TWIST BIOSCIENCE

Fully Customizable Combinatorial Libraries for Focused Screening of Variants

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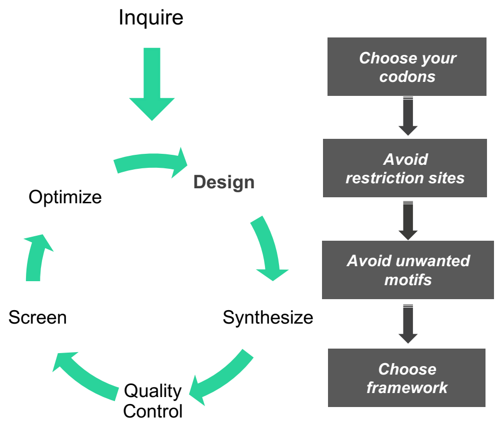
Abstract

Combinatorial DNA libraries are a collection of unique synthetic DNA sequences that are mutated simultaneously across targeted regions of a protein and assembled in precise ratios and combinations. These libraries can be cloned and propagated into microorganisms to perform high throughput screening for the identification of synergistic and beneficial mutations, that ultimately enhance organismal function. Combinatorial libraries are widely used in a variety of applications such as protein optimization (binding, stability, activity, and expression) via directed evolution or in vitro antibody affinity maturation via phage display libraries.

Scientist are often screening redundant sequences in their libraries, as a result of unavoidable side products generated by alternative fabrication technologies on the market. Utilizing Twist Bioscience's silicon DNA synthesis platform, precise mutations are individually synthesized and introduced via high quality oligo pools with low 1:2000 error rate and 90% within 5x of the Mean uniformity with no print limit. Coupled with our molecular biology expertise in gene synthesis and DNA assembly, Twist Bioscience can build full-length, highly diverse and precise libraries to maximize your screening capabilities.

Combinatorial DNA Libraries offered at Twist Bioscience are robust and uniquely customizable. By offering multiple combinatorial DNA library varieties, in-silico designs, codon optimization, NGS sequencing and numerous other customizations, we can help accelerate your design-test-build cycle.

Workflow



Inquire

Design

Synthesize

Quality Control

Screen

Optimize

Choose your codons

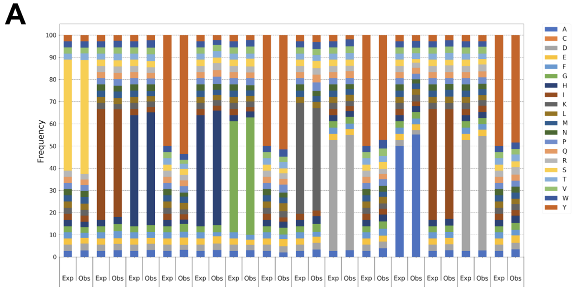
Avoid restriction sites

Avoid unwanted motifs

Choose framework

Domain Customization

A



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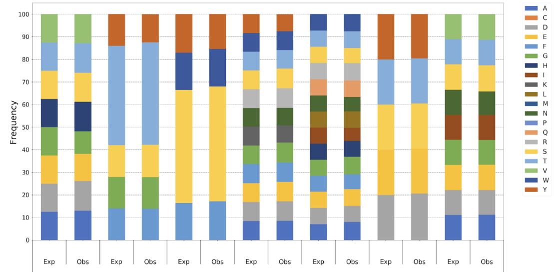

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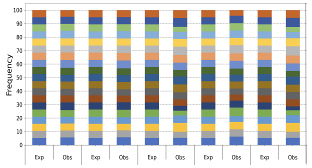

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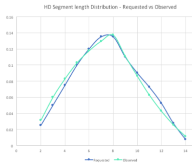
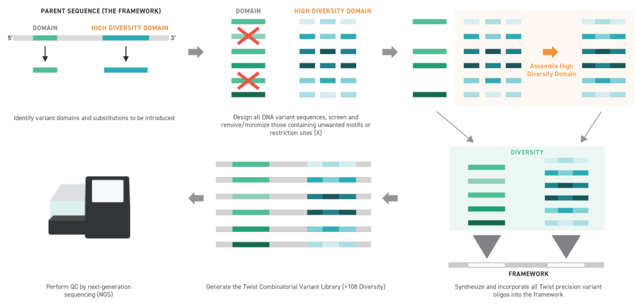


Length	Requested	Observed
2	2.5%	3.1%
3	5.0%	6.0%
4	7.5%	8.3%
5	10.0%	10.3%
6	12.0%	11.8%
7	13.5%	13.0%
8	13.5%	13.7%
9	11.0%	11.1%
10	9.0%	8.6%
11	7.3%	6.3%
12	5.3%	4.3%
13	2.8%	2.6%
14	0.8%	1.1%

Combinatorial Library Domains

(A) Wild Type Biased Ratio library. Traditional Method for reducing number of overall combinatorial substitutions from wild type. An alternative would be the precision library where users can select number of single/double/triple substitutions away from the wild type. **(B) Uniform Variant Library.** Equal Representation of each desired AA Change in each position with uniform distribution at all positions **(C) High Diversity Uniform Ratio Library.** Comprehensive sampling of combinatorial diversity in a domain **(D) Length Variation.** Exploration of variations in length at defined ratios and various amino acid profiles

In-Silico Removal of Liabilities



Identify variant domains and substitutions to be introduced

Design all DNA variant sequences, screen and remove/minimize those containing unwanted motifs or restriction sites (X)

Synthesize and incorporate all Twist precision variant oligos into the framework

Generate the Twist Combinatorial Variant Library (>108 Diversity)

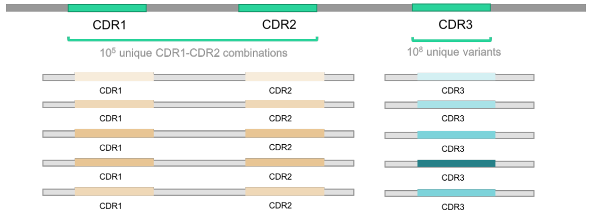
Perform QC by next-generation sequencing (NGS)

Motifs	Examples				
Glycosylation	NxS	NxP	NDS		
asparagine deamidation	NG	NS	NT	NH	
aspartate isomerisation	DG	DS	DT	DD	DH
Lysine glycation	KE	KD	KK		
Integrin binding aVb3	RGD	RYD	KGD	NGR	
Integrin binding a4b1	LDV				
Integrin binding a2b1	DGE				
CD11c/CD18 binding	GPR				
Fragmentation	DP	DQ			
Hydrophobicity	FF	FW	WW	WF	

Combinatorial Library Design and Synthesis with Twist

Sequence domains and frameworks identified for substitutions and all potential variation within those domains are designed. In-silico screening is done to remove unwanted motifs and restriction enzymes. Variants that pass design are synthesized and incorporated into framework to generate combinatorial library to be analyzed by NGS for quality control.

Leveraging Precision Synthesis



Define Explicit CDR1-CDR2 Pairings Along with Framework Mutations

Twist Bioscience uses machine learning tools to navigate through large sequence spaces. By coupling our silicon-based synthesis platform with our molecular biology expertise, we can effortlessly pair computational design with precision synthesis. Unique CDR1 & CDR2 combinations and framework mutations are identified from sequencing and machine learning tools can be incorporated with the high diversity CDR-H3, to create focused libraries. Our in-silico design platform can screen the libraries to avoid potential expression issues related to the display system and create opportunities for the desirable variants to be screened.

New Products

Spread-Out Low Diversity (SOLD) Library

A tool designed to enable the exploration of the sequence space encoded by mutating multiple positions simultaneously scattered across the parental sequence to generate a **diverse library of proteins for enzyme evolution, enzyme engineering, enzyme stabilization, protein engineering and protein stability**.

Standard CVL: Diversity is confined to **domains** along the WT sequence

SOLD Library: Diversity is **scattered** along the WT sequence

Cloned Oligo Pools

Cloned oligo pools enable the high-throughput screen of variants introduced from an oligo pool. Applications include sgRNA libraries, promoter and barcode libraries or single domain CDR3 libraries. Cloned oligo pools are amplicon sequenced to ensure tight uniformity and coverage with minimal dropouts. **(A/B) Post Cloning Distribution and GC Representation. (C) Read Counts before and after Cloning**