Peptide libraries: applications, design options and considerations

Laura Geuss, PhD
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Overview

1. Introduction
2. Peptide library basics
3. Peptide library design considerations
4. Service summary and resources
5. Q&A
Problem:
As progress continues to be made in drug discovery and development, we need higher-throughput and more accurate methods to discover important protein targets.

Solution
By spanning entire sequences of these important epitopes, peptide libraries ensure no potentially important sequences are missed.
Peptide libraries contain a systematic combination of a large number of different peptides that represent important bioactive regions or epitopes on a protein.
What is the difference between a phage-display and synthetic peptide library?

**Phage-Display Peptide Library**
- A library of phages that display peptides on their surface. Specific clones are selected based on binding affinity to a target.
- **Advantages**: simple assay, not limited by peptide length.
- **Disadvantages**: labor intensive, limited to natural, L-amino acids.

**Synthetic Peptide Library**
- Peptide sequences are synthesized by solid phase.
- Allows for library design flexibility.
- **Advantages**: can use D-amino acids, flexible design options. Less time needed to synthesize peptides in lab.
## Synthetic Peptide Library options

<table>
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<tr>
<th>Library type</th>
<th>Features</th>
<th>Typical applications</th>
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<tr>
<td>Micro-scale peptide library</td>
<td>0.2-0.5 mg, 5-20 AA</td>
<td>• Preliminary peptide screening</td>
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<td>• Proteomics</td>
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<td>• Mass Spectrometry</td>
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<td>Purified peptide library</td>
<td>1-4 mg, 5-25 AA</td>
<td>• Immune monitoring</td>
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<td></td>
<td>• Cell based assays</td>
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<tr>
<td></td>
<td></td>
<td>• Drug discovery</td>
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<tr>
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<td></td>
<td>• Clinical trials</td>
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<tr>
<td>Crude peptide library</td>
<td>1-20 mg, 5-25 AA</td>
<td>• Biomarker discovery</td>
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<td>• T cell binding assays</td>
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How do you design a peptide library?

1. **Identify the bioactive region you are interested in**
   - Online tools (ex: [http://bioware.ucd.ie](http://bioware.ucd.ie))
   - Input UniProt ID, can identify most likely bioactive regions

2. **Determine what peptide combinations to screen**
   - GenScript has free online tools to generate peptide library
   - Input the bioactive region peptide sequence to generate peptide combinations

3. **Choose the most appropriate library based on your end application**
   - Micro-scale peptide library or standard peptide library
In general, there are six common peptide library designs. The type you choose depends on the end application.
Option 1: Overlapping peptide library

Why it is useful

- Ideal for linear (or continuous) epitopes
- Span the entire epitope sequences

Research Applications

- Epitope Mapping: B and T cells
- Identify which sequences within the epitope are most important for activity
Case study: overlapping peptide libraries for ELISA design

- **Background**
  - There is no reliable biomarker to diagnose or predict the onset of osteoarthritis; however, the degradation products of cartilage oligomeric matrix protein (COMP) might serve as predictors for the disease.

- **How was the library designed?**
  - Overlapping peptides that cover the entire COMP epitope using GenScript’s services
    - 51 biotinylated peptides
    - 15 AA long, 10 AA overlap

- **What were the results?**
  - Authors were able to identify the exact epitope of an anti-COMP mAb and consequently developed a novel ELISA to predict osteoarthritis

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Option 2: Alanine scanning library

Why it is useful

- Alanine, the smallest AA, is substituted in for each non-Ala residue in a peptide sequence
- In some cases, substitution of a key residue with Ala will cause changes in epitope binding activity
- Can quickly determine each of AA’s contribution to peptide function

Research Applications

- Find protein binding sites and enzyme substrates
- Discover functional epitopes
Case study: Using alanine scanning to engineer T cells for immunotherapy

◆ **Background**
  • Naturally occurring T Cell receptors bind self (tumor) peptides with low affinity
  • Engineered T cells with higher MHC affinity may represent another immunotherapy option

◆ **How was the library designed?**
  • Each residue of the TCR region that mediates peptide specificity of T cell recognition was replaced with an Ala
  • Which residues are most important for the TCR-MHC binding interaction?

◆ **What were the results?**
  • Alanine substitutions to any region of the CDR3 region of the MHC significantly decreasing binding, identifying a key region that can be targeting for the discovery of immunotherapeutics

Option 3: Truncation peptide library

Why it is useful

- Peptides are systematically truncated from the N and C terminus

Research Applications

- Identify the minimum peptide length for activity
- Allows you to identify the peptides that have enhanced proteolytic stability
Case study: Truncation libraries for better antibody design

- **Background**
  - Antibody-based drugs are widely used for drug development, but their potential to aggregate can result in life-threatening side-effects.
  - What are the mechanisms behind aggregation? How do proteins A and G contribute?

- **How was the library designed?**
  - Peptides derived from protein A were sequentially truncated at GenScript to identify the peptide with the most dominant role in aggregation

- **What were the results?**
  - The authors identified protein A as being critical for mediating aggregation, providing a target for future antibody design

Option 4: Positional Scanning library

Why it is useful

- AAs at a specific area of interest are systematically substituted with other natural AAs
- Can identify which AAs may enhance binding

Research Applications

- Peptide sequence optimization
- Identify T cell epitopes from complex mixtures of proteins
Case study: positional screening of peptides important for enzyme catalytic activity

◆ Background

• What regions of an enzyme active site contribute the most to activity and specificity?

• Sought to understand functional constraints of an enzyme, LHE, which is used as a genome-editing agent.

◆ How was the library designed?

• Positional peptide library prepared at GenScript

• AAs were substituted at specific positions in non-catalytic and catalytic regions

◆ What were the results?

• The authors identified non-variant residues that are important for LHE activity

McMurrough et al. PNAS. 2014; 111(23): E2376-E2383.
Option 5: Random peptide library

Why it is useful

- Randomly and simultaneously generated variations with all other AA via a shotgun approach
- Can potentially generate peptides that have enhanced activity for a specific function

Research Applications

- Identify highly active and novel peptide sequences
- Peptide sequence optimization
Case study: random peptide library for tumor cell ligand screening

- **Background**
  - Targeted tumor treatment first requires identification of a specific, identifying ligand on the tumor cell.
  - The authors sought to isolate peptides that bound specifically to lung cancer cells.

- **How was the library designed?**
  - Used a phage display approach to create a library of cell-targeting peptides

- **What were the results?**
  - The authors were able to identify peptide sequences with high binding affinity to specific tumor cancer cells, which presents a potential target for downstream drug design.

Option 6: Scrambled peptide library

**Why it is useful**

- Provides the highest level of variation

**Research Applications**

- Create the ideal scenario for peptide sequence optimization
- Probe target molecules of interest including proteins, antibodies and DNA
**Background**

- The carbohydrate L2/HNK-1 is an important neural recognition molecule involved in many neural cell interactions.

- Further study of its biological role is important, but limited natural availability makes this difficult: finding a peptide mimic will make this possible.

**How was the library designed?**

- 15-mer candidate peptides were generated with a random, phage-display library.

**What were the results?**

- The authors isolated the peptides that bound to anti-L2/HNK-1 antibodies, opening up the open for future studies with the carbohydrate.

GenScript’s free online design tool

www.genscript.com/peptide_screening_tools

Step 1: Input your protein sequence
Step 2: Choose your library design
Step 3: Input the parameters and click the quote button to get a quote for the selected sequences.
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When designing overlapping peptide libraries, consider the offset number

- Libraries with longer offset numbers will cost less, but the chance of missing important AA combinations increases
- Choose shorter offset numbers (1/3 the peptide length) to ensure you make the most of your library

- Fewer peptides
  - Fewer possible epitopes

- More peptides
  - More possible epitopes
Important design considerations: hydrophobicity

- Highly hydrophobic residues are difficult to purify and also difficult to solubilize.

- If your library includes hydrophobic peptides, consider:
  - Choosing a lower purity;
  - Substituting the hydrophobic residues;
  - Choose different peptide sequences.

Replace with:
- Tryptophan
- Isoleucine
- Leucine
- Phenylalanine
- Methionine
- Valine
- Tyrosine
- Glutamic Acid
- Aspartic Acid
- Lysine
- Arginine
- Histidine
If you are designing a micro-scale peptide library to be combined with MS for reaction monitoring, choose:

- Peptide lengths around 10 AA
- No short hydrophilic or long hydrophobic sequences
- No residues that are prone to oxidation (such as Methionine or Tryptophan)

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## Peptide library summary

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<tr>
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<th>Standard Crude Peptide Library</th>
<th>Standard Purified Peptide Library</th>
<th>Micro-scale Peptide Library</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantity</strong></td>
<td>1-20 mg</td>
<td>1-4 mg</td>
<td>0.2-0.5 mg</td>
</tr>
<tr>
<td><strong>Peptide Length</strong></td>
<td>5-25 AA</td>
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<td>5-20 AA</td>
</tr>
<tr>
<td><strong>Purification</strong></td>
<td>~ &gt;20-30%</td>
<td>&gt;70%, &gt;75%, &gt;80%, &gt;85%, &gt;90%, &gt;95%, &gt;98%</td>
<td>Crude or &gt;70%</td>
</tr>
<tr>
<td><strong>QC</strong></td>
<td>MS only; HPLC, MS for each peptide</td>
<td>COA, HPLC and MS for each peptide</td>
<td>COA, HPLC and MS for each peptide; Analytical HPLC option</td>
</tr>
<tr>
<td><strong>Additional options</strong></td>
<td>TFA removal service</td>
<td>Extensive modification options</td>
<td></td>
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<tr>
<td><strong>Key applications</strong></td>
<td>Epitope discovery, T-cell assays</td>
<td>Drug development, immune monitoring, preclinical trials</td>
<td>Biomarker discovery Proteomics Reaction monitoring</td>
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[www.genscript.com/peptide-library.html](http://www.genscript.com/peptide-library.html)
Peptide services at GenScript

Standard peptide synthesis
• Starting at $3.2/AA
• Up to 200 AA
• Mg to kg quantity

Peptide Library
• Standard and micro-scale quantity
• Flexible purity options
• Peptide pooling available

Peptoid Synthesis
• Proteolytic resistant peptidomimetics
• Cost-effective, fast turnaround

Cosmetic Peptide Synthesis
• High batch-to-batch reproducibility
• High capacity

Click Peptide Synthesis
• O-acyl bond incorporation technology
• Increased peptide stability

www.genscript.com/peptide-services.html
Thank you for your participation
We wish you success with your research
Email me: Laura.Geuss@genscript.com

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Chaperone co-expression strategies for recombinant soluble protein production in *E.coli* – Bo Wu Ph.D
May 12, 2015, 2:00 pm EST

Analyzing antibody sequences for recombinant antibody expression – Hangxing Yu, Ph.D
May 20, 2015, 9:00 am EST

Expression vectors: how to choose, or customize, vectors for gene & protein expression – Rachel Speer, Ph.D
June 3, 2015, 11:00 am EST
If you have any other questions, visit www.genscript.com/faq_for_peptide
Or email: Laura.Geuss@genscript.com