Array-based antibody validation

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Development of PrEST-arrays
Protein fragment microarrays

for QUALITY ASSURANCE OF ANTIBODIES and AUTOIMMUNITY PROFILING

- 384 protein fragments in 21 subarrays
- New batch every second week (HPA production)
- Capacity: 84 samples in parallel
- > 50 000 array validated antibodies

- 58k spots on two slides
- 42k unique antigens
- 19k unique proteins
- Capacity: 4 samples in parallel
- Sjöberg et al, New Biotechnology, 2016

Autoantibody profiles

Antibody validations

World’s largest protein array printed at SciLifeLab

Protein fragments representing 34% of the human proteome have been printed on two glass slides at SciLifeLab’s facility Protein and Peptide Arrays making it the largest array ever printed worldwide.
Approximately 87\% of antibodies purified from sera within the Human Protein Atlas have been approved for further use by validating their selectivity on protein fragment microarrays.

<table>
<thead>
<tr>
<th></th>
<th>Passed antibodies</th>
<th>Failed antibodies</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>46,856 (87%)</td>
<td>7015 (13%)</td>
<td>53,871</td>
</tr>
<tr>
<td>Without comment</td>
<td>37,911 (81%)</td>
<td>0</td>
<td>37,911</td>
</tr>
<tr>
<td>Off-target</td>
<td>8945 (19%)</td>
<td>3250 (46%)</td>
<td>12,195</td>
</tr>
<tr>
<td>Weak or no</td>
<td>0</td>
<td>3765 (54%)</td>
<td>3765</td>
</tr>
</tbody>
</table>

One off-target >40\% of target
Four off-target >15\% of target
GAD1: glutamate decarboxylase 1

**Example 1**

<table>
<thead>
<tr>
<th>HPA arrays</th>
<th>HPA arrays</th>
<th>HPA arrays</th>
<th>HuProt arrays</th>
</tr>
</thead>
</table>

GAD1 (Protein fragment)

UTP6 (BC035325.1)

Identity: 10/34 (29.4%)
Similarity: 13/34 (38.2%)

14 RDARFRRRTETFNSNLFDQARDLLPAKENGEQTVQFL 47

| 347 RGKLERMTVFRKAEKLLACEYKQQLSVSLL 380 |
Anti-GAD1 (HPA058412) on 42k antigens
example II

HPA arrays

GOT1 analysis on 384 protein fragments

GOT1 (Protein fragment) 49 ASTLSNPEL 57
DCAF4 (Protein fragment) 6 AGTSSVPEL 14

Identity: 6/9 (66.7%)
Similarity: 6/9 (66.7%)

GOT1 analysis on 11,520 protein fragments

GOT1 analysis on 21,120 protein fragments

GOT1 analysis on 17,000 full-length proteins

GOT1: glutamic-oxaloacetic transaminase 1

HuProt arrays
Dependence on ab conc, fragment length and aa composition

Antibody Concentration

- 80 µg/ml
- 25 µg/ml
- 100 µg/ml

Fragment Length

- 76 aa
- 75 aa
- 81 aa

Amino Acids in Percent

Fragment Length Distribution

- All hPA Protein Fragments
- Protein Fragments in 21,120 array
- Protein Fragments in 11,520 array
Broad profiling of autoantibody repertoires in psychiatry
Plasma from four OCD patients on three global protein microarray formats