	0	5
DDX51	inframe	3	3	3	3	1	0	5
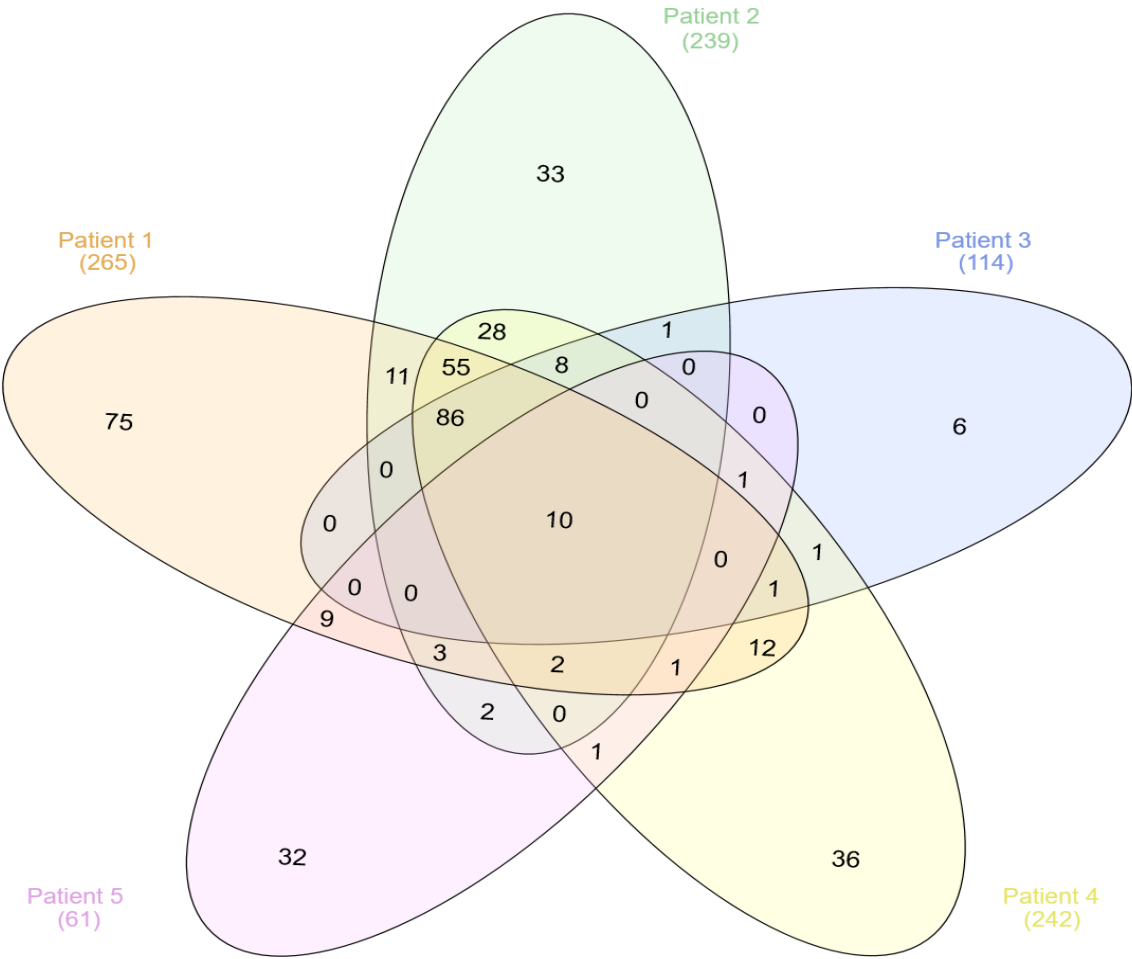
CONTACT

info@proteinarrays.bio

+49 (0)3302 55 199-0

www.proteinarrays.bio

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