

# A Genetic Engineering Strategy for the Production of Cancer-Therapeutic Compound Libraries

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Immunotoxins are synthetic protein-based targeted therapeutic compounds that have recently shown great promise for cancer treatment. NL11-PSA is one such immunotoxin peptide that has been shown in preliminary testing to exhibit selective toxicity to cancerous cells overexpressing the ERBB2 receptor. Current methods for generating mutant libraries of immunotoxins are costly and inefficient, hindering immunotoxin-based drug development. This project successfully develops and demonstrates a simple and industrially optimized strategy for building mutant libraries of small peptide-based immunotoxins via gene synthesis and DNA mutagenesis using NL11-PSA as a model. Small, overlapping oligo strands were combined in a PCR in order to synthesize the artificial gene coding for the NL11-PSA peptide. The resulting DNA was ligated into a cloning vector for isolation. After sequencing to confirm the synthesis method, the NL11-PSA insert was ligated into the pET-28b protein expression plasmid. The pET-28b plasmid containing the insert was transformed into BL-21 E. coli for production of the recombinant peptide. The peptide was purified and incubated on susceptible tissue culture cells and toxicity was assessed using phase-contrast microscopy. Cells incubated with the purified immunotoxin showed significant cytotoxicity over control samples, demonstrating the validity of the strategy. The data obtained in this study successfully demonstrates that peptide-based immunotoxins can be cloned and that recombinant bioengineering methods can be used to generate lower-cost libraries of active immunotoxin peptides, opening the door to development of peptide-based pharmaceuticals.