A New Twist on Antibody Library Engineering

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ABSTRACT

The precision of Twist Bioscience's technology allows for the sampling of large variant space with predefined base by base composition and ratio control. Other library fabrication methods, such as NNN and NNK, result in redundant codons that lead to poor control over diversity and uneven distribution of amino acids. Advancements in Twist's silicon-based DNA synthesis platform enables precise synthesis of high diversity domain libraries at uniform ratios. Every Twist library is validated by next-generation sequencing to determine variants that are present, which can confirm both positive and negative hits from screens.

WHAT CAN TWIST DO FOR YOU?

- ! Precisely controlled combinatorial diversity
- ! Ratio controlled amino acid distribution
- ! CDR length variation
- ! Codon usage control
- ! Base by base precision
- ! Avoid restriction sites and unwanted motifs
- ! Multiple germline scaffolds
- ! Library validation by next-generation sequencing

TYPES OF LIBRARIES: COMBINATORIAL & SITE SATURATION

Binary Substitutions Libraries

A single variant or wild-type at multiple positions

Humanization: minimizing immunogenicity risks and maximizing therapeutic potential

High Diversity Uniform Ratio Libraries

Uniform ratios at multiple positions

Comprehensive sampling of combinatorial diversity in a CDR

Ratio Bias: Multiple Substitutions Libraries

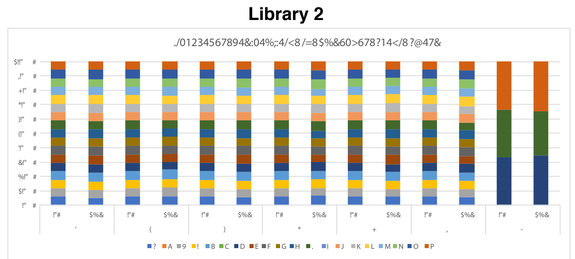
Ratio biased toward wild type amino acid with limited complexity at each variant position

Traditional method for reducing the number of overall combinatorial substitutions from wild-type. Twist precision individual substitution library provides a user-defined alternative with library composition consisting of all single, double, triple and/or more substitutions depending on domain diversity.

Library 1

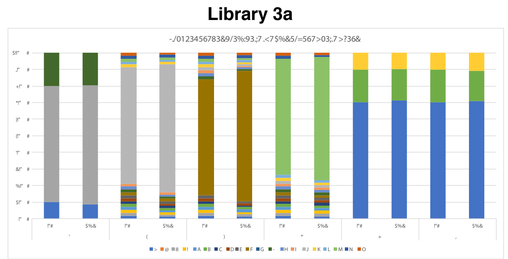
Wild-type: 50%, Variant: 50%

Library 2



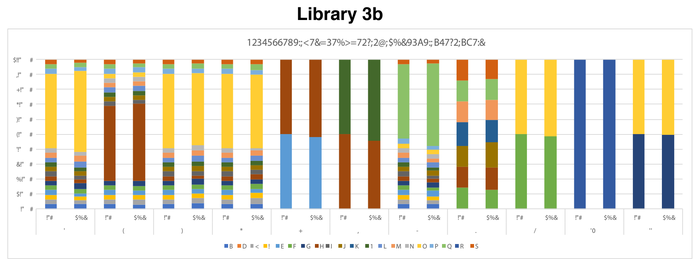
Wild-type: 5.3%, Variant: 5.3%

Library 3a

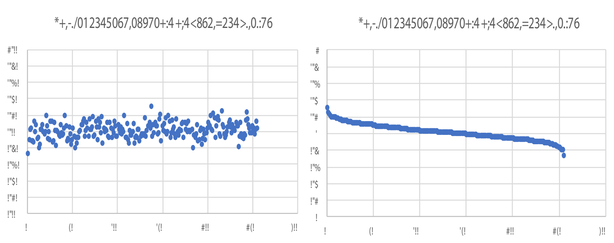


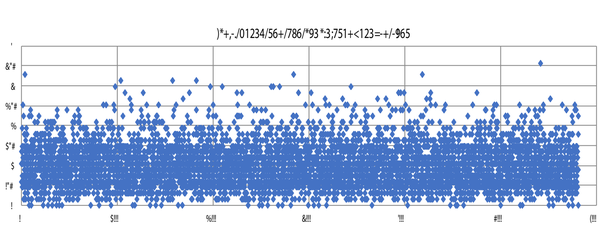
Wild-type: 70%, Variant: 1.8%

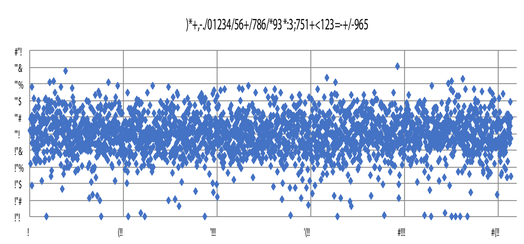
Library 3b

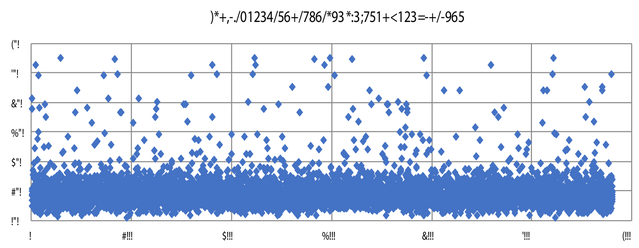


Wild-type: 50%, Variant: 3.1%









Length Variation Libraries

Explore variation in lengths and compositions of a domain

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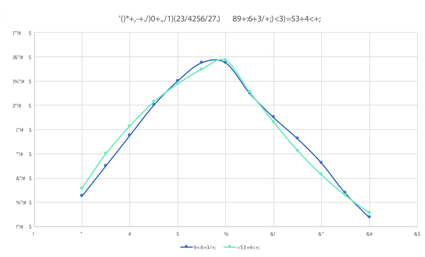
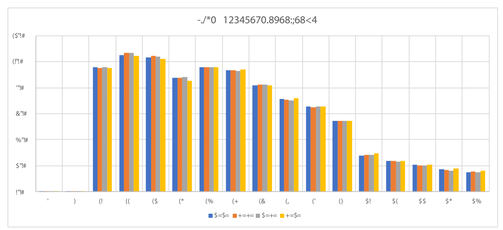
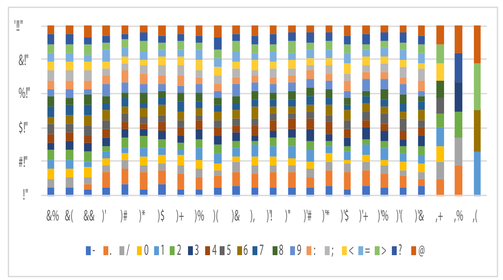




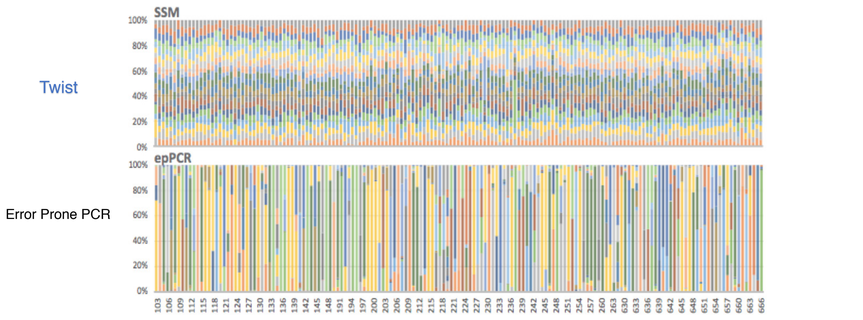


Twist Bioscience's DNA synthesis platform allows for the variation in both composition and length of variants at controlled ratios.

CDRH3 is often the domain of interest for length variation, either on its own or in combination with other CDRs and/or with multiple frameworks.

Site Saturation Libraries

Explore the sequence space one position at a time with precise control over codon usage

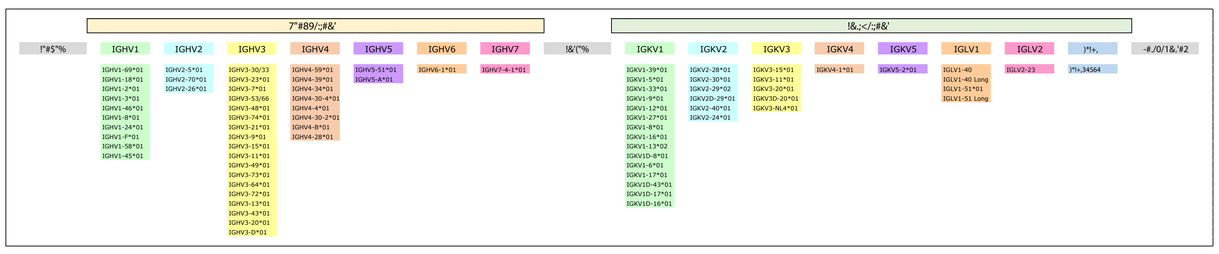


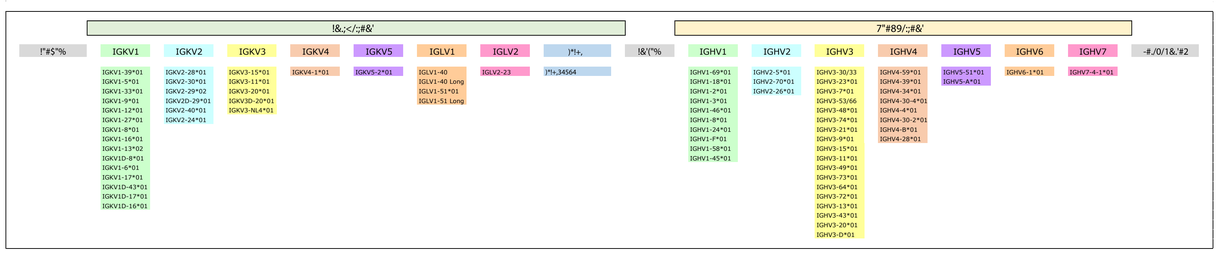
Site Saturation Libraries at Twist gives the user complete control over codon usage at each position with the choice of all 64 codons. Random mutagenesis via error prone PCR is a common tool used to generate variant libraries to investigate the relationship between sequence and protein structure and function. However, this method introduces deletions and frameshifts. Twist provides an alternative strategy to interrogate protein sequence with uniform representation of each amino acid.

Twist's Site Saturation Libraries provides unparalleled uniformity in a fast, cost effective manner.

SCAFFOLDS

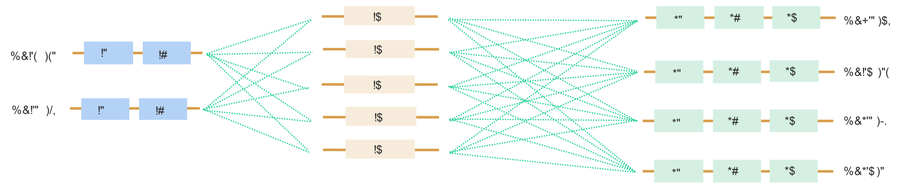
Our fabrication technology allows you to explore a larger variant space by creating similar libraries in multiple germline scaffolds or by mixing and matching multiple scaffolds with diversity cassettes.





Multiple Germlines

Discovery Libraries

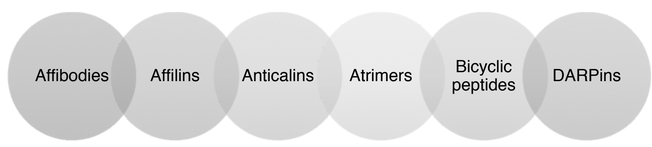


Non-human antibody scaffolds

Twist Bioscience's DNA synthesis technology can be applied to non-human antibody scaffolds such as Affibodies, Affilins, Anticalins, DARPins and Cathelicidins.

Accelerate the discovery of novel small protein therapeutics with controlled user-defined combinatorial libraries.

Domain Sized-Scaffolds



NEXT-GENERATION SEQUENCING (NGS) QC

- ! Know what you are screening
- ! Diversity less than 10^{10} can be incorporated in its entirety
- ! Each library is validated by NGS with >10^5 read coverage to ensure full diversity incorporation and uniform distribution of variants
- ! Negative screening results will be informative for the next design enabling an iterative exploration of antibody sequencing space