

CellFree Sciences

The natural power of wheat driving science



Antibody Validation - Protein array services for antibody testing

Motivation

- Reproducibility of scientific results has become a large concern undermining the trust in medical research
- This problem became even more pronounced as industry reduced discovery efforts and tries to rely more on academic research for new projects
- Low quality antibodies are considered one of the reasons for poor scientific data
- Thus, antibody validation is required for improving standards to conducting research and became essential for antibody development in industry
- There are new challenges to confirm antibody specificity during antibody engineering, selection and later in drug development
- CFS wants to support efforts for better antibody validation by offering dedicated antibody specificity testing services using our unique human protein bead array platform

Key elements to success

1. *Comprehensive human clone set:*

We are using the human cDNA clone set prepared at National Institute of Advanced Industrial Science and Technology (AIST) as part of a Japanese National Project (NEDO) to create the most comprehensive human full-length ORF clone set – licensed to CFS for commercial use in protein expression services

2. *Protein expression system:*

We are using our proprietary wheat germ cell-free protein expression system for reliable high-throughput protein synthesis – fully automated for expression and purification of nearly 20,000 human proteins for protein array production

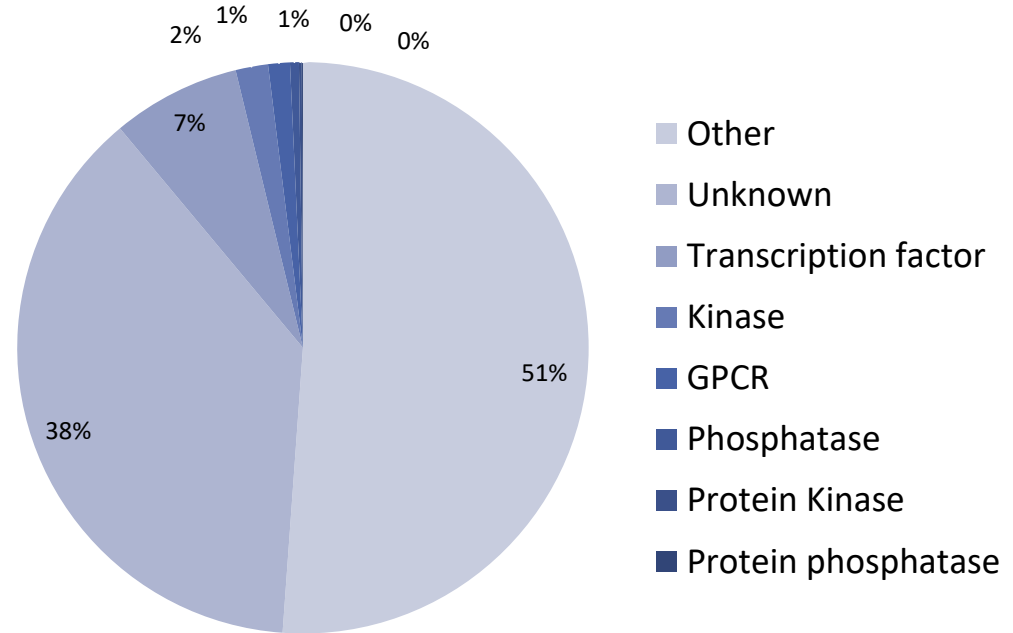
3. *Protein bead array platform:*

We are using a unique bead array format for our protein arrays developed together with researchers at AIST – immobilized proteins always stay in solution to avoid changes in protein conformation

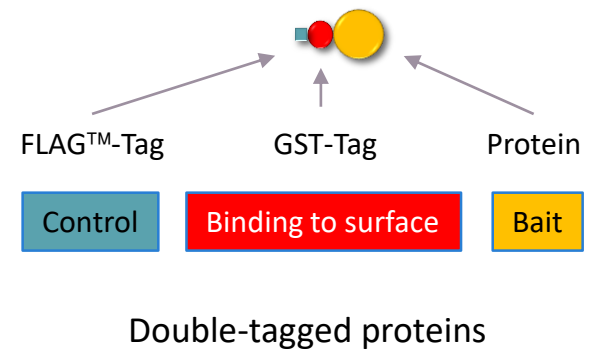
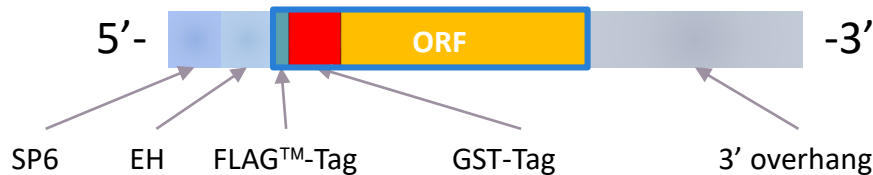
The AIST human ORF clone set

Human Gene and Protein Database (HGPD): <http://hgpd.lifesciencedb.jp>

Category	Clones
Other	10,080
Unknown	7451
Transcription factor	1434
Kinase	362
GPCR	242
Phosphatase	99
Protein Kinase	31
Protein phosphatase	13
Total	19,712

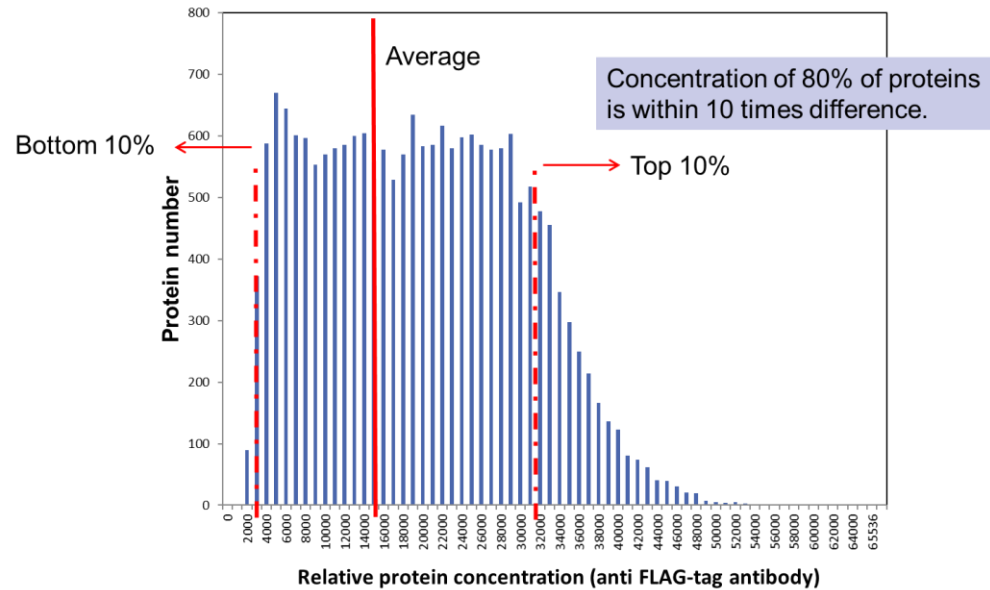
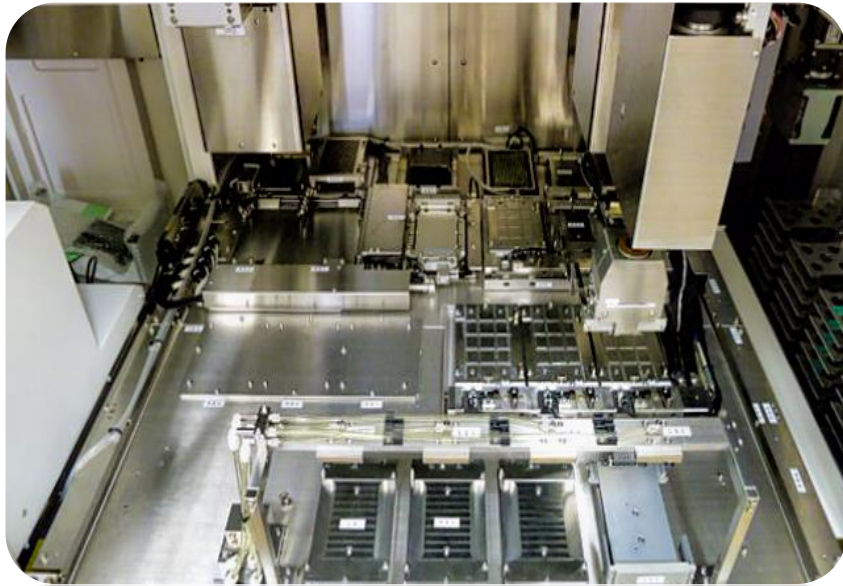


DNA templates for tagged proteins prepared from ORF clone set



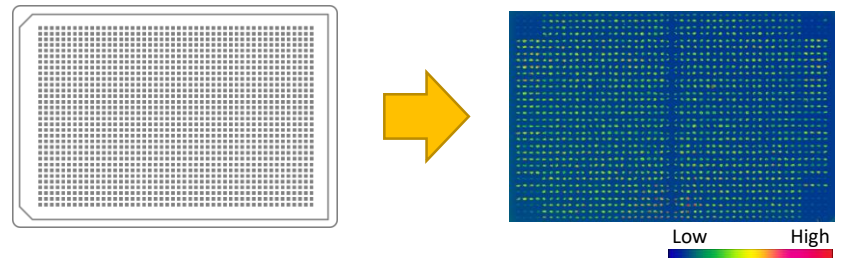
Automated protein production

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Confirming arrayed proteins with anti-FLAG™ antibody

Up to 14 proteins can be arrayed per well



Starting from PCR templates

RNA expression

Protein expression

Purification on beads

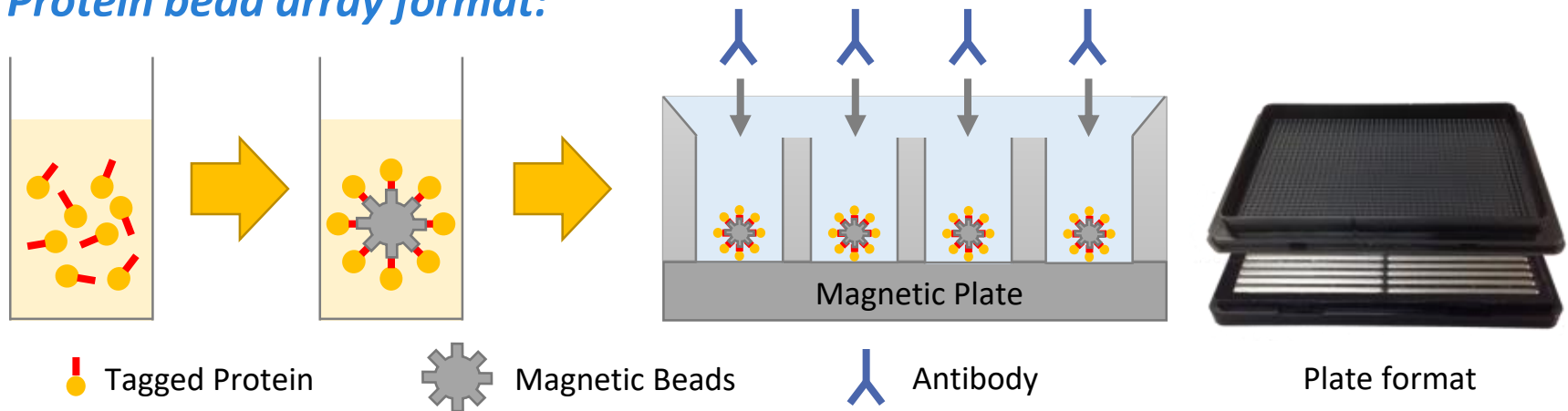
Unique bead array format

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1. Protein production:

CFS established an automated protein production pipeline for FLAGTM- and GST-tagged fusion proteins using a dedicated wheat germ extract precleared on a glutathione resin for higher protein purity - entire process has a ~99% success rate for making 19,712 proteins from the human clone set

2. Protein bead array format:

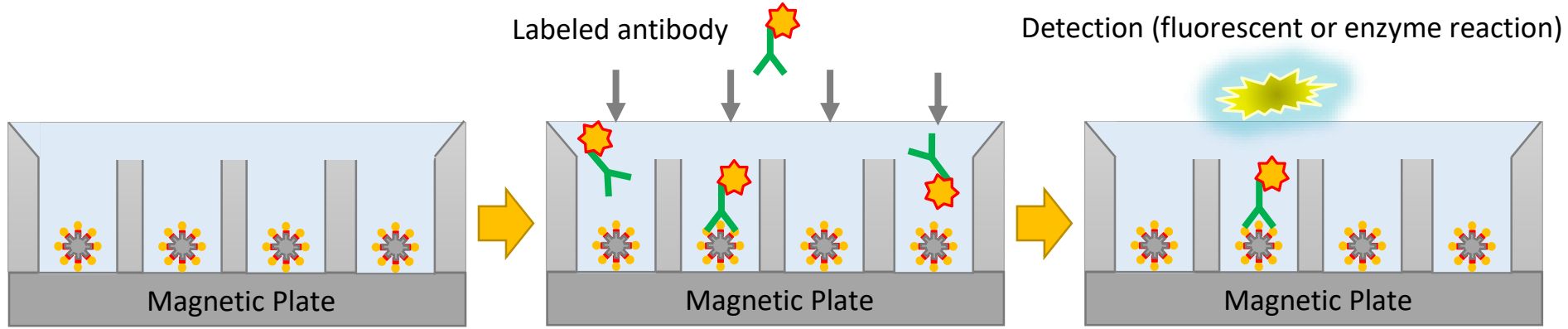


3. Quality control on produced bead arrays:

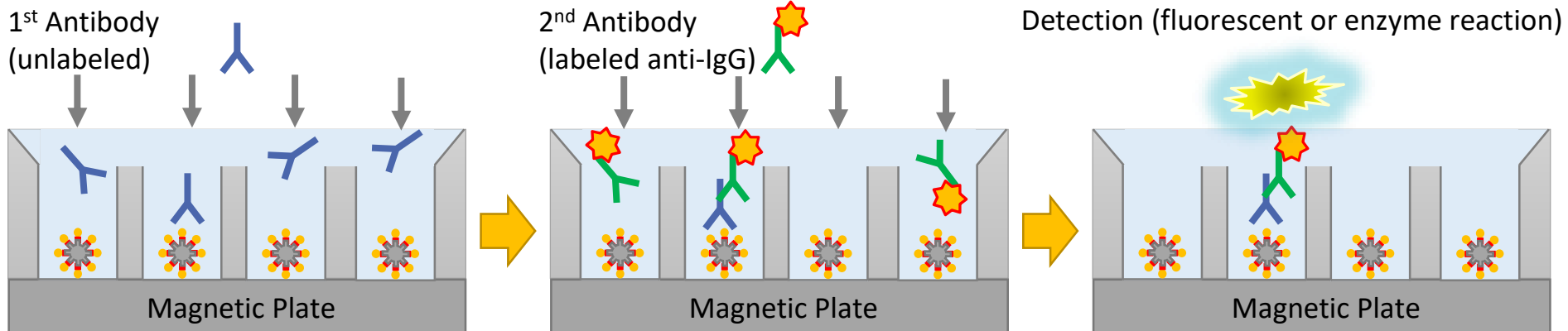
We perform a QC step on each batch using the FLAGTM-tag and an anti-FLAGTM antibody to confirm individual proteins on the arrays

Antibody detection on the array

1. Direct ELISA-like assay - Analyzing labeled antibodies:



2. Indirect ELISA-like assay - Analyzing common antibodies:



Service applications

1. *Screening clones during antibody selection:*

Test binding to 19,712 human full-length proteins arrayed onto one 1536 well plate with up to 14 proteins per well – quick overview doing a single experiment

2. *Production control:*

Test binding to 19,712 human full-length proteins arrayed onto one 1536 well plate with up to 14 proteins per well – quick overview doing a single experiment with option to use custom array formats for more dedicated searches

3. *Confirming specificity of given antibody:*

Screening binding to 19,712 human full-length proteins arrayed onto one 1536 well plate with up to 14 proteins per well followed by analysis of binding to individual proteins using custom array – identification of individual proteins recognized by antibody in question

Service overview

Customer Requirements:

Antibodies to test
Secondary antibody use
Use of controls

Entry QC:

Antibody titer check
Binding condition test
Secondary antibody test

Protein Array Screen:

Testing 19,712
Up to 14 proteins per well
Data analysis

Project Report:

Image
Interaction data

Option to prepare customized protein arrays

Protein Identification:

Custom array with all
proteins from each
selected well

Project Report:

Image
Interaction data
Full protein annotations

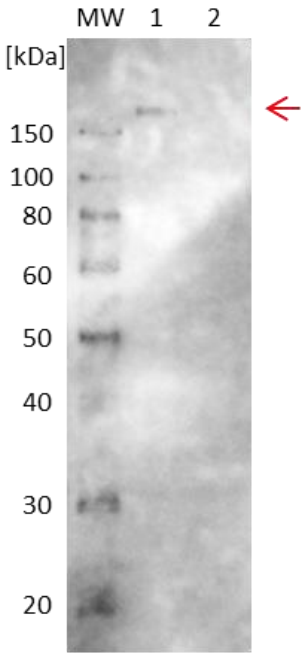
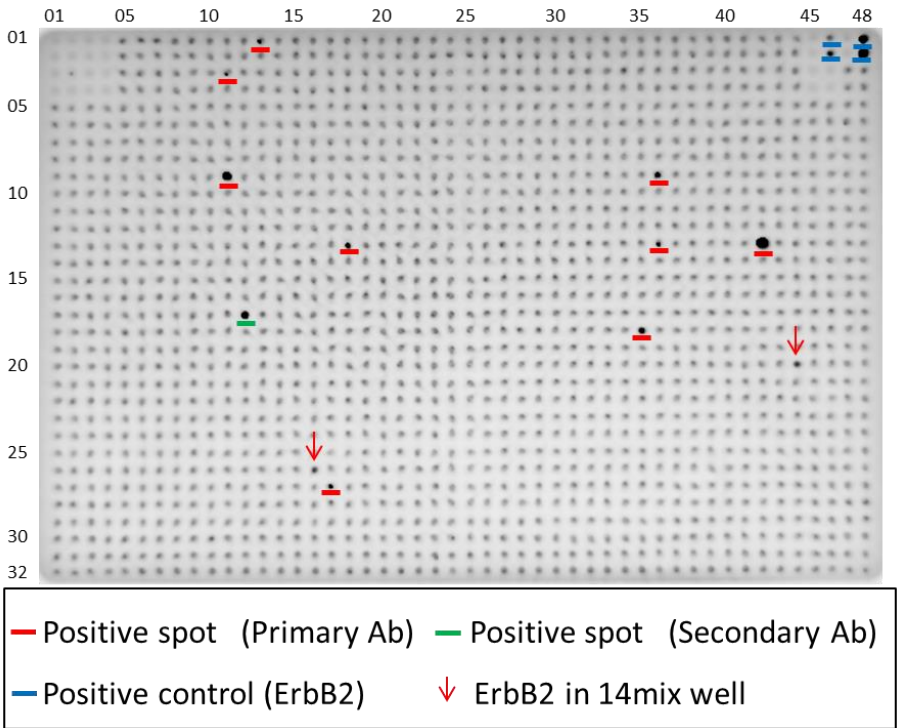
Other Services:

Providing human proteins
Full support on data
provided by CFS



How does it compare

Testing commercial anti-ErbB2/HER2 antibody doing protein array screen with 19,712 proteins and Western Blot with commercial cell lysates from wild-type (wt) and knock-out (ko) cells



Antibody detects ErbB2 in wt (lane 1) but not in ko cell extract (lane 2); MW = size marker

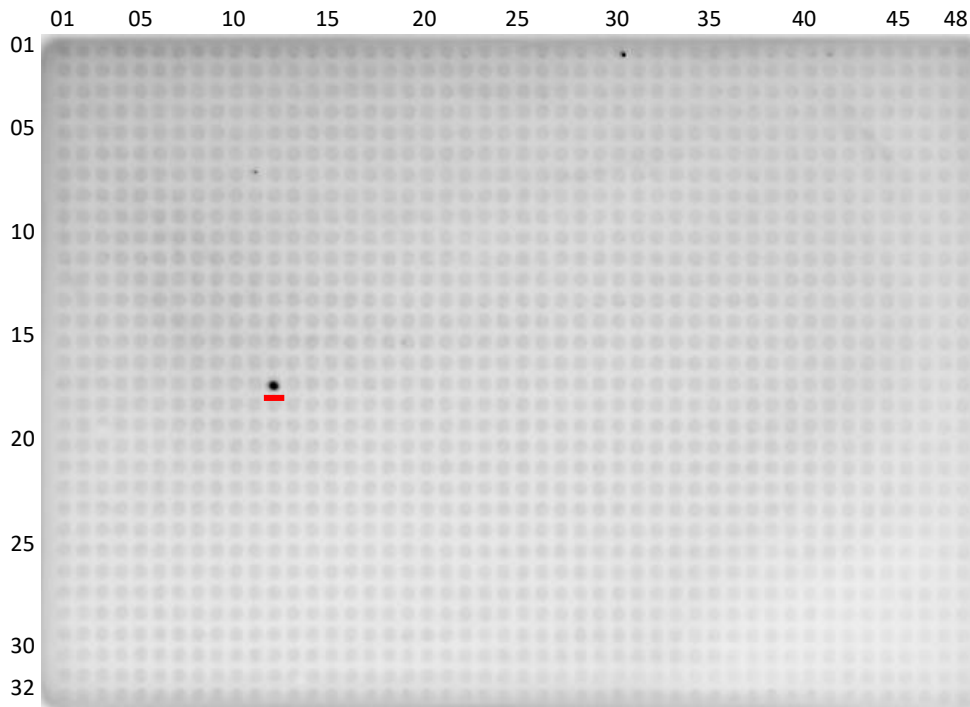
Positive signals for 16 wells including 2 containing ErbB2 (positive controls indicated in blue)

No one cell line allows for genome-wide profiling on all human proteins!

Signal detection

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- Secondary antibodies used for signal detection are pretested for binding to proteins on the bead array
- Several antibodies can bind to the human (tripartite motif) TRIM21 protein, a known cytoplasmic Fc receptor having a broad isotype specificity binding IgG, IgM and IgA

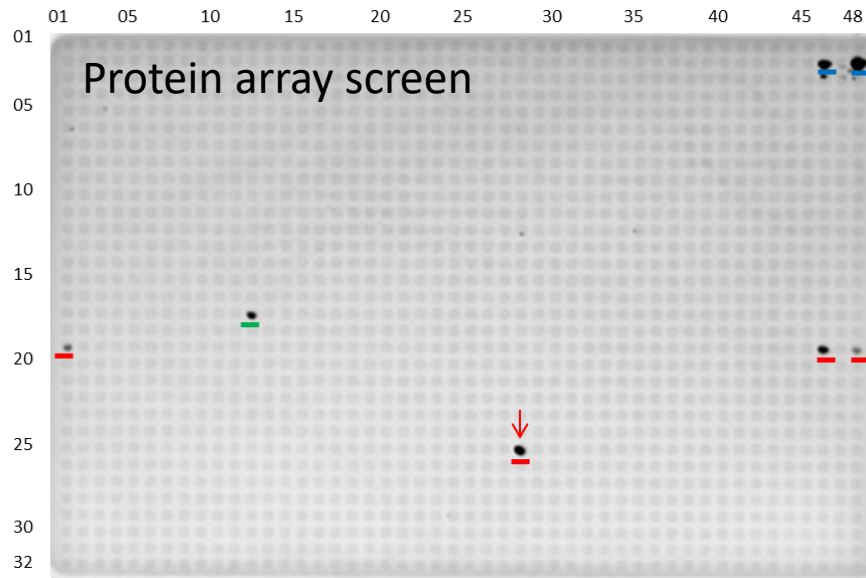


Example: Anti-Rabbit IgG, HRP-Linked Whole Ab from Donkey [1/5000 dilution, GE Healthcare (NA934)] used for testing anti-PD-1 antibody shown in this presentation

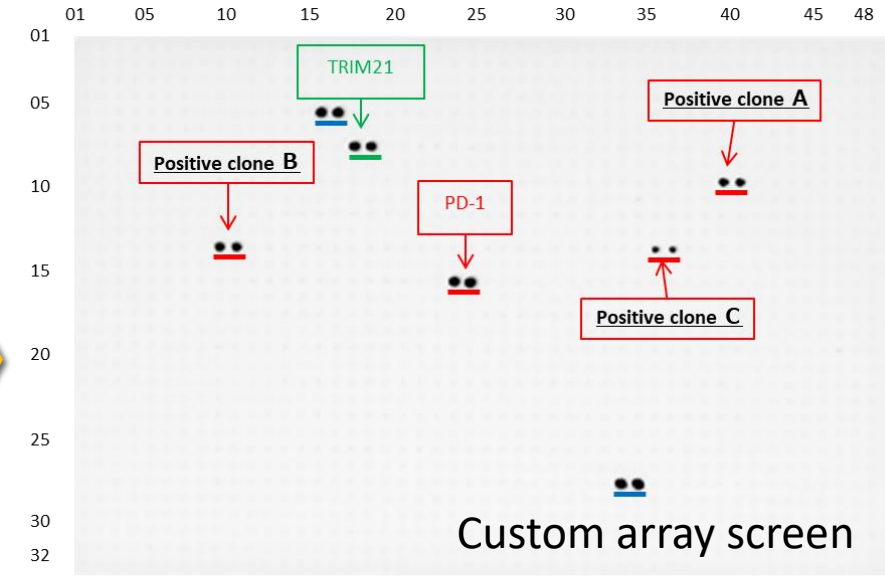
Identifying cross reactivity

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Testing a commercial anti-PD-1 antibody using protein bead array with 19,712 proteins on one 1536 well plate, followed by detection of individual proteins on custom array



- Positive spot (Primary Ab)
- Positive spot (Secondary Ab)
- Positive control (PD-1)
- ↓ PD-1 in 14mix well



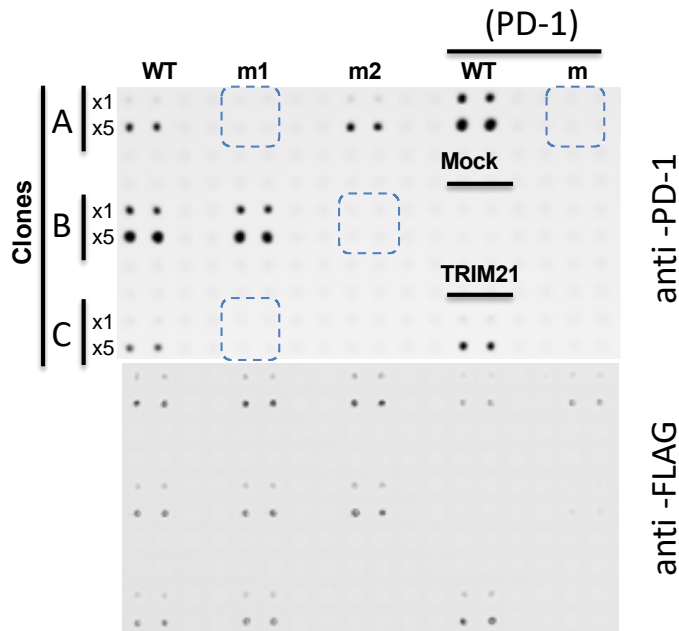
- Positive spot (Primary Ab)
- Positive spot (Secondary Ab)
- Positive control (PD-1)

Identifying PD-1 within clone set plus 3 unrelated proteins (positive controls indicated in blue)

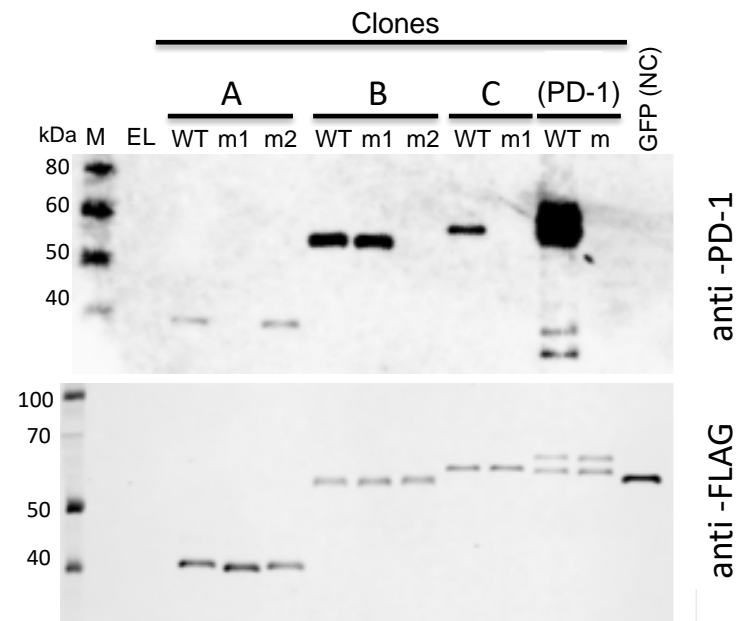
Specific epitope recognition

Loss of antibody binding caused by mutations introduced into binding sites:

Protein array



Western Blot



- PD-1** (m) 257- MGTSSPARRGSADGPRSAQPLRPEDGHCSWPL -288 [**Ala274 in peptide**]
- Positive clone A** (m1) 1- MSVCPVDLPRRAQRLWLRAPARLRRKPPVLDPLPAHSGMCGNCRLAS -47
- Positive clone A** (m2) 61- HTAMVLPPDALLWCGLSAQYLV – 82 (not recognized by AB)
- Positive clone B** (m1) 234- LGDTADARASSPTTPRSAPSSDSFPR SAQK – 263 (not recognized by AB)
- Positive clone B** (m2) 244- SPTTPRSAPSSDSFPRSAQK -263
- Positive clone C** (m1) 240- NQANSSGRESKVPGARSANLPGGGPASH SNPRSLSSGHLQGKPWK -285

- Unique protein bead array format allows to keep protein conformation intact after protein synthesis
- ELISA-like assay designs allow for robust signal detection using standard methods
- Experiments with anti-PD-1 antibody show that our protein bead array can accurately detect antibody specificity for linear epitopes
- Similar results were obtained for directly testing the specificity of a labeled anti-HA-tag antibody (data not shown)
- Many proteins on the bead array are recognized by natural antibodies when testing human sera; similar results obtained using AlphaScreen™ and human protein sets indicating a high immunoreactivity of proteins made in our system (data not shown)
- Detection of the TRIM21 protein shows that many proteins on the array could be correctly folded and thus should also allow the detection of conformational epitopes

At your service

1. *Discuss your needs and chose your service:*

Select the best service option for your needs
Defined milestones as the project proceeds

2. *CFS services and deliveries:*

Established experimental procedures
Detailed project reports including full annotation on identified proteins

3. *After services:*

We want to make sure you get meaningful data to support your projects
Use other CFS services and products for additional experiments on protein targets

We are looking forward to be at your service!

Thank you for working together with us!

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