REPORT

Analysis of binding pattern of commercially available antibodies on hEXselect Arrays

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DETERMINATION OF ANTIBODY SPECIFICITY by analysis of 10,000 antigens in one shot. In an internal project we have examined three commercially available antibodies with the engine hEXselect arrays (product No. 1003) .

We analyse following antibodies:

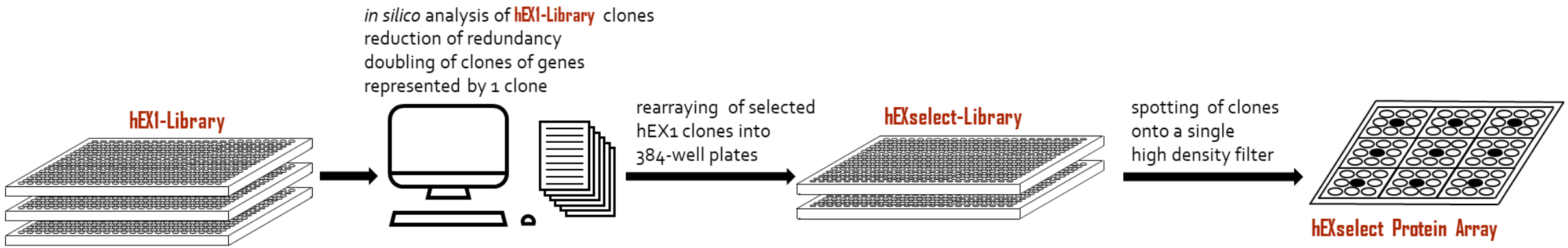
- (polyclonal goat) anti-mouse IgG (Fc specific)-AP, Sigma A2429
- (monoclonal mouse) anti-human PP2A-Aα/β (clone A5), Santa Cruz sc74580
- (monoclonal mouse) anti-human Karyopherin β1, Santa Cruz sc365299

We identified specific and several unspecific bindings.

Properties of our hEXselect Array

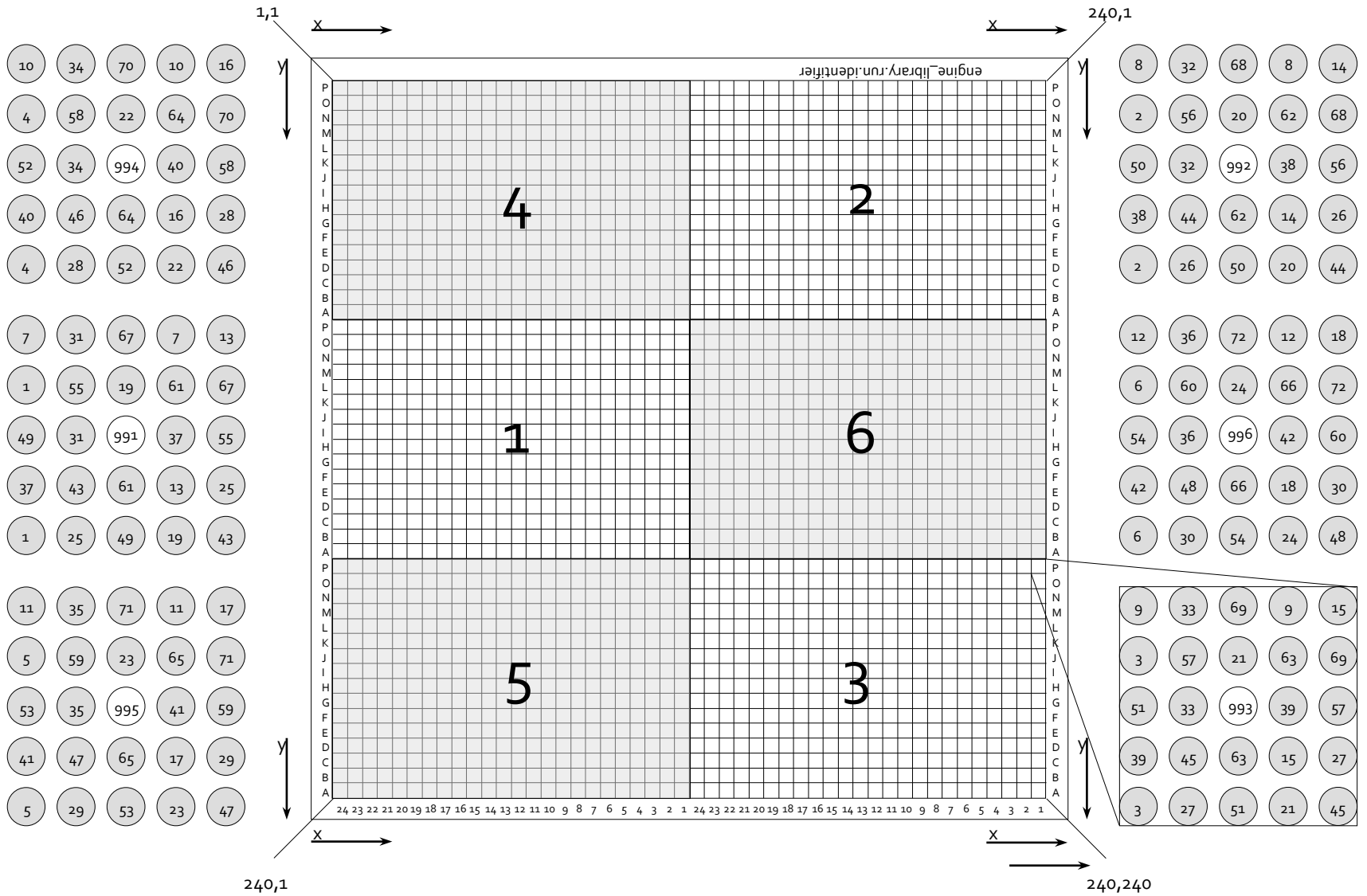
- Proteins are expressed by clones derived from a human fetal brain cDNA library
- Mixture of partially de- and renatured, full length proteins and peptides
- ORF: Mix of in-frame and out-of-frame proteins, 5‘ and 3‘ UTRs
- Proteins and peptides are expressed with an n-terminal RGS-His6-tag
- Spotting in 5x5 pattern
- 23,806 clones representing 6,909 different human proteins
- Product Number: 1003

Creation of hEXselect Array

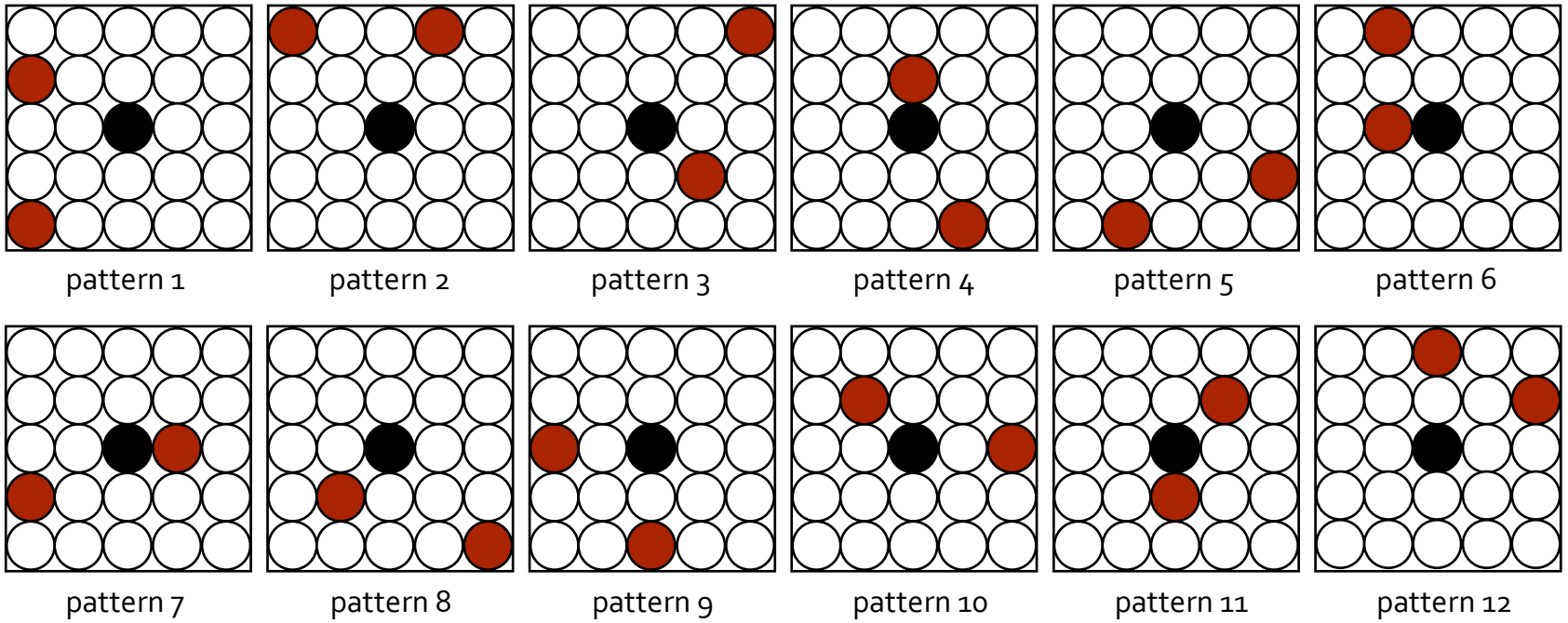


- hEX1-Library clones were analysed in silico
- Redundancy was reduced
- For unique Genes (represented by only one clone), the clone was doubled
- In order to fit onto one Array, the total number of clones was reduced.
- Clones were rearrayed into the final hEXselect-Library

ArrayDesign_240x240



Scoring Pattern on hEXselect Protein Arrays

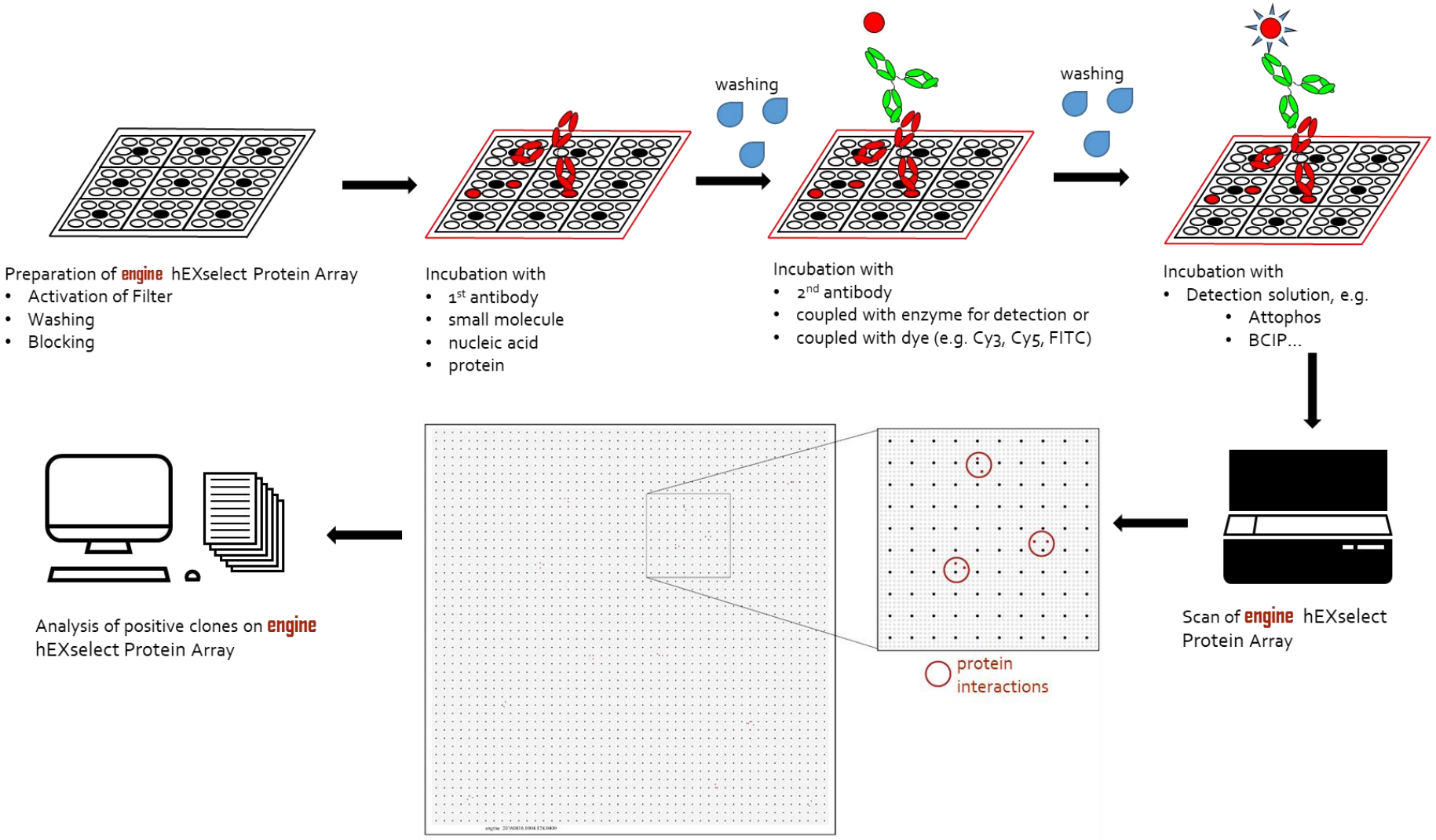


- Spots **higlighted in red** show the corresponding pattern, in which clones were spotted
- A clone is only regarded as positive, if both spots can be detected
- Black spots are guiding dots

engine Protein Arrays – as simple as a Western Blot

- Activation of array (PDVF 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



Detection of positives hits at a glance

Example for Scoring:

Scoring (circle colour):

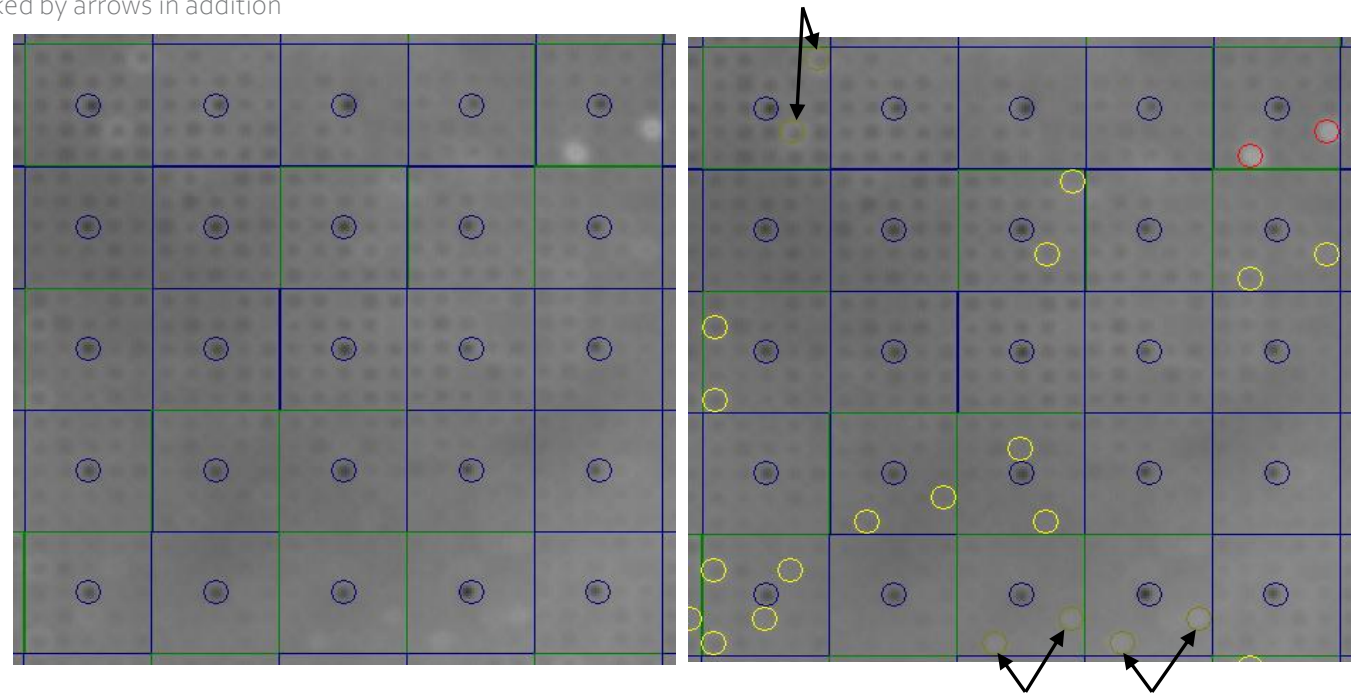
Red – 3 = strong

Brown – 2 = moderate

Yellow – 1 = low

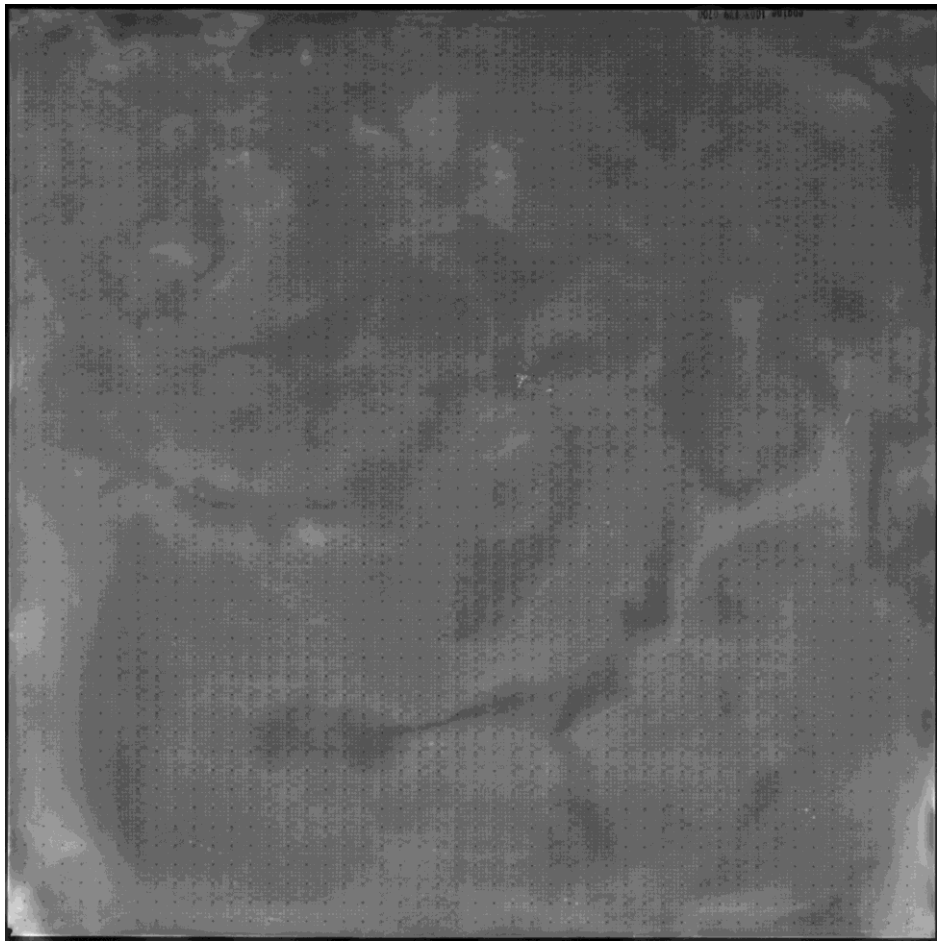
As brown circles of score 2 are poorly visible, these are marked by arrows in addition

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, which were clearly detectable by the analysis software at a zoom of 50%, were marked with a score of "3"
- Hits, which were clearly detectable by the analysis software at a zoom of 200%, were marked with a score of "2"
- Hits, which were detectable by the analysis software but not standing out prominently at a zoom of 200%, were marked with a score of "1"

Results – Secondary antibody control

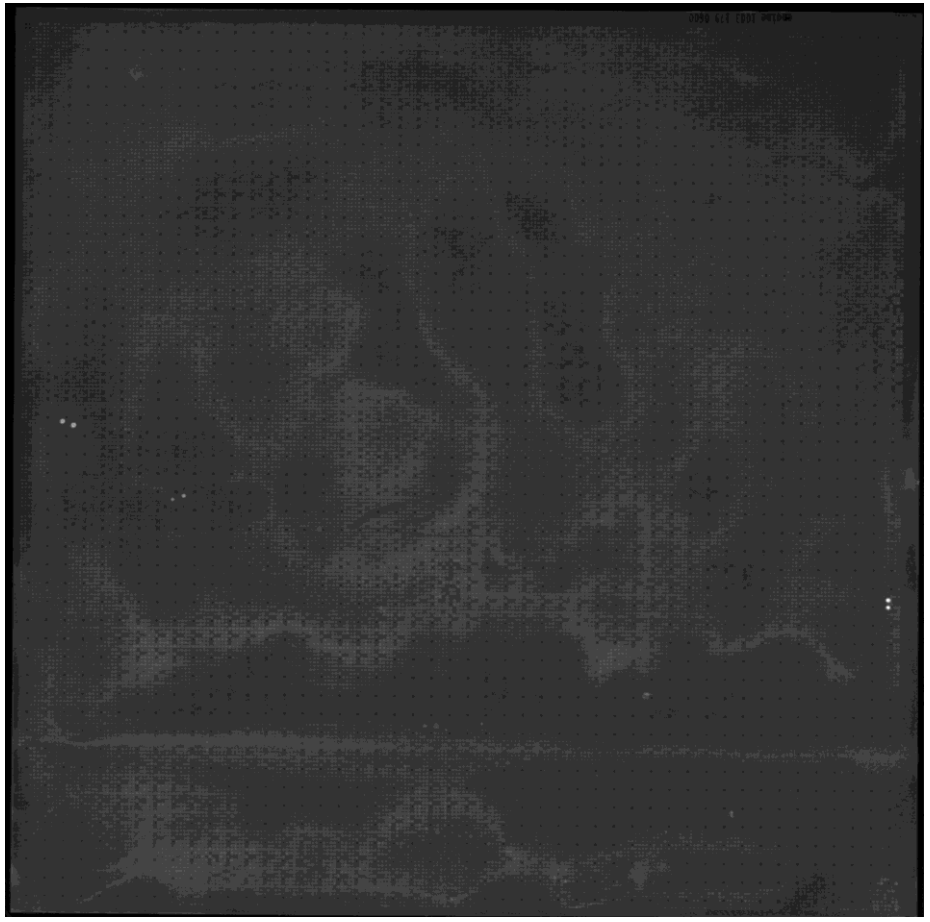


- Sample: none – secondary antibody control
- Sec antibody: anti mouse IgG (Fc spec)-AP 1:10.000
- Array: engine_1003_179_0700

- Positives total: 33

visual score of spot intensity		#
Intensity 1	low	24
Intensity 2	moderate	9
Intensity 3	strong	0

Results – anti human KPNB1

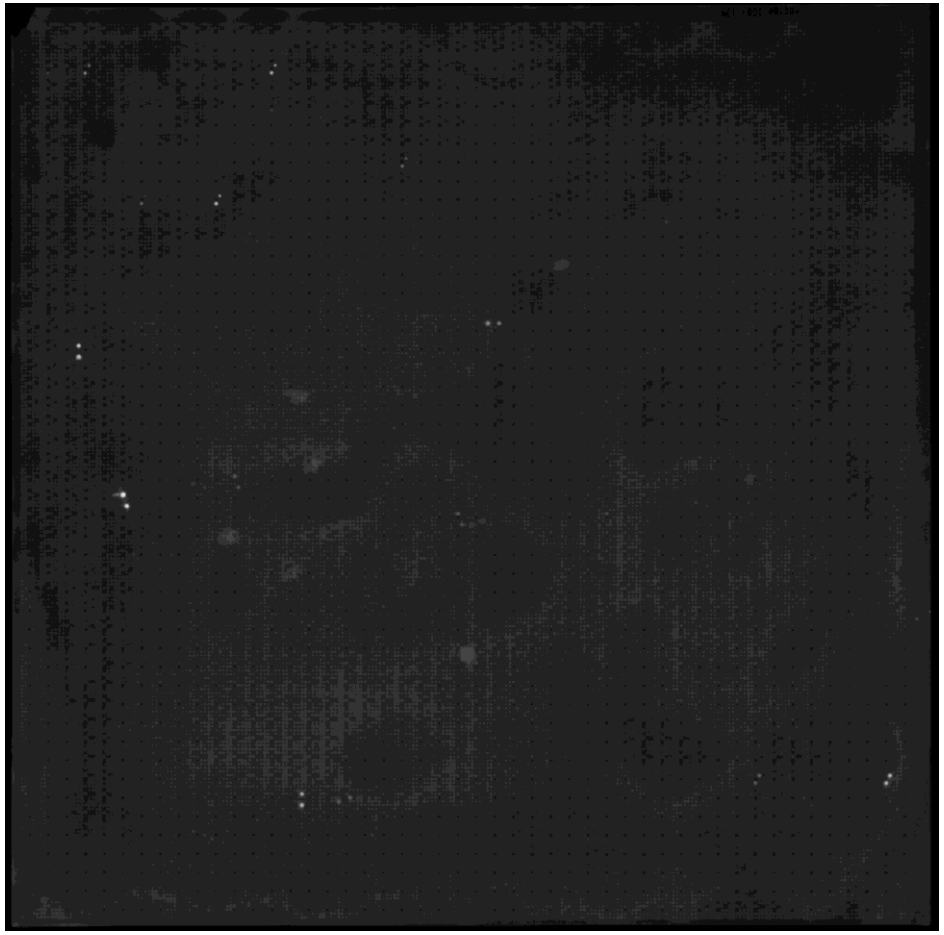


- Sample: anti human KPNB1 antibody, 3 µg total (15 µl/50 ml)
- Sec antibody: anti mouse IgG (Fc spec)-AP 1:10.000
- Array: engine_1003_179_0600

- Positives total: 114

visual score of spot intensity		#
Intensity 1	low	38
Intensity 2	moderate	54
Intensity 3	strong	22

Results – anti human PPP2R1A



- Sample: anti human PPP2R1A (clone A5) antibody, 3 µg total (15 µl/50 ml)
- Sec antibody: anti mouse IgG (Fc spec)-AP 1:10.000
- Array: engine_1003_179_0300

- Positives total: 39

visual score of spot intensity		#
Intensity 1	low	21
Intensity 2	moderate	10
Intensity 3	strong	8

Summary

For all samples, antibody binding to antigens at the protein array could be detected.

Distribution of hits and intensity of hits:

		visual score of spot intensity		
	total	1 low	2 moderate	3 strong
anti mouse IgG (Fc-spec)	**33**	24	9	0
anti Karyopherin β1	**17**	12	2	3
anti PP2A-Aα/β	**39**	21	10	8

Summary **anti mouse IgG**

Clone	Gene	anti mouse IgG	anti human Karyopherin β1	anti human PPP2A-Aα/β (A5)
ELIB1003p43H15	CDKN2C	2	1	2
ELIB1003p57B19	NUDT16	2	1	1
ELIB1003p14E8	NUDT16	2	1	1
ELIB1003p51G6	ZNF358	2	1	1
ELIB1003p4F3	MEGF6	2	1	0
ELIB1003p47E14	CUEDC2	2	0	1
ELIB1003p24F17	FAM239A	2	0	1
ELIB1003p59E15	LOC389906	2	0	1
ELIB1003p19M24	FADD	2	0	0
ELIB1003p11J2	CFL1	1	2	0
ELIB1003p47E2	H2AFY	1	0	1
ELIB1003p14F14	DDAH2	1	0	1
ELIB1003p35I23	SYT5	1	0	0
ELIB1003p37O11	LINGO1	1	0	0
ELIB1003p43C11	RRP1	1	0	0
ELIB1003p37K4	H2AFY	1	0	0
ELIB1003p58L4	TLCD2	1	0	0
ELIB1003p11B3	CACNG3	1	0	0
ELIB1003p41J2	PIN4	1	0	0
ELIB1003p30K24	CEBPA	1	0	0
ELIB1003p18H21	SYT5	1	0	0
ELIB1003p8J14	RPS15	1	0	0
ELIB1003p33P13	HDAC10	1	0	0
ELIB1003p36G12	EBNA1BP2	1	0	0
ELIB1003p42O11	TRIM27	1	0	0
ELIB1003p8K8	GNAS	1	0	0
ELIB1003p62C7	THAP10	1	0	0
ELIB1003p18L7	RBFOX3	1	0	0
ELIB1003p6M6	DIS3L2	1	0	0
ELIB1003p2M6	EXT1	1	0	0
ELIB1003p47K19	THAP10	1	0	0
ELIB1003p34C18	H2AFY	1	0	0
ELIB1003p7P17	H2AFY	1	0	0

Table shows all antigens which reacts with secondary anti mouse IgG **and** overlapping reactions with anti KPNB1 and anti PPP2R1A

Determination of 33 off-target activities for commercial Fc specific anti mouse IgG !

Summary **anti KPNB1**

Clone	Gene	anti human Karyopherin b1	anti human PPP2A-Aa/b (A5)
ELIB1003p36A1	**KPNB1**	3	0
ELIB1003p25G16	**KPNB1**	3	0
ELIB1003p16O2	**KPNB1**	1	0
ELIB1003p43K22	DNAJC2	3	0
ELIB1003p9H22	ZNF511	2	0
ELIB1003p44H2	ANKRD16	1	1
ELIB1003p35I14	CCDC130	1	0
ELIB1003p20H24	RTN1	1	0
ELIB1003p21I21	WASHC1	1	0
ELIB1003p33I20	HDAC10	1	0
ELIB1003p20E3	HIST1H1C	1	0
ELIB1003p64L11	**KPNB1**	0	2
ELIB1003p41G7	**KPNB1**	0	2

Table shows all antigens which reacts with anti KPNB1 antibody **and** overlapping reactions with anti PPP2R1A, **excluding secondary antibody binding.**

Determination of

- 3 specific reactions (three different KPNB1 clones at the array) but there is no reaction with 2 other KPNB1 clones
- 8 off-target activities (strong spot intensity with DNAJC2 and moderate spot intensity with ZNF511)
- 1 overlapping reaction with anti PPP2R1A (ANKRD16)

Summary **anti PPP2R1A**

Clone	Gene	anti human PPP2A-Aα/β (A5)	anti human Karyopherin β1
ELIB1003p64G14	**PPP2R1A**	3	0
ELIB1003p64N11	**PPP2R1A**	3	0
ELIB1003p5G9	**PPP2R1A**	3	0
ELIB1003p12P23	**PPP2R1A**	3	0
ELIB1003p1O21	**PPP2R1A**	3	0
ELIB1003p64N21	**PPP2R1A**	3	0
ELIB1003p63H2	**PPP2R1A**	3	0
ELIB1003p19G19	**PPP2R1A**	3	0
ELIB1003p64N23	**PPP2R1A**	2	0
ELIB1003p19H13	**PPP2R1A**	2	0
ELIB1003p64I4	**PPP2R1A**	2	0
ELIB1003p19F1	**PPP2R1A**	2	0
ELIB1003p64G18	**PPP2R1A**	2	0
ELIB1003p30F24	TSPOAP1	2	0
ELIB1003p63H9	KPNB1	2	0
ELIB1003p64L11	KPNB1	2	0
ELIB1003p41G7	KPNB1	2	0
ELIB1003p44H2	ANKRD16	1	1
ELIB1003p19P14	FADD	1	0
ELIB1003p11I11	IGFBP7	1	0
ELIB1003p58G11	MINDY3	1	0
ELIB1003p10K8	PRAP1	1	0
ELIB1003p10I21	PRAP1	1	0
ELIB1003p24L17	n.d.	1	0
ELIB1003p48G12	FAM161A	1	0
ELIB1003p16H20	AMER2	1	0
ELIB1003p14D6	UQCRFS1	1	0
ELIB1003p62E4	FAM161A	1	0
ELIB1003p56J4	RAI1	1	0
ELIB1003p9F1	RBFOX3	1	0

Table shows all antigens which reacts with anti PPP2R1A antibody and overlapping reactions with anti KPNB1, excluding secondary antibody binding.

Determination of

- 13 specific reactions (13 different PPP2R1A clones at the array)
- 17 off-target activities (4 with moderate spot intensity)
- 1 overlapping reaction with anti KPNB1 (ANKRD16)

Conclusion

We could detect **specific reactions** for anti KPNB1 and anti PPP2R1A.

All antibodies have **off-target activities**, which can lead to non-specific reactions, e.g., in an ELISA, because the antibodies do not only bind their specific antigen. Therefore, the investigation of antibodies via the protein arrays is a good tool for the selection of antibodies to **avoid false-positive** results e.g., in diagnostics.

The hEXselect Array (product no. 1003) has >45,000 protein-coding E. coli clones which represents > 19,000 human antigens and > 4,000 different human genes. Because we have the proteins **not only in full-length, but also as protein isoforms & peptides** (different clones, same gene) we can specify the binding domain in more detail, like **epitope mapping**.

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