Why Compromise? Welcome to the Future of Peptide Library Synthesis with the Overture™

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INTRODUCTION

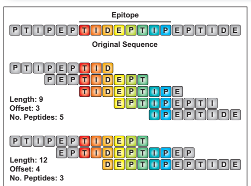
Peptide libraries are groups of peptides with systematic combinations of amino acids that are an extremely useful tool for drug discovery. Peptide libraries can be used to identify target ligands and then optimize them for potency, selectivity, and stability [1]. Automation plays a pivotal role in increasing the speed and efficiency of this process. The Overture™ Robotic Peptide Library Synthesizer was designed to generate and synthesize peptide libraries in an easy to use, high-throughput format. The Overture's™ flexible reaction vessel configurations and software allow libraries from 4 to 96 compounds to be made, including overlapping peptides for epitope mapping, alanine-scanning libraries, positional scanning libraries, combinatorial 2- and 3-positional scanning libraries, truncation and T-cell truncated peptide libraries and scrambled peptide libraries for sequence optimization. Overviews and examples of each type of library generated from the acyl carrier protein (ACP) sequence (Figure 1) are shown.

STIEERVKKIIGEQLGVKQEEVTDNASFVEDLGADSLDTVELVMALEEEFDTEIPDEEAEKITT
VQAAIDYINGHQA

Figure 1: Acyl carrier protein sequence.

Once generated, the Overture Windows Utility can break up peptide libraries into individual syntheses of 4 to 16 sequences each for running on 2 x 2 or 4 x 4 reaction vessel blocks, respectively. The Overture can run six reaction vessel blocks independently, so different scales and reaction conditions can be run on each block simultaneously.

OVERLAPPING LIBRARY



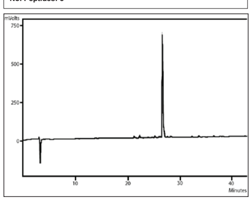


Figure 2: 65-74 fragment of ACP.

Overlapping peptide libraries are used for T-cell epitope determination and mapping linear, or continuous B-cell epitopes. Select a peptide length and overlap amount to create a library of equal-length overlapping fragments of the original sequence. In the example to the right, a modified version of the ACP sequence is used to create a library of 10-mers overlapping by 5 amino acids. An HPLC of sequence 14 is shown in Figure 2.

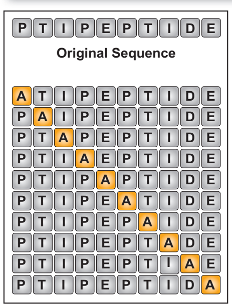
Original Sequence: ASTIEERVKKIIGEQLGVKQEEVTDNASFVEDLGADSLDTVELVMALEEEFDTEIPDEEAEKITTVQAAIDYINGHQA

Length: 10

Overlap: 5

	Sequence	MW (g/mol)
1	ASTIEERVKK	1159.34
2	ERVKKIIGEQ	1198.42
3	IIGEQLGVKQ	1083.29
4	LGVKQEEVTD	1116.23
5	EEVTDNASFV	1109.15
6	NASFVEDLGA	1021.09
7	EDLGADSLDT	1034.03
8	DSLDTVELVM	1120.28
9	VELVMALEEE	1160.34
10	ALEEEFDTEI	1194.25
11	FDTEIPDEEA	1164.18
12	PDEEAEKITT	1131.20
13	EKITTVQAAI	1072.26
14	VQAAIDYING	1062.18
15	AIDYINGHQA	1100.19

ALANINE SCANNING LIBRARY



Alanine scanning is used to identify the amino acids that are important to the functionality of the original sequence. Systematically substitute alanine into each amino acid position of the original sequence. Or, substitute other amino acids or groups of amino acids. Decreased activity shows the relative importance of the substituted amino acid. In the example to the right, the 65-74 fragment of ACP is substituted first with alanine, and then with isoleucine to create a library of 16 sequences.

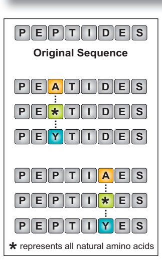
Original Sequence: VQAAIDYING

Length: 10

Overlap: 5

	Sequence	MW (g/mol)
1	AQAAIDYING	1034.13
2	VAAAIDYING	1005.13
3	VQAAAIDYING	1020.10
4	VQAAIAYING	1018.17
5	VQAAIDAING	970.08
6	VQAAIDYANG	1020.10
7	VQAAIDYIAG	1019.16
8	VQAAIDYINA	1076.21
9	IQAAIDYING	1076.21
10	VIAAIDYING	1047.21
11	VQIAIDYING	1104.26
12	VQAIIDYING	1104.26
13	VQAAIIYING	1060.25
14	VQAAIDIING	1012.16
15	VQAAIDYIIG	1061.24
16	VQAAIDYINI	1118.29

POSITIONAL SCANNING LIBRARY



Use positional scanning libraries for sequence optimization. Select one or more positions, and the software will substitute each one with all of the remaining natural amino acids one at a time. Each selected position will result in 20 sequences. An increase in activity will reveal the preferred amino acid residue for each position. Alternatively, specific amino acids may be chosen as substitutions if all 20 amino acids are not necessary. In the example to the right, amino acids A, C, D, E, F and G are substituted one at a time in positions 1 and 2.

Original Sequence: VQAAIDYING

Substitutions: ACDEFG

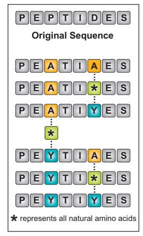
Positions: 1, 2

	Sequence	MW (g/mol)
1	AQAAIDYING	1034.13
2	CQAAIDYING	1066.19
3	DQAAIDYING	1078.14
4	EQAAIDYING	1092.16
5	FQAAIDYING	1110.23
6	GQAAIDYING	1020.10
7	VAAAIDYING	1005.13
8	VCAAIDYING	1037.19
9	VDAAIDYING	1149.14
10	VEAAIDYING	1063.17
11	VFAAIDYING	1081.23
12	VGAAIDYING	991.10

COMBINATORIAL 2- AND 3- POSITIONAL SCANNING LIBRARIES

Use combinatorial 2- or 3-positional scanning libraries for sequence optimization. Select two or three positions, and the software will systematically substitute each position with all of the remaining natural amino acids one at a time. Defining two positions will result in 20 x 20 = 400 sequences. Defining three positions will result in 20 x 20 x 20 = 8000 sequences. An increase in activity will reveal the preferred amino acid residues for each position. Alternatively, specific amino acids may be chosen as substitutions if all 20 amino acids are not necessary. In the first example to the right, amino acids A, C, and D are substituted combinatorially in positions 1 and 2. In the second example to the right, amino acids A and C are substituted combinatorially in positions 1, 2 and 5.

Ex. 1: Combinatorial 2-Positional Scanning Library



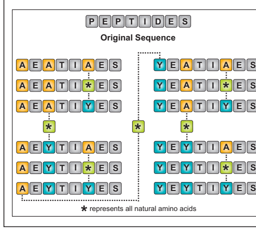
Original Sequence: VQAAIDYING

Substitutions: ACD

Positions: 1, 2

	Sequence	MW (g/mol)
1	AAAAIDYING	977.08
2	ACAAIDYING	1009.14
3	ADAAIDYING	1021.09
4	CAAAIDYING	1009.14
5	CCAAIDYING	1041.20
6	CDAAIDYING	1053.15
7	DAAAIDYING	1021.09
8	DCAAIDYING	1053.15
9	DDAAIDYING	1065.10

Ex. 2: Combinatorial 3-Positional Scanning Library



Original Sequence: VQAAIDYING

Substitutions: AC

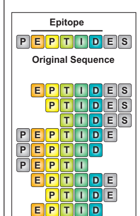
Positions: 1, 2, 5

	Sequence	MW (g/mol)
1	AAAAADYING	934.99
2	AAAACDYING	967.06
3	ACAAADYING	967.06
4	ACAACDYING	999.12
5	CAAAADYING	967.06
6	CAAACDYING	999.12
7	CCAAADYING	999.12
8	CCAACDYING	1031.19

TRUNCATION AND T-CELL TRUNCATED LIBRARIES

Use truncation peptide libraries to identify the shortest amino acid sequence needed for activity. Select the number of residues to systematically remove from each terminus of the original peptide. The truncation direction can be tailored to maintain important residues determined by alanine scanning. In the first example to the right, a truncation library was created from the 65-74 fragment of ACP with an N-terminal truncation of 1 and C-terminal truncation of 2 selected.

Ex. 1: Truncation Library



Original Sequence: VQAAIDYING

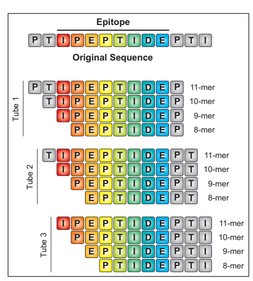
N-Terminus Truncation: 1

C-Terminus Truncation: 2

	Sequence	MW (g/mol)
1	VQAAIDYING	1062.18
2	QAAIDYING	963.05
3	AAIDYING	834.92
4	AIDYING	763.84
5	IDYING	692.76
6	VQAAIDYI	891.02
7	VQAAID	614.68
8	QAAIDYI	791.89

T-cell truncated peptide libraries allow all possible T-cell epitopes across a protein of interest to be tested. Enter a peptide length ("n"), and the n, n-1, n-2, and n-3 peptides for each position across the sequence will be generated. Peptides are shortened in each position from the N-terminal end. For T-cell epitope mapping, a length of 11 should be used. Use other lengths for different mapping strategies. In example 2 above, a T-cell truncated library is generated from the 65-77 fragment of ACP with length 11 selected.

Ex. 2: T-Cell Truncated Library



Original Sequence: VQAAIDYINGHQA

Length: 11

	Sequence	MW (g/mol)
1	VQAAIDYINGH	1199.32
2	QAAIDYINGH	1100.19
3	AAIDYINGH	972.06
4	AIDYINGH	900.98
5	QAAIDYINGHQ	1228.32
6	AAIDYINGHQ	1100.19
7	AIDYINGHQ	1029.11
8	IDYINGHQ	958.03
9	AAIDYINGHQA	1171.27
10	AIDYINGHQA	1100.19
11	IDYINGHQA	1029.11
12	DYINGHQA	915.95

SCRAMBLED LIBRARY

Scrambled libraries are made up of permutations of the original peptide sequence. They are used as random screening libraries for the identification of new leads, or as negative controls to show that the specific sequence and not the amino acid composition is required for activity. In the example to the right, a library of 10 scrambled sequences was created from the 65-74 fragment of ACP.

Original Sequence: VQAAIDYING

Number: 10

	Sequence	MW (g/mol)
1	NQGIVAIDYA	1062.18
2	YAAIDGVING	1062.18
3	INDYIGVQAA	1062.18
4	DVQIIGANAY	1062.18
5	IINDAYAGQV	1062.18
6	AGNAIIVQDY	1062.18
7	DAYQINGVIA	1062.18
8	QGYAIIDANV	1062.18
9	NQIAYGIVAD	1062.18
10	IINDGQYAVA	1062.18

REFERENCE

[1] Marasco D, Perretta G, Sabatella M, Ruvo M. Past and Future Perspectives of Synthetic Peptide Libraries. *Curr. Protein Pep. Sci.* 2008; **9**: 447-467.