Novel Technologies for Difficult Neoantigen Peptide Synthesis

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Introduction

Even with standard methods of care, 55% of the 2 million people diagnosed with cancer this year will unfortunately pass. With this devastating statistic in mind, researchers and clinicians worked together to stray from one size fits all treatments and moved to precision medicine. Personalized immuno-oncology utilizes next generation sequencing technologies (NGS) to determine the genome of a patient's tumor in order to identify unique biomarkers known as neoantigens which can be used to initiate an anti-tumor immune response.

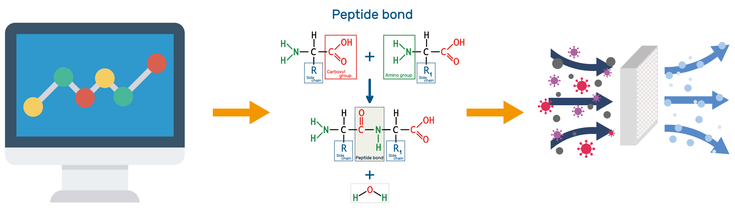
Regardless of the therapeutic avenue, one commonality linking all forms of neoantigen treatments is the requirement for reliable neoantigen peptides as they are the only reagent required for therapeutic discovery, development, and efficacy screening. However, neoantigen peptides are commonly quite difficult to be synthesized as they can be highly hydrophobic, vary significantly in length and charge, and have a strong tendency to aggregate. Therefore, neoantigen peptide synthesis can be a significant bottleneck in the timeline of clinicians generating personalized immunotherapies. In order to help the neoantigen community circumvent this bottleneck, GenScript has launched their neoantigen peptide synthesis platform featuring their proprietary NeoPre™ algorithm which identifies synthetic difficulty and recommends the best synthetic technology based on each individual peptides intrinsic characteristics. On top of NeoPre™, we have developed our platform for difficult peptide synthesis, utilizing our HiSyn, advanced coupling and purification platforms. This poster will review the technologies behind GenScript's novel platform to achieve a 95% success rate for difficult peptide synthesis.

What Makes Neoantigen Peptides so Difficult to Produce?

Quality	Description	Difficulty
Length	The number of amino acids within a peptide	Neoantigen peptides range from 8-50 amino acids. Peptides over 20 AA are difficult to synthesize
Charge	The isoelectric point (pI) of a peptide chain	Many purification steps only work with specific charges, the further from neutral a peptide is, the synthesis will be more difficult
Hydrophobicity	Amino acids which naturally repel water due to their charge	The more hydrophobic a peptide is, the more difficult purity and synthesis will be due to its insolubility in water
Yield	The amount of one peptide in weight (mg)	It is very difficult to find a provider who can generate a range of yields for difficult peptides
Purity	The percentage of an individual peptide in a solution of peptides	The charge and hydrophobicity (solubility & aggregation) make standard purification difficult

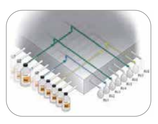
How Does GenScript Get Around These Difficulties?

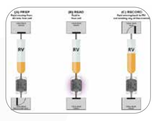
Streamlined Workflow



Industry-leading Instruments


Rapid Heating


Matrix Liquid Transfer Technology


UV Monitoring


Super Vibration & Inert Gas Protection

Results

GenScript has developed NeoPre™, a predictive algorithm which is able to determine peptide synthesis difficulty through machine learning (LinearSVM). NeoPre™ analyzes six intrinsic characteristics of each peptide in order to predict synthetic difficulty, including length, yield, charge (pI), aggregation, hydrophobicity, and purity. NeoPre™ can then recommend the most efficient approach to successfully synthesize peptides using one of GenScript's many synthesis platforms.

Length	Yield	Purity	pI	Aggre-gation	Hydropho-bicity	Prediction	Protocol
27	20mg	>98%	7.4	24.4	-0.25	Difficult	Automatic synthesis
25	4mg	>98%	10.2	52.2	0.65	Difficult	Mankind handling with improved coupling reagent, monitored under all process, involving Mid-control process to detect MS results
25	24mg	>98%	8.6	48.3	0.15	Difficult	Semi-automatic synthesis, monitored under all process to ensure coupling efficiency. "O-Acyl isopeptide method" for peptide synthesis
25	4 mg	>98%	6.4	39.3	0.05	Difficult	R&D

Example Read Out of NeoPre™. NeoPre™ will analyze individual peptide sequence and assign numerical difficulty values for aggregation, isoelectric point (PI), and hydrophobicity before determining synthetic difficulty and methodology.

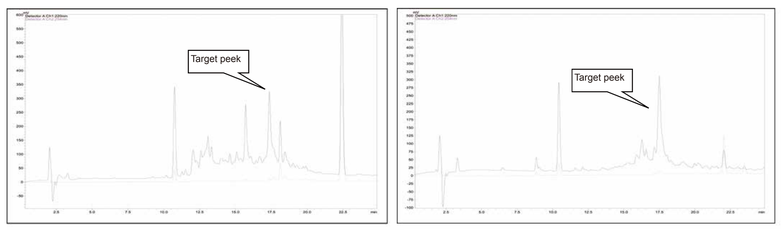
	Total	Delivered	Success rate
Total	3665	3528	96.26%
Difficult	1673	1593	95.22%
Easy	1992	1935	97.14%

Success Rate of Peptide Synthesis Using NeoPre™. GenScript has >96% success rate for neoantigen peptide synthesis utilizing our NeoPre™ bioinformatics tool. Overall, we were able to synthesize 95% of predicted difficult peptides.

GenScript offers two synthetic technologies specific for hydrophobic peptide production on top of our standard five synthetic methodologies in order to reach out 95% success rate for difficult peptides. One of them is our HiSyn technology which utilizes an optimized reaction temperature and condensation reagents to increase coupling efficiency. GenScripts advanced coupling technology uses coupling reagents from our HiSyn technology in optimized reaction conditions for our microwave synthesis method. GenScript is the first CRO in the field who can apply such optimized reaction technology utilizing microwave technology.

	Attempted	Synthesized	Synthesis success rate
Total	234	206	88%

Increasing The Success Rate of Difficult Neoantigen Peptides Using HiSyn. Utilizing advanced coupling methods, HiSyn is a manual technology which has allowed GenScript to synthesize 88% of previously un-producible peptides!

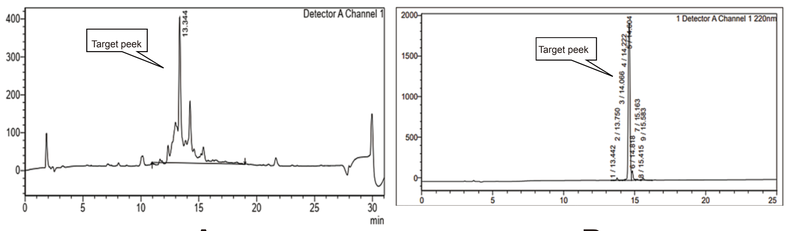


A B

Increasing Purity of Peptides Using HiSyn. The same peptide was unable to be synthesized at 50 mg & 95% purity using our standard (A) technologies, however, utilizing HiSyn (B), we were able to produce the peptide at the required yield & purity as shown through the comparing HPLC chromatograms.

	Total	QC Passed	Synthesis Success Rate
Advanced Coupling	385	342	88.83%

Increasing The Success Rate of Difficult Peptides Using Advanced Coupling. Utilizing advanced coupling methods has allowed GenScript to synthesize 88.83% of previously un-producible peptides!



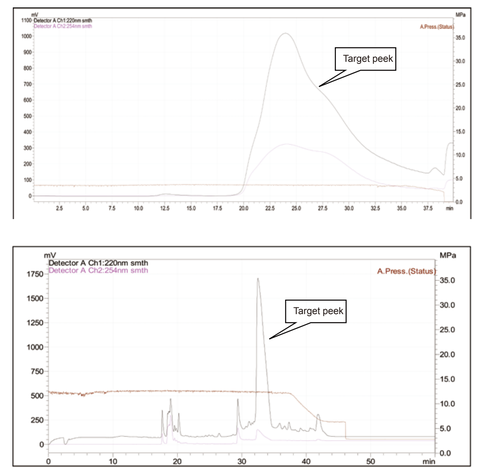
A B

Increasing Purity of Peptides Using Advanced Coupling. The same peptide was unable to be synthesized at 19 mg & 90% purity using our standard technologies (A), however, utilizing advanced coupling method (B), we were able to manufacture the peptide at the required yield and purity as shown through the comparing HPLC chromatograms.

Rather than relying on traditional peptide purification protocols, GenScript has optimized standard methods to enhance peptide purity. Based on our expertise in purifying both peptides and proteins of different intrinsic properties, we have designed an enhanced purification protocol utilizing numerous different reagents and purification columns. This allows us to reach peptide purities of even the most difficult neoantigen peptides of up to 98%.

	Total	QC Passed	Synthesis Success rate
Purification	67	58	86.6%

Increasing The Purification of Difficult Peptides Using GenScript's Optimized Purification Technology. GenScript's optimized purification methodology has allowed us to purify 86.6% of peptides which were not able to be synthesized at desired purity.



Increasing Purity of Peptides Using Optimized Technology. The same peptide was unable to be purified at 19 mg & 98% purity using our standard manual and automatic technologies, however, utilizing HiSyn, we were able to not only produce the peptide at the required yield, but also at the requested purity as shown through the comparing HPLC chromatograms.

Conclusion

With over 17 years of peptide production experience, GenScript's neoantigen peptide synthesis service offers the technical means & experience to synthesize large numbers of challenging neoantigen peptides and provide tailored optimization tools for your downstream applications, allowing to generate reliable neoantigen peptides for the personalized immunotherapy solution.