

Mixture Sciences, Inc. has developed a technology, positional scanning libraries, for rapidly generating and screening millions of different peptides. Positional scanning libraries are composed of systematically arranged mixtures. In the case of single-position-defined positional scanning libraries, each compound present in a given mixture has a common individual building block at a given position, while the remaining positions are composed of mixtures of all the building blocks used to prepare the library; a common single building block defines each relevant mixture.¹



Hexapeptide Positional Scanning Library

Sublibrary 1: ○ X X X X X }
 Sublibrary 2: X ○ X X X X
 Sublibrary 3: X X ○ X X X
 Sublibrary 4: X X X ○ X X
 Sublibrary 5: X X X X ○ X
 Sublibrary 6: X X X X X ○

Sublibrary 1

○ X X X X X
 Sample 1: A X X X X X
 Sample 2: C X X X X X
 Sample 3: D X X X X X
 Sample 4: E X X X X X
 Sample 5: F X X X X X
 ...
 Sample 20: Y X X X X X

For example a hexapeptide library can be illustrated as above as six separate sublibraries. Sublibrary 1 contains 20 different samples. The first sample contains Alanine, represented by an A, at the first position and a mixture, represented by an X, at the other five positions. The mixture represents an equal molar amount of 19 amino acids (the 20 natural minus Cysteine). This means that Sample 1 contains a mixture of $19^5 = 2.4$ million hexapeptides all with the first position fixed with Alanine. Sample 2 contains a mixture of 2.4 million hexapeptides all with the first position fixed with Cysteine, C. This format is continued through the length of the peptide forming the 6 sublibraries. This means that $19^6 = 47$ million different hexapeptides can be screened using only $6 \times 20 = 120$ samples.

| Library | Receptor | Activity of lead Compound(s) |
|--------------|----------------------|---|
| Tetrapeptide | Opioid | $IC_{50} = 2 \text{ nM}^2$, $K_i = 0.4 \text{ nM}^2$ |
| Hexapeptide | Opioid | $IC_{50} = 17 \text{ nM}^3$, 45 nM^4 |
| Hexapeptide | Antipeptide/Antibody | $IC_{50} = 2 \text{ nM}^5$ |
| Decapeptide | Antipeptide/Antibody | $IC_{50} = 0.6 \text{ nM}^6$ |
| Hexapeptide | Enzyme Inhibitors | $K_i = 3.2 \text{ nM}^7$, 360 nM^7 |
| Hexapeptide | Melittin Inhibitors | $K_i = 3.0 \text{ ug/mL}^4$ |
| Decapeptide | T-cell | $IC_{50} = 18 \text{ nM}^8$ |
| Decapeptide | T-cell | $EC_{50} = 0.0002 \text{ ug/mL}^{9,10}$ |

Our peptide positional scanning libraries have been used in a wide range of bioassays by both pharmaceutical and academic institutions in order to identify novel enzyme inhibitors, receptor agonists and antagonists, antimicrobial, antifungal, and antiviral compounds. Above is a list of some of the libraries used in different bioassays as well as the activity of leads generated from testing those libraries.

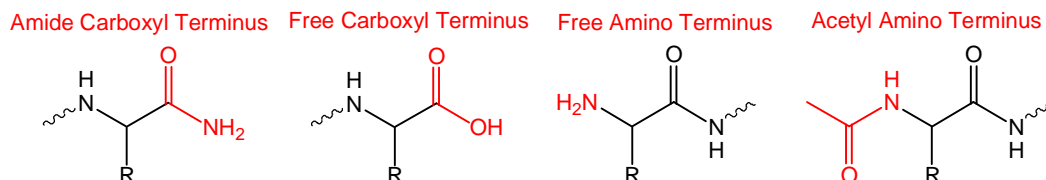
The following is a list of some of the peptide positional scanning libraries Mixture Sciences, Inc. has available.

| Library | # of Compounds | # of Samples | Amino Terminus | Carboxyl Terminus |
|---------------------------|----------------|--------------|-------------------|-------------------|
| Hexapeptide | 47 million | 120 | Both ^a | Both ^a |
| Decapeptide | 300 billion | 180 | Both ^a | Both ^a |
| Dodecapeptide | 6 trillion | 200 | Both ^a | Amide |
| Hexapeptide ^b | 47 million | 120 | Acetyl | Amide |
| Decapeptide ^b | 300 billion | 180 | Both ^a | Amide |
| Tetrapeptide ^c | 12 million | 240 | Both ^a | Amide |

^aBoth means that the Amino Terminus can be either free or an acetyl group and the Carboxyl Terminus can be either free or an amide group. This means for the first Hexapeptide Library we actually have four different libraries; a library with a free amino and carboxyl terminus, a library with a free amino terminus and an amide carboxyl terminus, a library with acetyl amino terminus and a free carboxyl terminus, and a library with an acetyl amino terminus and an amide carboxyl terminus.

^bThese libraries contain only the D versions of the natural amino acids.

^cThese libraries contain L, D, and unusual amino acids.



Reference: (1) Houghten, R.A., et al. *J. Med. Chem.* 42:3743 **1999**. (2) Dooley, C.T., et al. *J. Biol. Chem.* 273:18848 **1998**. (3) Dooley, C.T., et al. *Life Sci.* 52:1509 **1993**. (4) Pinilla, C., et al. *Drug. Dev. Res.* 33:133 **1994**. (5) Pinilla, C., et al. *Biotechniques* 13:901 **1992**. (6) Pinilla, C., et al. *Biochem. J.* 301:847 **1994**. (7) Apletalina, E., et al. *J. Biol. Chem.* 273:26589 **1998**. (8) Pinilla, C., et al. *Cancer Research* 61:5153 **2001**. (9) Hemmer, B., et al. *J. Immun.* 12:375 **2000**. (10) Hemmer, B., et al. *Nature Med.* 5:1375 **1999**.

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