From Epitope Mapping to Autoimmune, Infectious Disease and Vaccine Research
Who we are

History
• Founded & based in Heidelberg, Germany
• Privately held, independent company
• Market launch in 2011
• ISO 9001 certified QMS

Scientific Background
• Experienced team of executives
• Proven track record (e.g. Science, Nature)
• Strong IP protection

Products for Academia & Pharma
• Linear & conformational epitope mapping
• Serum antibody & protein profiling
• Antigen, epitope & peptide discovery
• Development of IVD & CDx tests

Customers & Partners

[Logos of various universities and companies]
Laser-Printed Peptide Microarrays and Analysis Services
Competitive Advantage

**Standard**

1. Pre-synthesis of peptides
2. Spotting of peptides on chip
3. Standard peptide array

- Expensive peptide pre-synthesis
- Small peptide libraries
- Mostly standard peptide content

**On-chip peptide synthesis**

PEPperCHIP® Peptide Microarray

- High number of different peptides
- Highly customizable
- Highly economic
Peptide Microarray Technology

**Benefits**
- Very low material consumption
- High content (1,000 peptides/cm²)
- Digital printing flexibility & fast production times
- High peptide quality with routine double couplings
PEPperCHIP® Product Portfolio

<table>
<thead>
<tr>
<th>Format</th>
<th>Number of Peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery Format</td>
<td>75,460</td>
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<tr>
<td>Standard Format</td>
<td>11,280</td>
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<tr>
<td>Mapping Format</td>
<td>5 x 1,632</td>
</tr>
<tr>
<td>Multiplexed Format</td>
<td>16 x 273</td>
</tr>
</tbody>
</table>

PEPperCHIP® Peptide Microarrays with Accessories
send to customer

PEPperMAP® Services
full analysis service
Why PEPperPRINT?

**Speed**
Library set-up in one week

**Flexibility**
Easy library maturation

**Quality**
ISO 9001 standards

**Size**
Biggest antigen libraries

**Structure**
Linear & conformational peptides

**Resolution**
Epitope biomarker discovery
Why PEPperPRINT?

Would you recommend PEPperPRINT to a colleague?

- Yes: 92%
- Unsure: 6%
- No: 2%

92% of PEPperPRINT customers would recommend us to a colleague

Result of Customer Survey 2017
PEPperCHIP® Applications

...read-out a patient's immune profile in a day.

...select the best drug for each patient in a single assay.

...identify neutralizing antibodies for malaria vaccine research.

...differentiate Zika and Dengue virus infections with a drop of blood.

...translate sequencing data of cancer patients into proteomics.

...dramatically improve the efficiency of peptide drugs.

Cancer Research  Autoimmune Research  Antibody Analysis  Peptide Target Binder  Vaccine Development  Infectious Diseases
PEPperCHIP® Applications

- Antibody Analysis
- Vaccine Development
- Peptide Target Binder
- Autoimmune Research
- Infectious Diseases
- Cancer Research
Antibody Analysis

PEPperMAP® Epitope Mapping

• linear and conformational epitope mapping service with max. epitope resolution
• including a custom peptide microarray with single or multiple antigens

PEPperMAP® Epitope Substitution Scans

• in-depth analysis of epitopes for the identification of essential and variable amino acids
• ideal solution for specificity and/or cross-reactivity analyses of monoclonal antibodies

PEPperMAP® Antibody Cross-Reactivity Analysis

• identification of unknown antigens and epitopes with the Human Epitome Microarray
• cross-reactivity profiling of antibodies
Epitope Mapping

Antigen Sequence

Overlapping Peptides

Peptide Microarray

Target Antibody

Secondary Antibody

Monoclonal Sample

Polyclonal Sample

Epitope = Consensus Motif

Data Analysis

Microarray Scan
(Double Spots)
PEPperPRINT uses a maximum peptide-peptide overlap (shift 1) to get high-resolution epitope data.

Possible Samples

- monoclonal or polyclonal antibodies
- synthetic or purified antibodies, hybridoma supernatant
- plasma or serum samples
- species: human, mouse, rabbit, goat, chicken, pig, bovine…
- isotypes: total Ig, IgG, IgM, IgE, IgY, IgA…
Epitope Mapping

- clear separation of two adjacent epitopes
- easy determination of epitope length
- exact identification of conserved core motifs

NO

YES
• **model:** therapeutic mAb Rituximab directed against the extracellular domain of human CD20
• **data basis:** X-ray Analysis of a co-crystal of Rituximab Fab fragment and CD20 peptide (163-187 aa)
• **observation:** conformational epitope due to disulfide bond formation
• **outcome:** 15 amino acid loop NIYCEPANPSEKNSPSTQYCYSIQ covers the epitope of Rituximab
**Conformational Epitope Mapping**

- **Goal:** epitope mapping of Rituximab (Rituxan®), a chimeric monoclonal anti-CD20 antibody
- **Microarray:** CD20 as linear and **cyclic constrained** overlapping peptides (7 aa, 10 aa and 13 aa)
- **Sample:** monoclonal antibody Rituximab
- **Outcome:** high resolution data with **EPANPSEK** as Rituximab epitope
**Epitope Substitution Scan**

- **goal:** in-depth epitope analysis to determine conserved and variable amino acids
- **microarray setup:** full substitution scan of looped peptide **NIYNCEPANPSEK** with all 20 amino acids
- **data analysis:** antibody binding relative to the wild type epitope at 100%
- **outcome:** detailed information about essential, conserved and variable amino acids

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**Microarray Scan**

- **Heatmap**
- **Signal intensity (wild type epitope set to 100%)**

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**Part of extracellular domain of CD20**
Applications

- epitope mapping and analysis of therapeutic antibodies
- cross-reactivity analysis with response matrix
- patient stratification: point mutations vs. essential amino acids
- specificity testing of diagnostic antibodies
Content

- covers all human epitopes of the Immune Epitope Database (www.iedb.org)
  - 8,504 epitopes of infectious diseases
  - 3,808 epitopes of autoimmune diseases
  - 4,661 epitopes of the most common vaccines
  - 199 cancer epitopes, 3,451 epitopes of allergens
  - 28,895 different peptides of 2,542 proteins and 468 organisms

Applications

- epitome-wide antibody screening
- differentiation between IgG and IgM or IgA response profiles
- high throughput serum biomarker discovery
- identification of prognostic epitopes
- cross-reactivity analysis of mono- and polyclonal antibodies
- patient stratification
- investigation of links between autoimmune and infectious diseases
- cross-reactivity analysis of therapeutic and diagnostic antibodies
One-Step Cross-Reactivity Analysis

Human Monoclonal Antibody anti-c-Myc (chi9E10), 2 µg/ml

- **microarray setup**: PEPperCHIP® Human Epitome Microarray
- **goal**: cross-reactivity analysis with 28,895 human database epitopes on one chip
- **sample**: chimeric human anti-Myc (chi9E10) antibody
- **data analysis**: few but clear peptide hits (red) with 2 µg/ml antibody
- **outcome**: cross-reactions against epitopes of nucleoprotein, erythrocyte membrane-associated giant protein antigen 332 and dihydrolipoamide S-acetyltransferase
### One-Step Cross-Reactivity Analysis

<table>
<thead>
<tr>
<th>Peptide</th>
<th>anti-c-Myc (ch9E10), 2 µg/ml</th>
<th>Epitope ID</th>
<th>Source Molecule Accession</th>
<th>Source Molecule Name</th>
<th>Source Organism ID</th>
<th>Source Organism Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGITAEDARLVSEIAMH</td>
<td>2.335,0</td>
<td>98874</td>
<td>127900</td>
<td>Nucleoprotein</td>
<td>11235</td>
<td>Measles virus strain Edmonston</td>
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<td>EEVGEKLVSEEEVT</td>
<td>1.960,5</td>
<td>98874</td>
<td>127900</td>
<td>Dihydrolipoamide S-Acetyltransferase (E2 Component Of Dihydrolipoamide S-Acetyltransferase (E2 Component Of Nuclear Protein</td>
<td>11235</td>
<td>Measles virus strain Edmonston</td>
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<tr>
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**Cross-reactivity Profile**

- Cross-reactivity profile over 28,895 known human epitopes with database annotations
- Identification of conserved and variable amino acid positions by MEME motif discovery

**Example Peptides**

- **Epitope:** LGITAEDARLVSEIAMH
  - **Source Molecule:** Nucleoprotein
  - **Accession:** 98874
  - **Accession:** 127900
  - **Source Organism:** Measles virus strain Edmonston

**Additional Information**

- **Methodology:** One-Step Cross-Reactivity Analysis
- **Database:** Immune Epitope Database and Analysis Resource

**Cross-reactivity Profile**

- **Cross-reactivity profile over 28,895 known human epitopes with database annotations
  - **Identification of conserved and variable amino acid positions by MEME motif discovery**
PEPperMAP® Service Process

Curious about how the PEPperMAP® Service process looks like? Here is the answer!

**Quote Request**
Just send us a quote request along with your antigen/peptide sequences.

**STEP 01**
Based on your request, we provide you a quote within 48 hours.

**STEP 02**
We send an order confirmation along with a microarray layout for approval.

**Purchase Order**
If you agree with the quote, please send us a purchase order.

**Microarray Production**
After confirmation of the microarray layout, we initiate peptide microarray production. This process takes about 4 weeks.

**STEP 03**
Meanwhile, you send samples with all requested sample information to us.
Service Process Overview – Part 2

**Quality Control**
When the array production is completed, we perform a routine quality control. This process usually takes 2 days.

**Lab & Computer Work**
After the quality control, we start the immunoassays in our lab. This process takes about 1-2 weeks.

**Delivery**
Done! The report is sent to you. We are available for further questions and support.

www.pepperprint.com
PEPperCHIP® Applications

- Antibody Analysis
- Vaccine Development
- Peptide Target Binder
- Autoimmune Research
- Infectious Diseases
- Cancer Research
Autoimmune Research

PEPperCHIP® Autoimmune Epitope Microarray
- covering 4,287 linear autoimmune epitopes of the Immune Epitope Database
- including 286 citrullinated peptides and the corresponding arginine controls

PEPperCHIP® Human Epitome Microarray
- covering all linear human B-cell epitopes of the Immune Epitope Database
- most comprehensive human epitope microarray with 28,895 different peptides

PEPperCHIP® Lupus Microarray
- 28 lupus antigens translated into overlapping peptides for a multiplexed epitope mapping
- including 761 lupus epitopes of the Immune Epitope Database and literature

PEPperCHIP® Cyclic Citrullinated Peptide Microarray
- covering 577 different cyclic peptides of the Immune Epitope Database and literature
- including 337 different cyclic citrullinated peptides and the corresponding arginine controls
PEPperCHIP® Infectious Disease Epitope Microarray
• covering 3,857 linear infectious disease epitopes of the Immune Epitope Database
• epitopes of 192 different pathogens including bacterial, fungal, parasitic and viral pathogens

PEPperCHIP® Human Epitome Microarray
• covering all linear human B-cell epitopes of the Immune Epitope Database
• most comprehensive human epitope microarray with 28,895 different peptides

PEPperCHIP® Standard Peptide Microarrays
• PEPperCHIP® Dengue Virus Proteome Microarrays
• PEPperCHIP® MERS-CoV Proteome Microarray
• PEPperCHIP® Influenza Virus H1N1 Proteome Microarray
• PEPperCHIP® Oncovirus Epitope Microarray and many more…
PEPperCHIP® Applications

- Antibody Analysis
- Vaccine Development
- Peptide Target Binder
- Autoimmune Research
- Infectious Diseases
- Cancer Research
PEPperCHIP® Oncovirus Epitope Microarray
- covering 3,653 linear B-cell epitopes of human oncoviruses
- including epitopes of hepatitis B and C, human herpesviruses, human papillomavirus etc.

PEPperCHIP® Tumor Antigen Microarray
- covering 22 selected tumor antigens as overlapping peptides (p53, NY-ESO-1 etc.)
- multiplexed and isotype-specific epitope mappings against general tumor antigens

PEPperCHIP® Melanoma Microarray
- covering 21 melanoma-associated antigens as overlapping peptides (e.g. MAGEA1, 2, 3)
- multiplexed and isotype-specific epitope mappings against melanoma antigens

PEPperCHIP® Custom Peptide Microarrays
- covering any epitope or antigen collection with linear or cyclic constrained peptides
- isotype-specific screening of antibody responses against linear or conformational epitopes
Cancer Neoepitope Microarrays

Translate **cancer point mutations** into **peptide microarrays**

Select neoepitopes from databases & sequencing
 Translate point mutations into peptide microarray
 Incubate sera from cancer patients & healthy controls
 Identify patient specific cancer antibody profiles

Applications

- **Validate** therapeutic antibodies against tumor associated antigens
- **Target discovery**: Identify immunogenic epitopes for immunotherapy
- **Develop** personalized cancer vaccines
- **Monitor** patient-specific anti-tumor antibody responses
- **Discover** prognostic cancer neoepitopes
PEPperCHIP® Cancer Neoepitope Microarray Specifications:

- **Microarray Content** – cancer neoepitopes, sequencing data, custom peptide lists etc.
- **Peptide Length** – 15 amino acids by default (adjustable)
- **Peptide Numbers** – up to 5,500 different peptides printed in duplicate
- **Control Peptides** – HA epitopes by default, optional custom controls
- **Timeline** – 4 weeks for microarray delivery, 2 weeks for data analysis & reporting
- **Microarray Example** – 2,750 wild type / neoepitope peptide pairs printed in duplicate
Peptide Pools for T-Cell Stimulation

- unpurified & purified custom peptides with varying QC levels (MS, HPLC)
- from 6 - 25 amino acids in 2-10 µmol scale
- T-cell stimulation: 8 - 15 amino acids each peptide with free amino and carboxyl termini
- individual peptides and peptide pools, optional modifications like acetylation, phosphorylation etc.

General Capacities

- 8 peptide synthesizers, 4 RP-HPLC units (analytical & preparative)
- more than 20 RP-HPLC columns
- Bruker Ultraflex MALDI-TOF

Delivery Times

- set of 384 peptides, < 2µmol scale (research grade): 6 weeks
- set of 192 peptides, 2-10 µmol scale (research grade): 4-5 weeks
- for higher peptide purities (>80% purity), delivery times may vary

→ about 100 customers all over the world
PEPperMAP® Immune Monitoring
- linear and conformational epitope mapping of immunogens with max. epitope resolution
- fingerprint analysis of B-cell responses at various time points before and after immunization

PEPperMAP® HLA Class II Screening
- linear mapping of HLA class II binding peptides for peptide vaccine design
- identification of HLA II DR specific epitopes with stable recombinant HLA proteins

Peptide Pools for T-Cell Stimulation
- unpurified & purified custom peptides with varying QC levels from 6 - 25 amino acids
- peptide pools with hundreds of peptides from 2-10 μmol scale in few weeks
PEPperCHIP® Applications

- Antibody Analysis
- Vaccine Development
- Peptide Target Binder
- Autoimmune Research
- Infectious Diseases
- Cancer Research
Screening Options

PEPperMAP® Protein Interaction Analysis
  • linear and conformational protein mapping service with max. peptide resolution
  • including a custom peptide microarray with single or multiple target proteins

PEPperMAP® Hit Discovery
  • target binder screening with ~35,000 custom or random peptides (linear or cyclic)
  • initial peptide hit discovery for target proteins

PEPperMAP® Peptide Display Hit Validation and Optimization
  • in-depth analysis of hit peptides of peptide display screening
  • peptide hit validation and high-throughput optimization by peptide substitution scans

Full Combinatorial Constrained Tripeptide Libraries
  • target binder screening with all possible cyclic constrained tripeptides
  • small-molecule-like peptide hit discovery for target proteins
For **detailed presentations** including case studies

**please contact:** info@pepperprint.com
Customization:
- most flexible peptide microarray platform with any content
- from up to 35,000 different peptides per microarray to prototype test format
- linear and cyclic constrained peptides
- incorporation of peptides with posttranslational modifications

Quality:
- high peptide quality and chip reproducibility, ISO 9001-certified quality
- custom and adjusted glass slide coatings for best signal-to-noise ratios

Service:
- extensive knowhow from peptide selection to bioinformatics
- custom and scientific reporting instead of machine-made reports
- thorough scientific support and fast response times
Antibody Characterization


Autoimmune Research

• Bicaudal D2 is a novel autoantibody target in systemic sclerosis that shares a key epitope with CENP-A but has a distinct clinical phenotype. Fritzle MJ, Hudson M, Choi MY, Mahler M, Wang M, Bentow C, Milo J, Baron M., Autoimmunity Reviews 2018; doi: 10.1016/j.autrev.2018.01.006 (Epub)


Key Publications

Cancer Research

- **Non-invasive glioblastoma immunoprofiling by printed peptide arrays.** Mock A, Herold-Mende C., OncoImmunology 2015; doi: 10.1080/2162402X.2015.1069941 (Epub)

Infectious Diseases and Vaccines

- **Mapping Putative B-Cell Zika Virus NS1 Epitopes Provides Molecular Basis for Anti-NS1 Antibody Discrimination between Zika and Dengue Viruses.** Freire MCLC, Pol-Fachin L, Coelho DF, Viana IFT, Magalhães T, Cordeiro MT, Fischer N, Loeffler FF, Jaenisch T, Franca RF, Marques ETA, Lins RD., ACS Omega 2017; 2(7): 3913-3920. doi: 10.1021/acsomega.7b00608 (Epub)
Peptide Target Binder Development


PEPperPRINT Technology

More Information

Tutorial Video & Webinar

- Webinar: High Density Peptide Microarrays - A Versatile Platform for Autoimmune Research
- Webinar: Antibody Validation and Cross-Reactivity Analysis by High-Density Peptide Microarrays


Application Notes

- Application Notes: Validation of a Novel Recombinant Human anti-<r>4-Myc Tag Antibody</r>
- Application Notes: Anti-body Cross-Reactivity Profiling of Human Monoclonal Antibody with the PEPepMAB®-human Eppitope Microarray (PEP-MAbS)
- Application Notes: Characterization of Recombinant Epitope Mapping and Epitope Substitution Scan (Hi. 1342)
- Application Notes: Comparison of Specificity between PEPepMAB® Epitopes

https://www.pepperprint.com/updates/application-notes/

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