Redesign Your Research Funnel

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@TwistBioscience #WeMakeDNA

Introduction

What is a research funnel - and why does it matter?

Start with a large number of possibilities

Screen

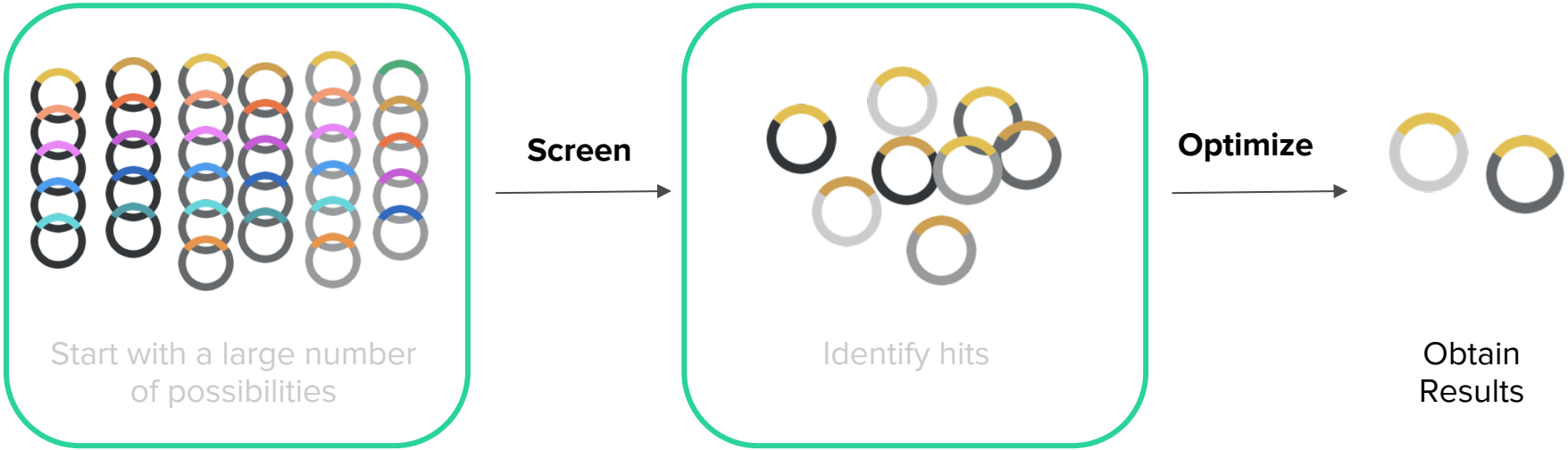
Identify hits

Optimize

Obtain Results

Eliminate Your Bottlenecks

How Twist can Help Redesign Your Research Funnel



"Too Expensive!"
"Hard to Build!"

"Too Complicated!"

TWIST BIOSCIENCE

Founded in 2013

San Francisco

South San Francisco

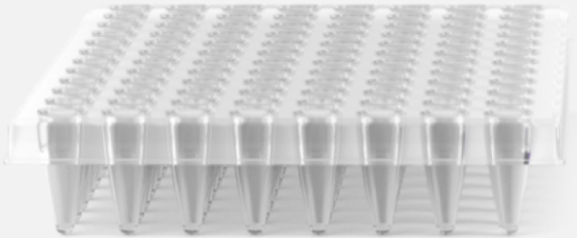
Tel Aviv

San Diego

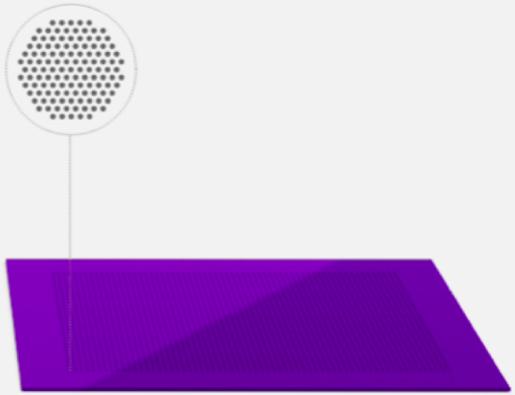
Singapore

Guangzhou

ReWriting DNA with the Power of Silicon



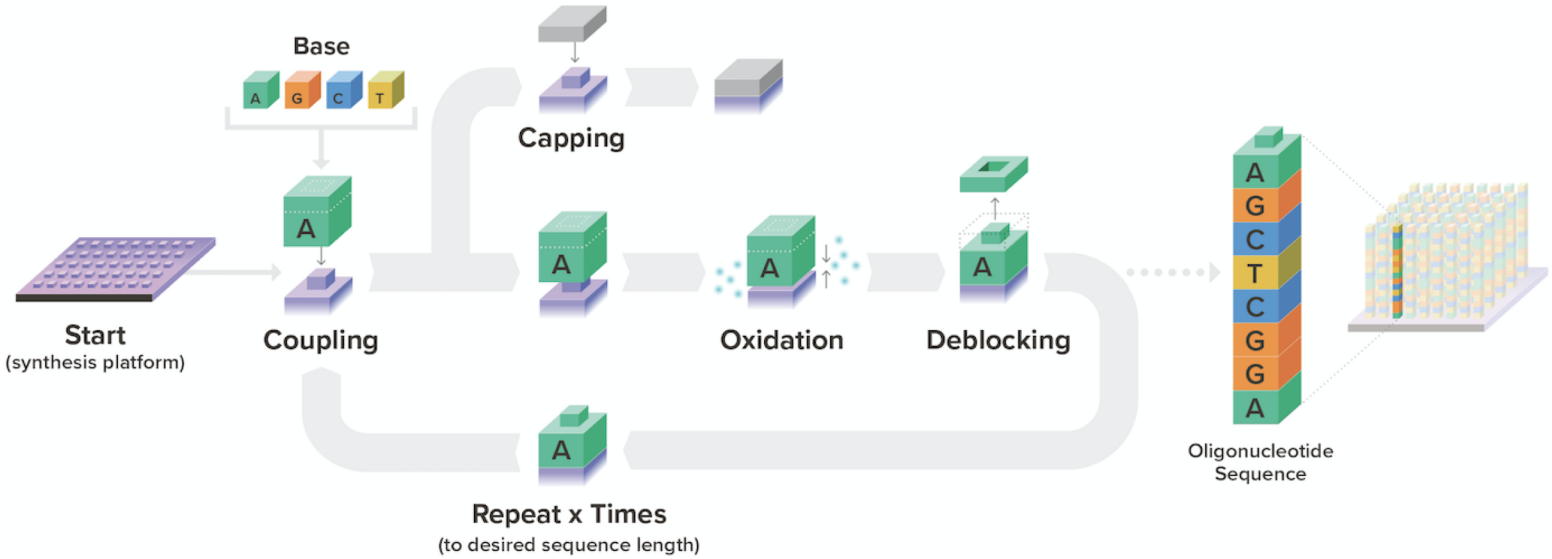
Traditional 96 WELL PLATE
makes 1 gene



TWIST SILICON PLATFORM
makes 9,600 genes

Developing Game Changing Throughput and Cost
through Quality and Speed at Scale

Oligonucleotide Synthesis



What can Twist do for you?

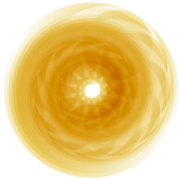
Precision DNA Synthesis at Scale

Genes

Oligo Pools

Libraries

NGS



Data Storage

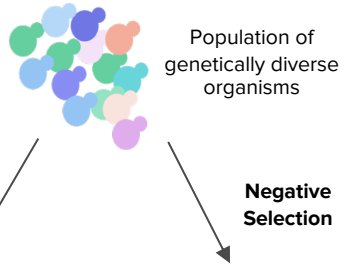
Defining a Research Funnel

Building a High Throughput Research Funnel

Challenge: There are too many potential inputs to test one by one.

Solution: Channel inputs through a research funnel starting with a high throughput (HTP) screen

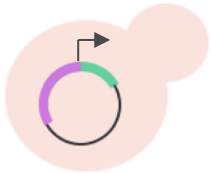
In Vivo Selection Screens



Isolate organisms and sequence.

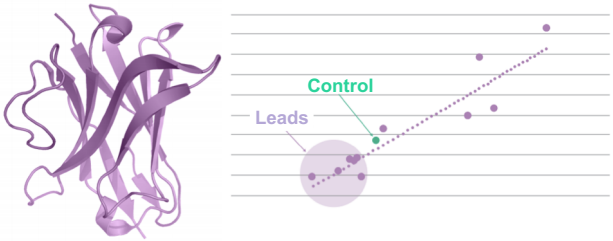
Sequence organisms before/after screen. Look for repressed populations

In Vivo Reporter Screens



Input triggers a measurable reporter, i.e. a fluorescent protein. Cells with measurable response are isolated and inputs are sequenced.

In Vitro Reporter Screens



Typically performed in 96 or 384 wp, inputs trigger a measurable colormetric or absorbance change.

"Hit" is a term commonly used to characterize inputs that perform well in the screen

Understanding the power of scale

Screen

Optimize

Starting pool diversity:

10^6 x 800bp dsDNA genes

100,000 x 175nt ssDNA

Cost of *de novo synthesis for each individual sequence*:

$7,200,000 (0.09c/bp)

Cost of a Twist Oligo Pool: ~$15,000

When designing your research funnel, it's important to know the different DNA tools available.

Redesign your Research Funnel

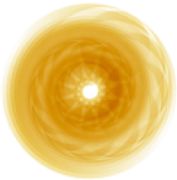
Goal: Identify the DNA tools appropriate for your funnel

Genes

Oligo Pools

Libraries

NGS

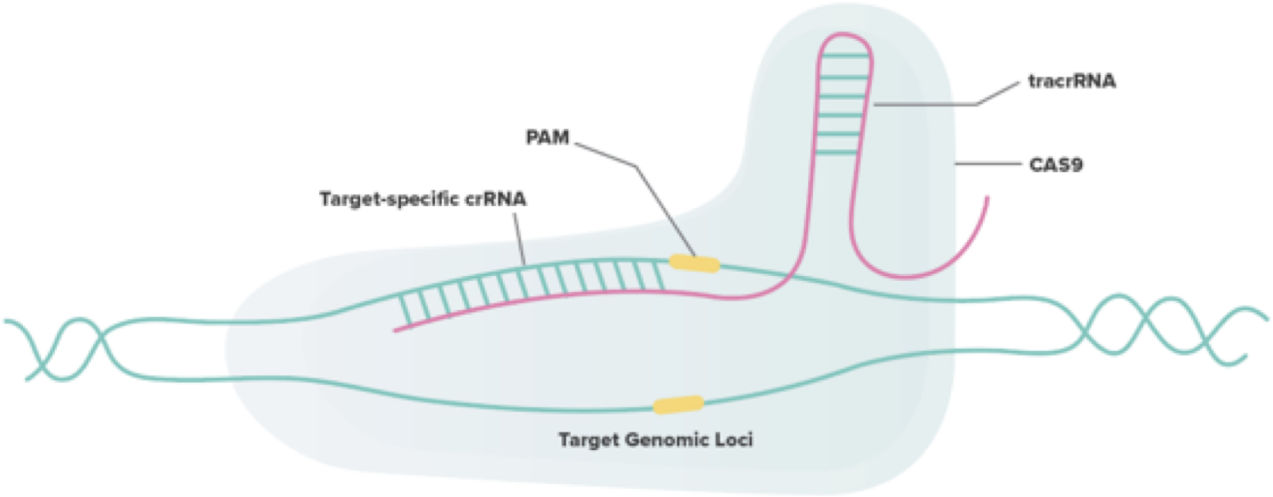


Data Storage

In this workshop we'll go through different DNA solutions for common research funnels.

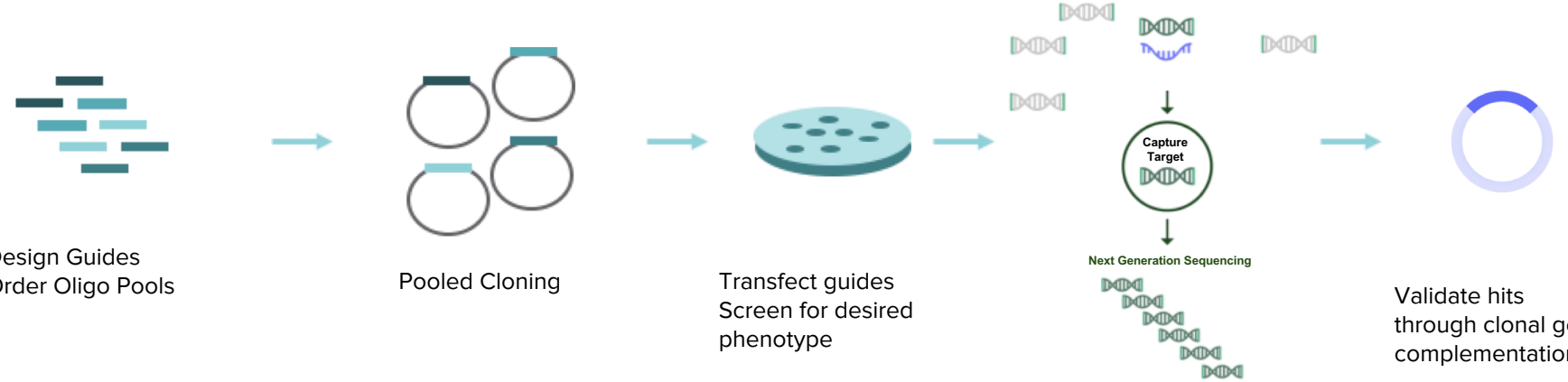
CRISPR Workflow

CRISPR enables precise DNA modifications



CRISPR is used to target specific sites in the genome for cutting or base modification. A guide RNA (gRNA) directs the CAS enzyme to the site of modification.

CRISPR Workflow



Design Guides
Order Oligo Pools

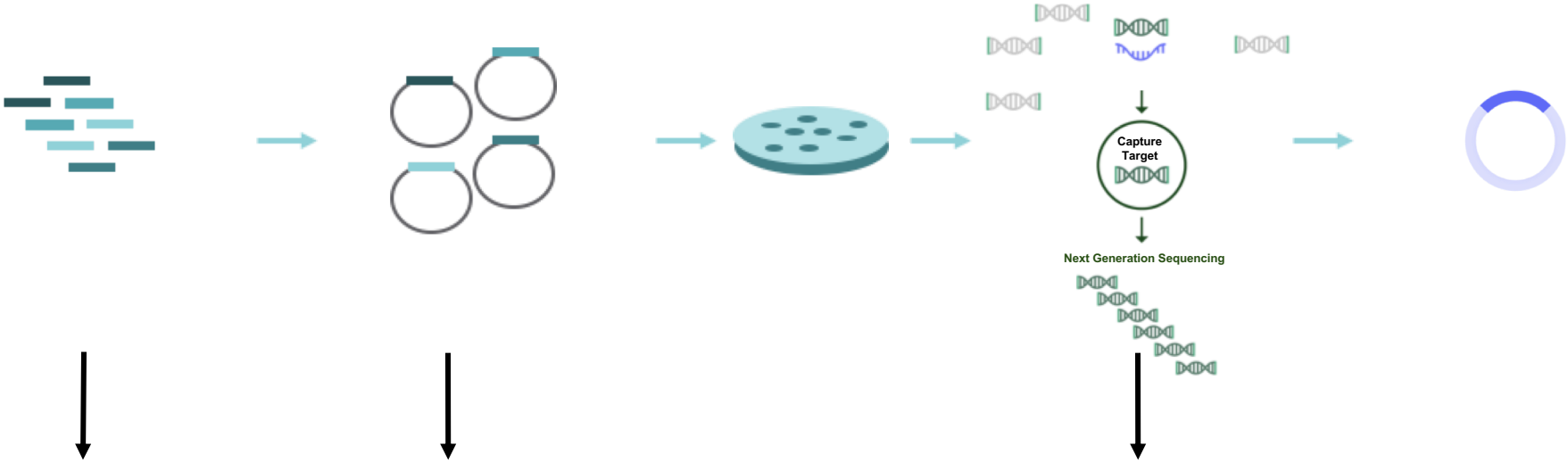
Pooled Cloning

Transfect guides
Screen for desired phenotype

NGS TE to ID mutations and off-targets

Validate hits through clonal gene complementation

Challenges with a CRISPR Workflow



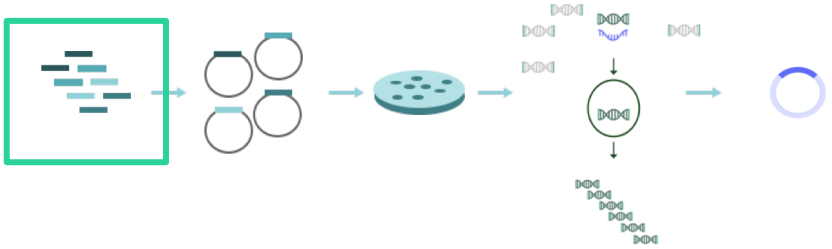
Hard to find access to highly diverse oligos with low error rates and high uniformity.

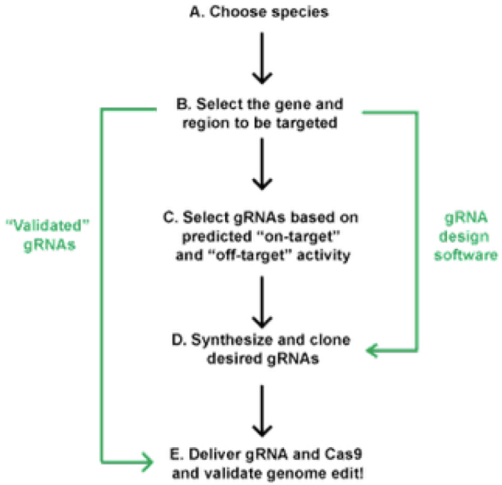
Amplifying and cloning oligo pools can lead to sequence uniformity bias.

Identifying on and off target mutations are time consuming, requires expensive sequencing, and lack comprehensiveness.

Step 1: Design Guide

Twist offers Oligo pools to simplify your workflow





Design: There are many online design tools to design guide RNA (gRNA) sequences.

Twist Handy Tip: To ensure efficient CRISPR targeting, design 3+ guides for each gene.

DNA Input: Use Twist Oligo Pools to code for gRNA sequences.

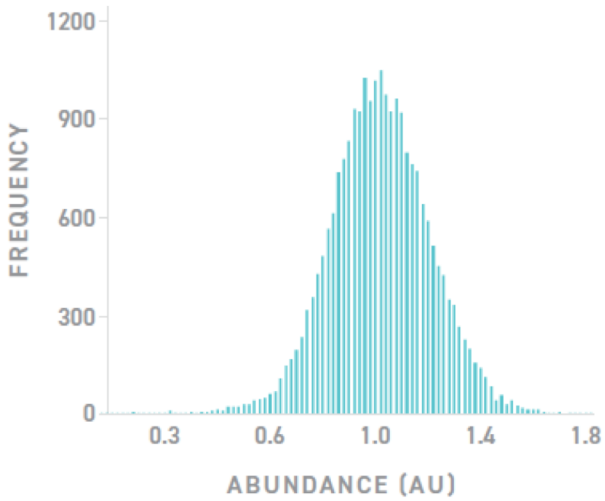
https://www.addgene.org/crispr/guide/

Oligo Pools - Uniformity and Error Rate Matter

Twist Oligo pools provide sequence flexibility with high uniformity

Flexibility matters.
Design your pool with the exact number of sequences you need.

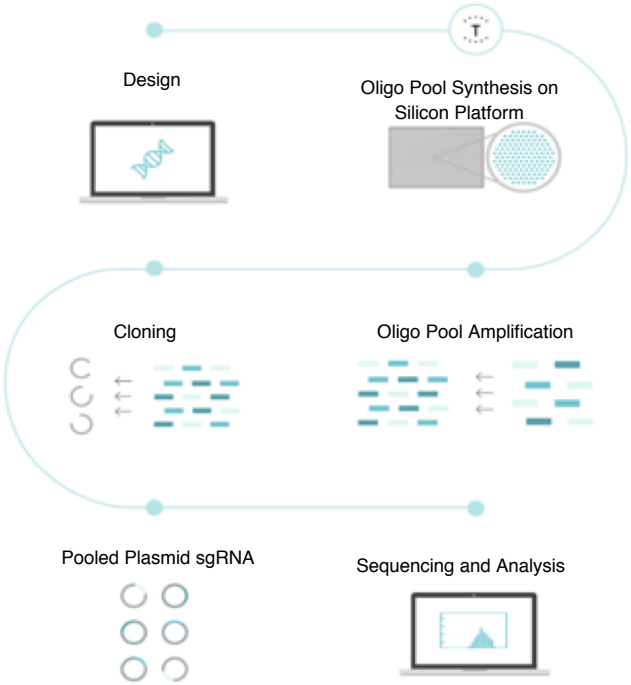
100% OLIGO REPRESENTATION
HIGH UNIFORMITY

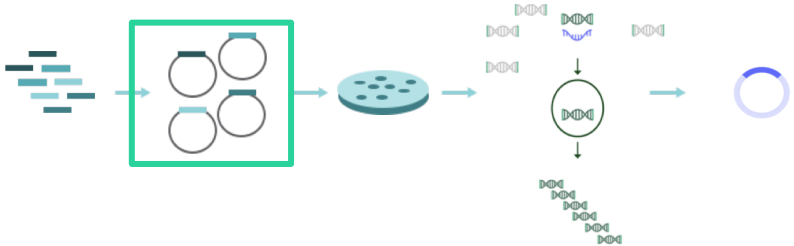


1:2000 BP ERROR RATE

Step 2: Pooled Cloning

Twist offers Cloned Oligo Pools to simplify your workflow





Potential Pitfall: Cloning gRNA into vectors for delivery is a step that can potentially introduce bias and can be time consuming.

Twist Handy Tip: Check out Twist's white paper on how to amplify pools to limit bias.

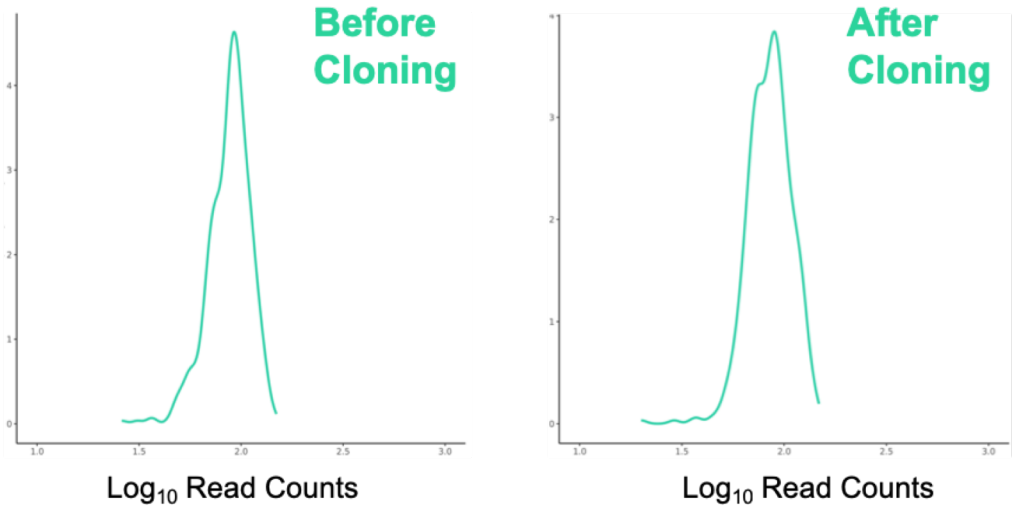
DNA Input: Use Twist's Cloned Oligo Pools. (Innovation Lab!)

Friends don't let friends clone.

Step 2: Pooled Cloning

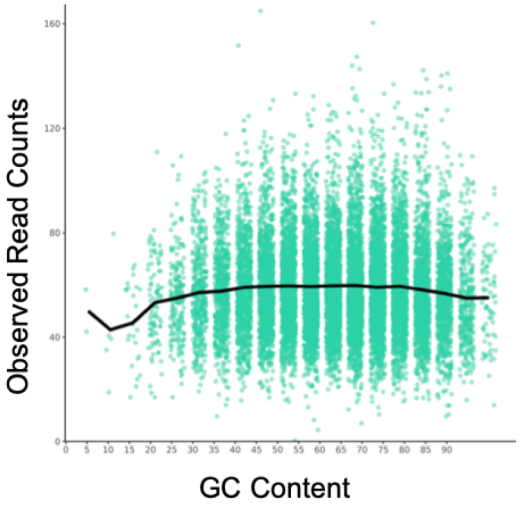
Twist's Cloned Oligo Pools maximize experimental efficiency, maintain diversity and ensure uniformity

Sequence Distribution



Uniformity is maintained before and cloning

GC Uniformity

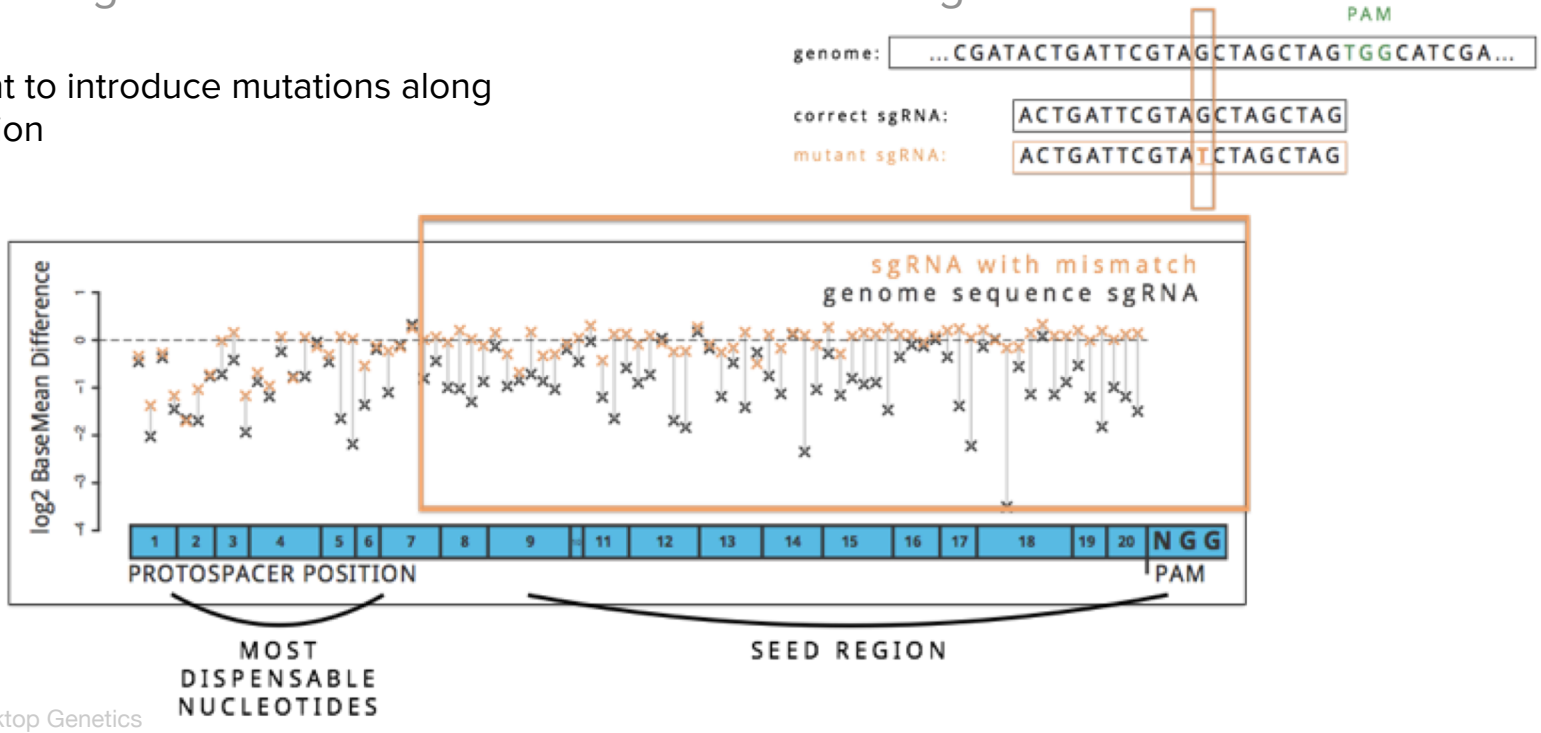


GC% does not impact uniformity

Importance of High Quality Synthesis

sgRNA containing mutations results in less efficient cutting

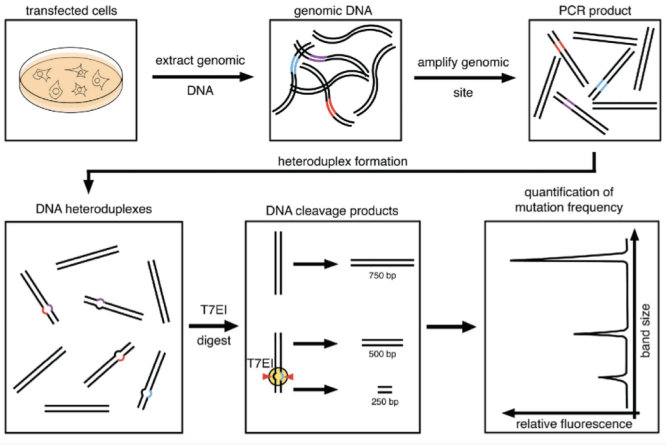
Tiling experiment to introduce mutations along protospacer region



Slide courtesy of Desktop Genetics

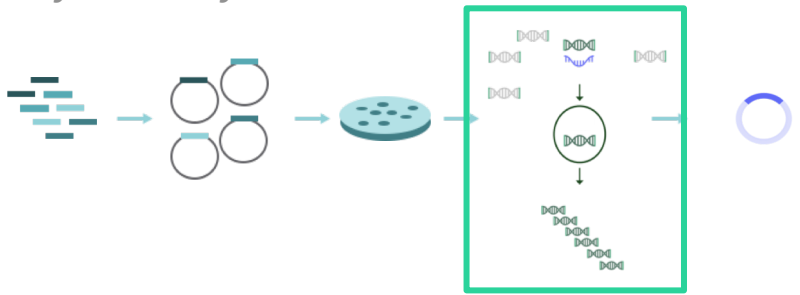
Step 3: Identifying On and Off Target Modifications

Twist's Target Enrichment Custom Panels can rapidly identify modifications



Unlike NGS Target Enrichment, other common methods for determining on and off target mutations are often limited in scope and highly complex.

DOI: 10.1002/0471142727.mb3103s112



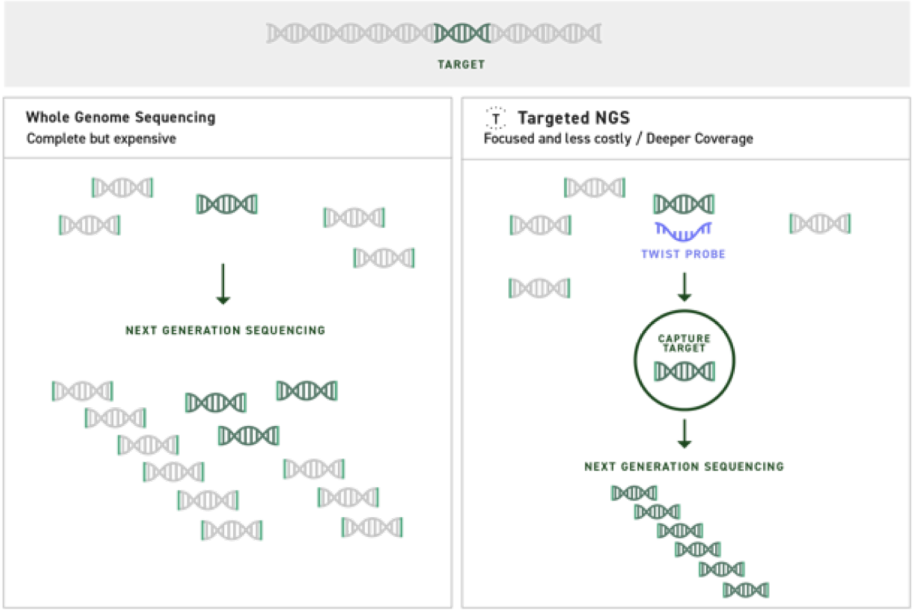
Potential Pitfall: While accurate, CRISPR systems can lead to off-target mutations at unpredictable sites.

DNA Input: Use Twist's Target Enrichment Kits.

Twist Handy Tip: Use the Exome kit or design your own custom panel

Twist provides a variety of Target Enrichment tools

From Exomes to Custom Panels - Twist has you covered



EXCEPTIONAL PERFORMANCE

- dsDNA probes, high capture efficiency
- Exceptional uniformity
- NGS QC of final probe library

GREATER FLEXIBILITY

- Easy customization, rapid optimization
- Scalable: small panels to large exomes
- Modular kits, seamless integration into any workflow

MAXIMIZED SEQUENCING EFFICIENCY

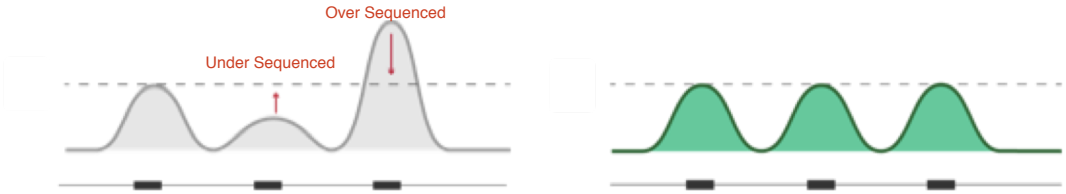
- Increased depth per sample or more samples per run
- Lower sequencing costs

Why design tomorrow's experiments with yesterday's tools?

Measuring the Quality of Target Enrichment

Uniformity: Fold 80 Base Penalty

Fold 80: how much more sequencing is needed to bring 80% of the bases to the observed mean value



Specificity: On Target Rate

Library Complexity: HS Library size and Duplication Rate

Twist's Target Enrichment Performance

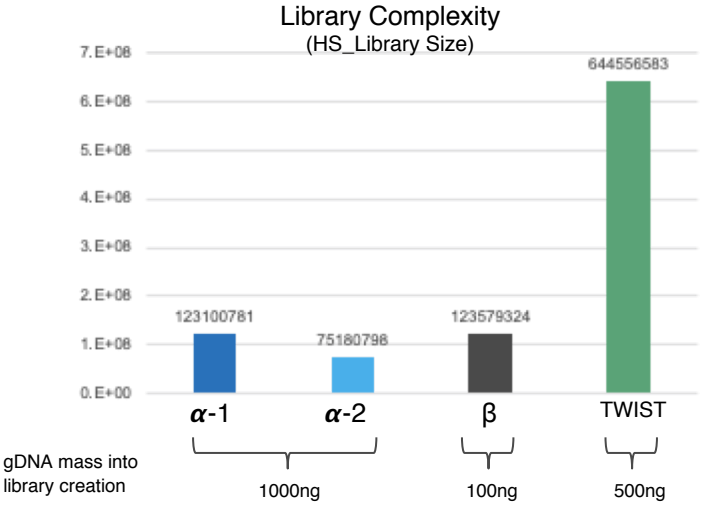
Recover more unique molecules with higher uniformity

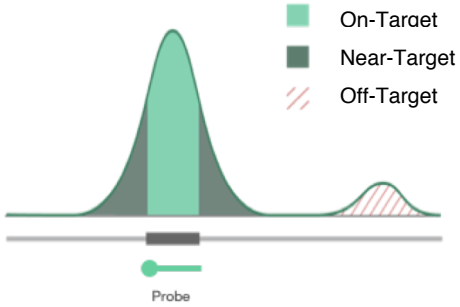
Implication of capturing fewer molecules

- High coverage affected by fewer molecules
- Duplicates are discarded from analysis
- Diminishing return on sequencing directly affects multiplexing

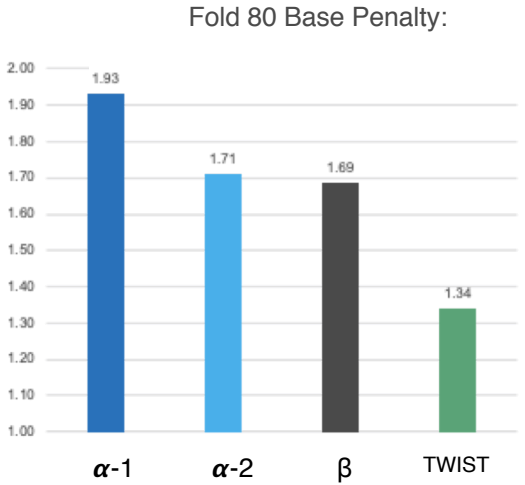
How does Twist capture more with lower Fold 80?

- Optimized probe panels through data driven
- A lower value depicts higher uniformity performance.
- Design-Build-Test-Learn cycle
- Finely tuned buffers and workflow





Twist uniformity increases target enrichment efficiency by 35-60% in comparison to competitor kits.



Challenges with a CRISPR Workflow

Hard to find access to highly diverse, oligos with low error rates and high uniformity.

Amplifying and cloning oligo pools can lead to sequence uniformity bias.

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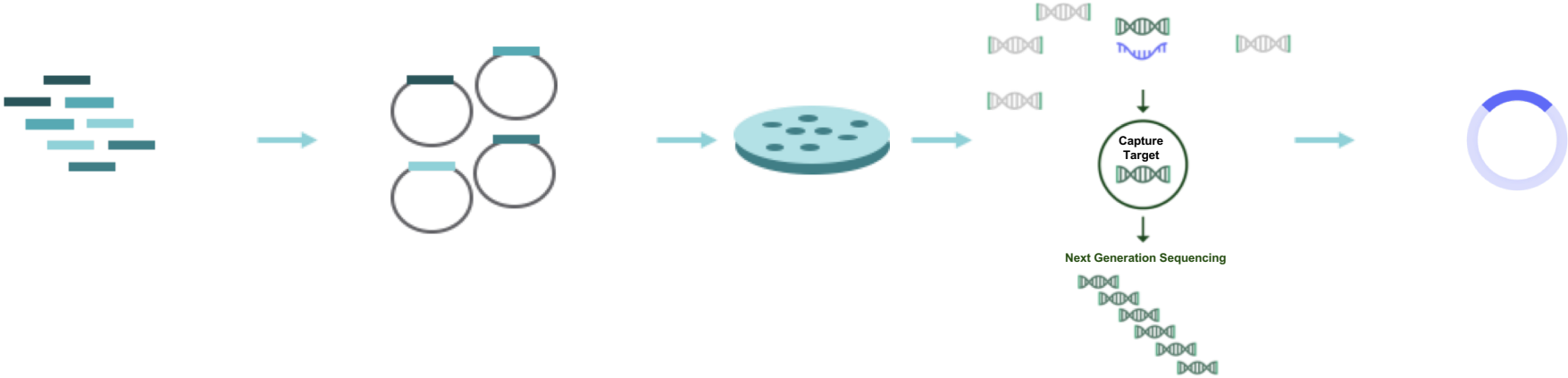
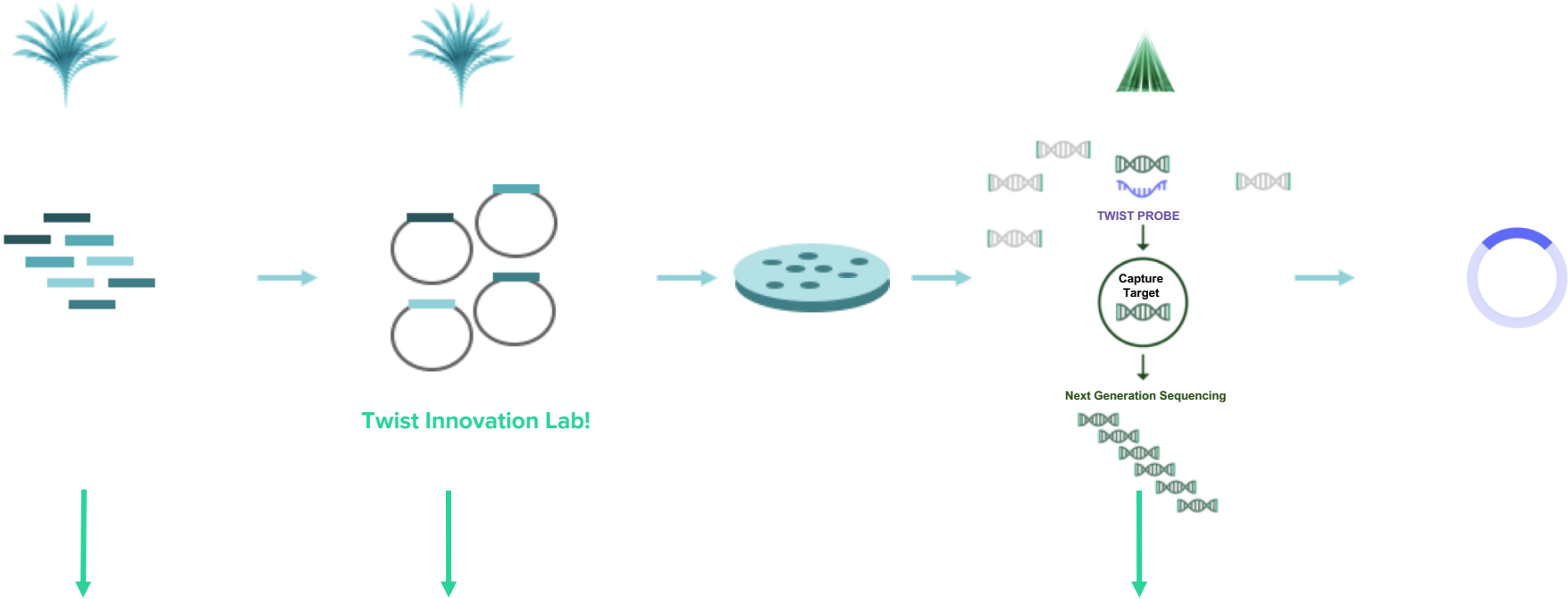


DNA Solutions for CRISPR Workflows



Twist Oligo Pools

Twist Cloned Oligo Pools

Twist NGS Target Enrichment

Protein Characterization

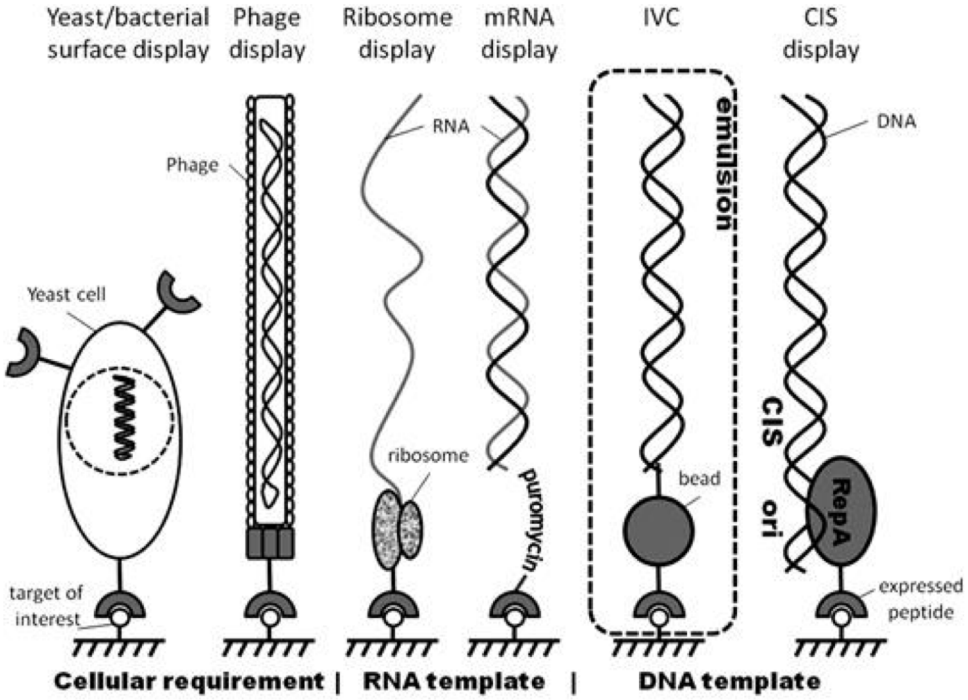
Protein Characterization

Peptide Displays allow for rapid protein screening

DNA inputs are transcribed and translated.

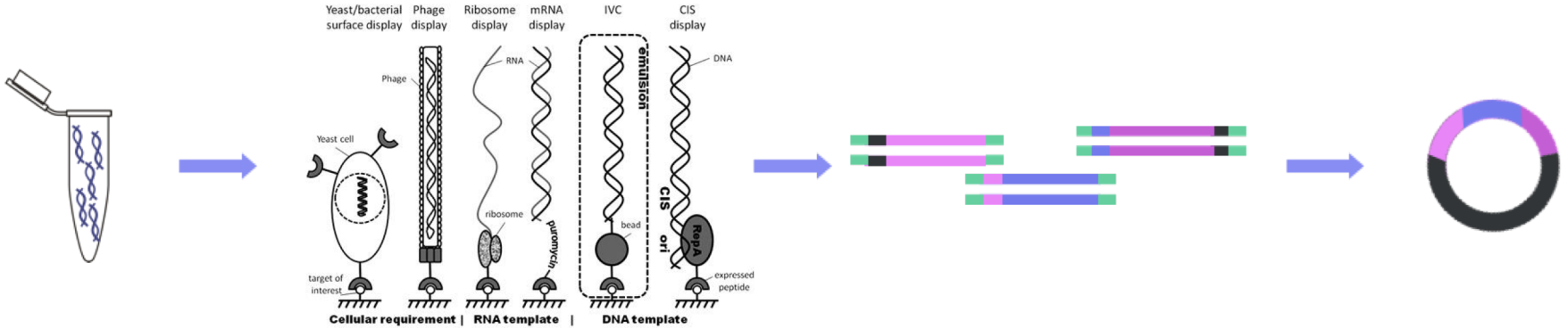
The subsequent peptides can be used for characterizing:

- Protein:Protein Interactions
- Protein:Substrate Interactions
- Enzymatic Functions



https://doi.org/10.1093/bfgp/elr010

Protein Characterization



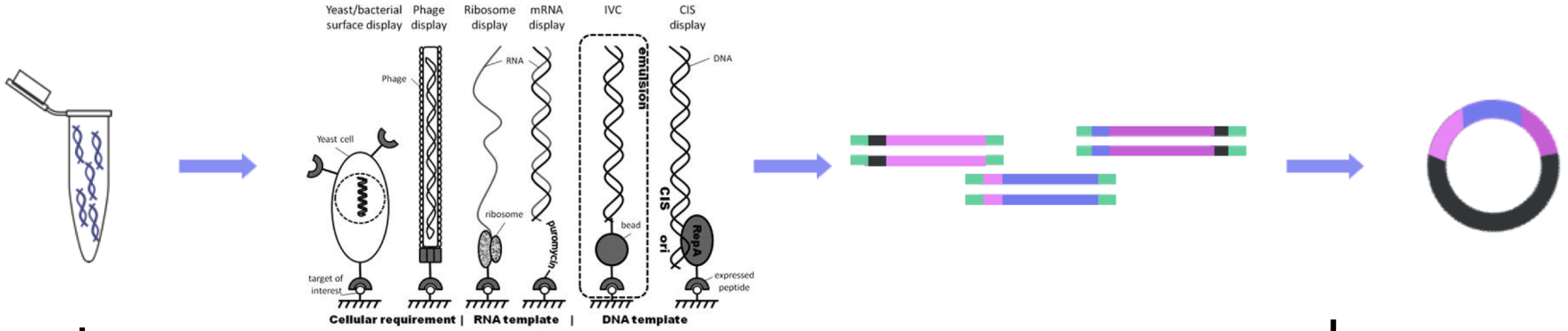
Diverse Collection of Genes

Screen with *in vivo* or *in vitro* display

Gene Fragments

In House Cloning

Protein Characterization

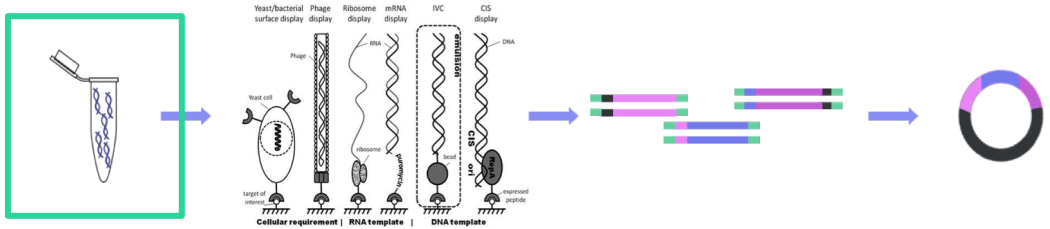


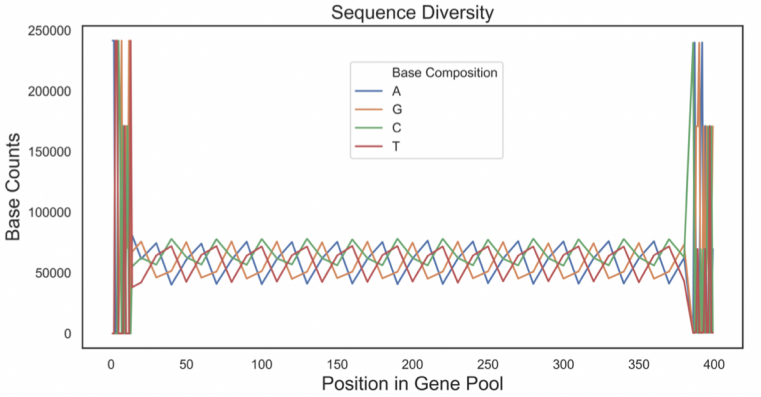
Diverse Peptide libraries are limited by cost of gene synthesis or length of oligo synthesis

Current DNA assembly methods either lack efficiency, accuracy or are hard to scale with many sequences

Step 1: Generating a Peptide Library

Introducing Gene Pools





Example of a highly diverse gene pool of 400bp in length

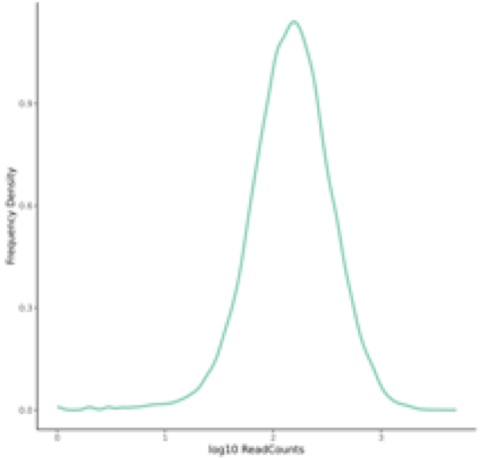
Potential Pitfall: Gene libraries are expensive to source input DNA. ssDNA oligonucleotide libraries can be assembled into longer genes, but it's complicated to construct and hard to QC.

DNA Input: Use Twist's Gene Pools

Gene Pools Code for Diverse Peptide Libraries

High-quality gene pools minimize wasted time and resources

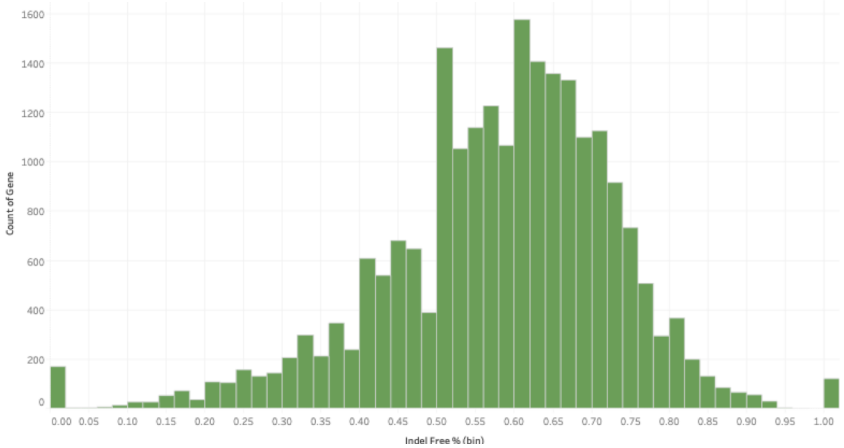
Sequence Uniformity



Maximize screening efficiency

Sequence Quality

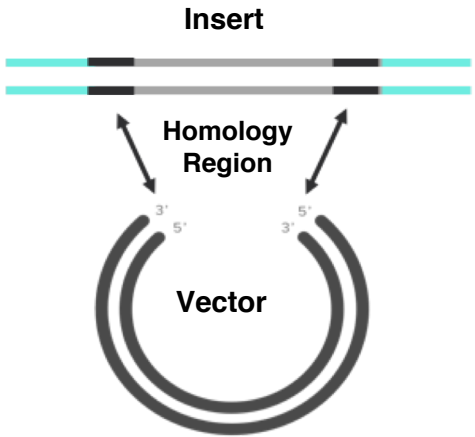
%Indel Free Sequences of 400bp Gene Pool



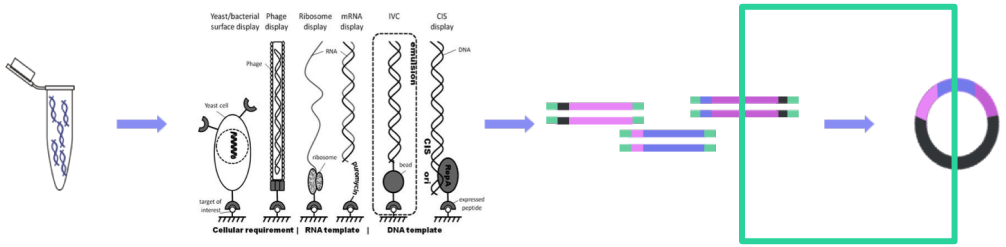
Access every sequence

Step 3: Cloning

Introducing NUGE Assembly



Next **U**niversal **G**uided **E**nzymatic Assembly: NUGE



Potential Pitfall: Seamless Assembly Cloning methods either lack accuracy or aren't efficient enough.

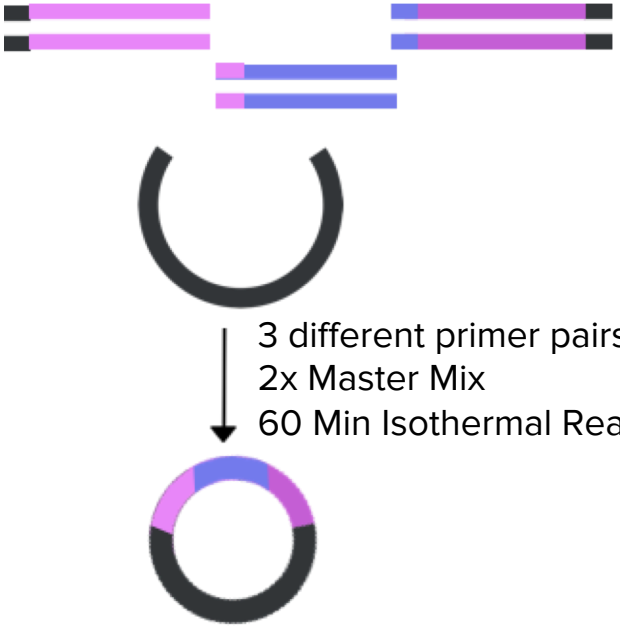
DNA Assembly Tool: NUGE Cloning Mix

Twist Handy Tip: Universal Adapters on Twist's Gene Fragments can be used as PCR primer binding sites.

NUGE: A Cloning Mix for Twist Fragments

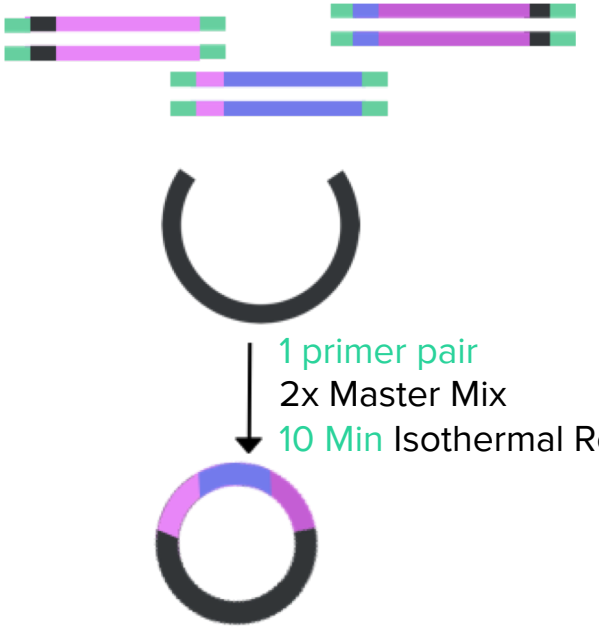
The NUGE is a drop in replacement for currently available cloning methods.

Current Seamless Assembly Methods



3 different primer pairs
2x Master Mix
60 Min Isothermal Reaction

NUGE

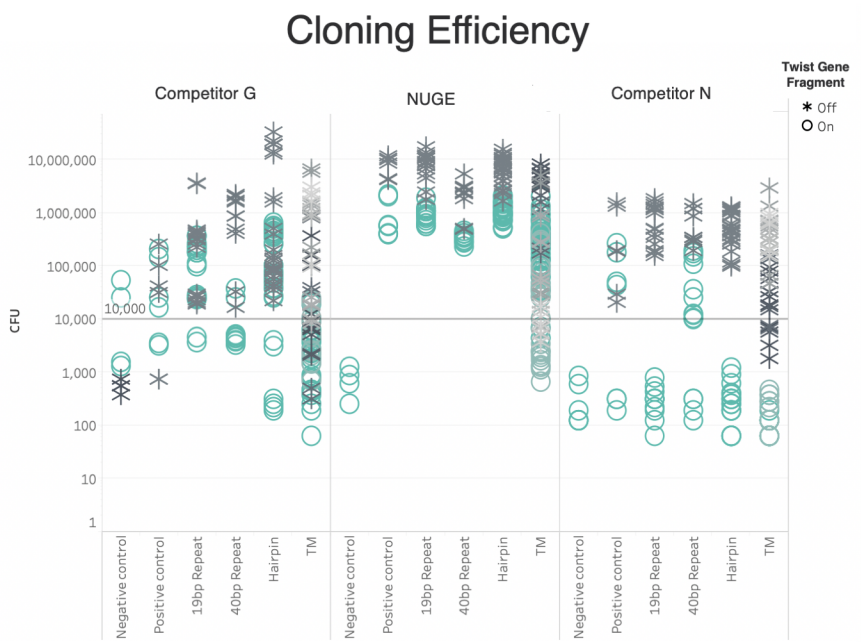


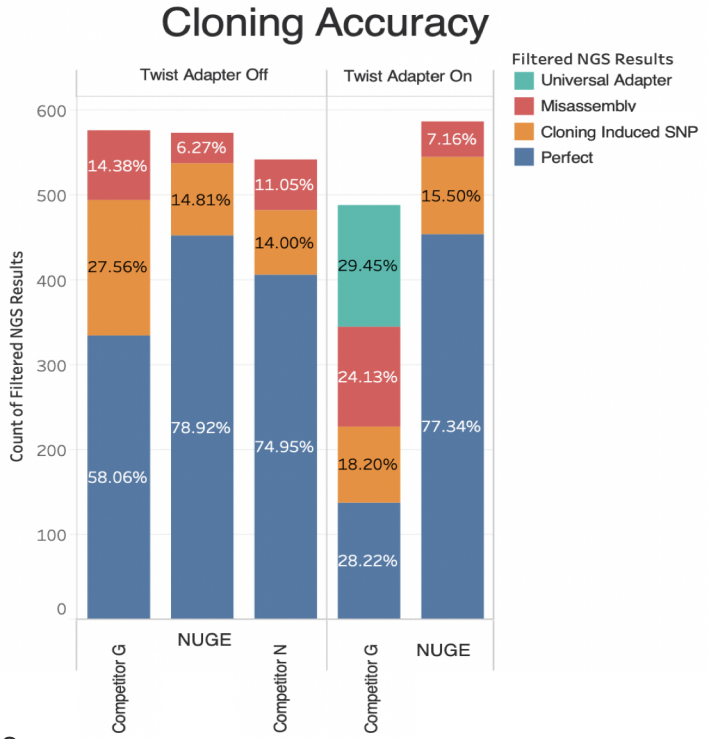
1 primer pair
2x Master Mix
10 Min Isothermal Reaction

NUGE Assembles Difficult Sequences Accurately

The NUGE is capable of assembling 10 fragments together in a single reaction

NUGE Assembles Difficult Sequences Accurately

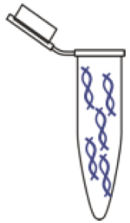


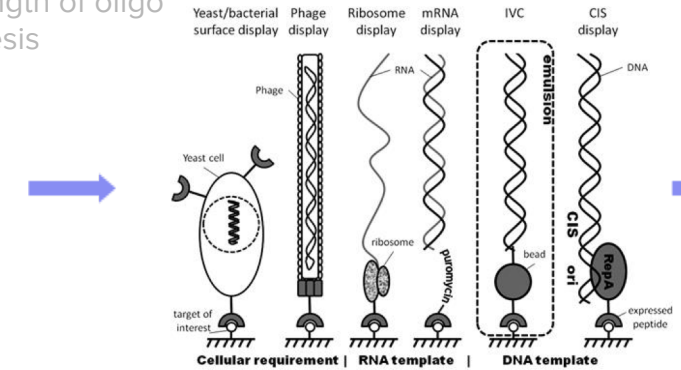


Conclusion: NUGE is as accurate and more efficient than current methods.

Challenges with Protein Characterization

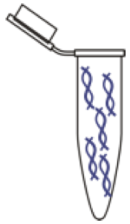
Diverse peptide libraries are limited by cost of gene synthesis or length of oligo synthesis





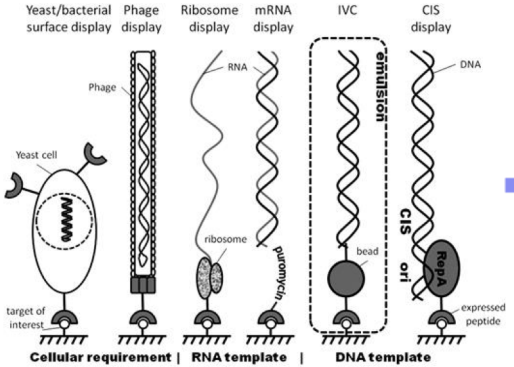
Current DNA assembly methods either lack efficiency, accuracy or are hard to scale with many sequences

DNA Solutions for Protein Characterization



Twist Innovation Lab!

Twist Gene Pools



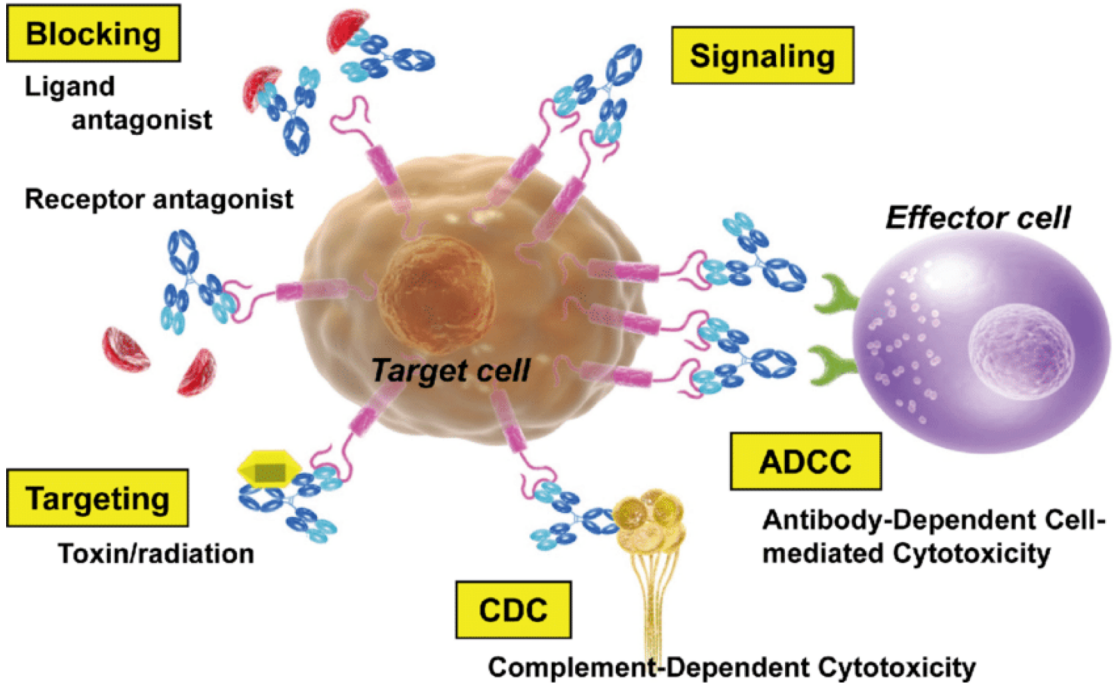
Twist Innovation Lab!

Twist NUGE cloning mix

Therapeutic Biologics Workflow

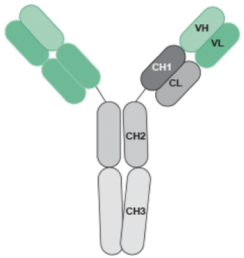
Therapeutic Biologics Background

Therapeutic Biologics like antibodies are the core of many promising treatments.



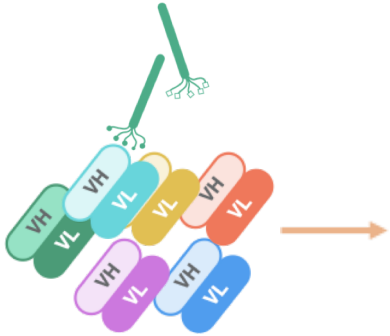
J Toxicol Pathol. 2015 Jul;28(3):133-9. doi: 10.1293/tox.2015-0031.

Therapeutic Proteins



Protein Target

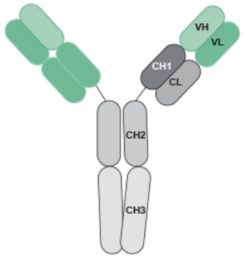
Design protein variants
DNA libraries

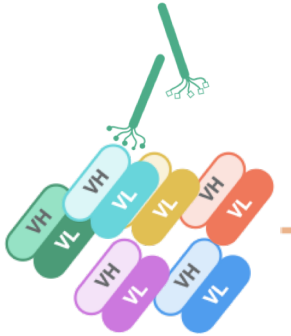


Phage display for
binding to
therapeutic target

Characterize
hits with
clonal gene

Challenges with Therapeutic Protein Screens



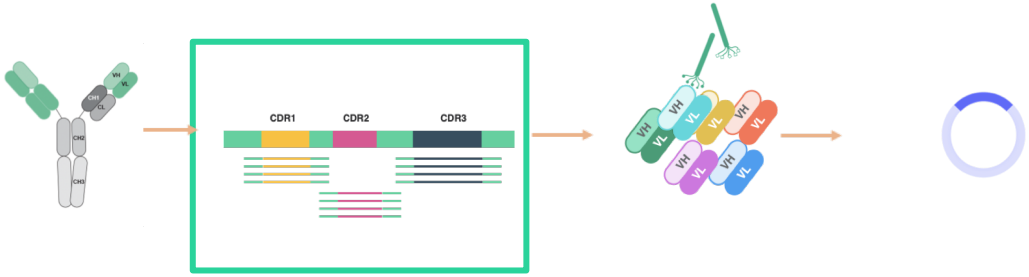


Creating highly diverse, uniform variant libraries are complicated and time consuming to create.

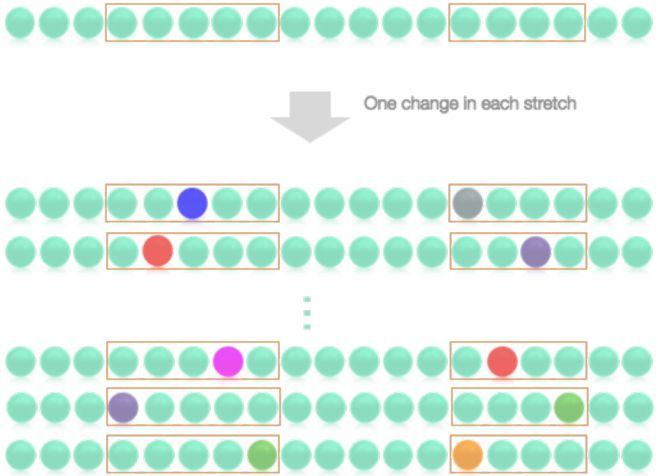
Gene synthesis is often too costly to screen many hits

Step 1: Design your Diversity

Twist's Library Offering



WT



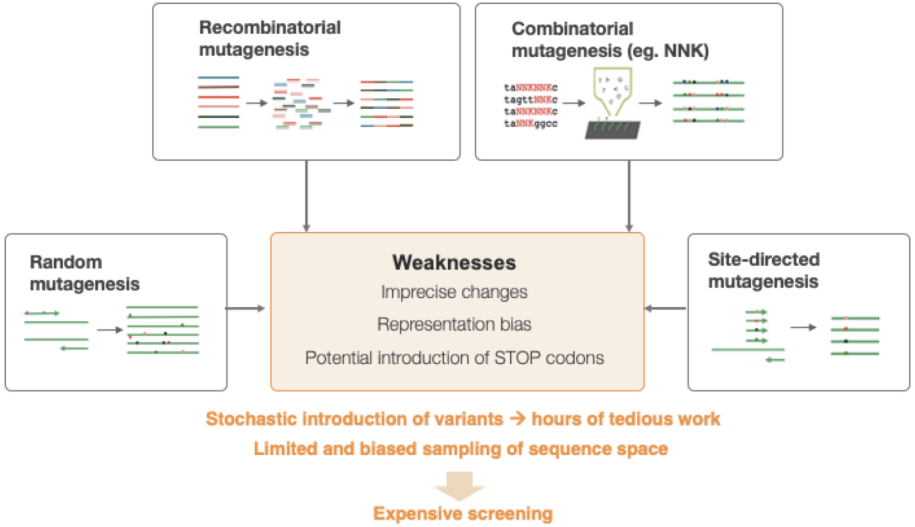
Multiple Site Mutagenesis

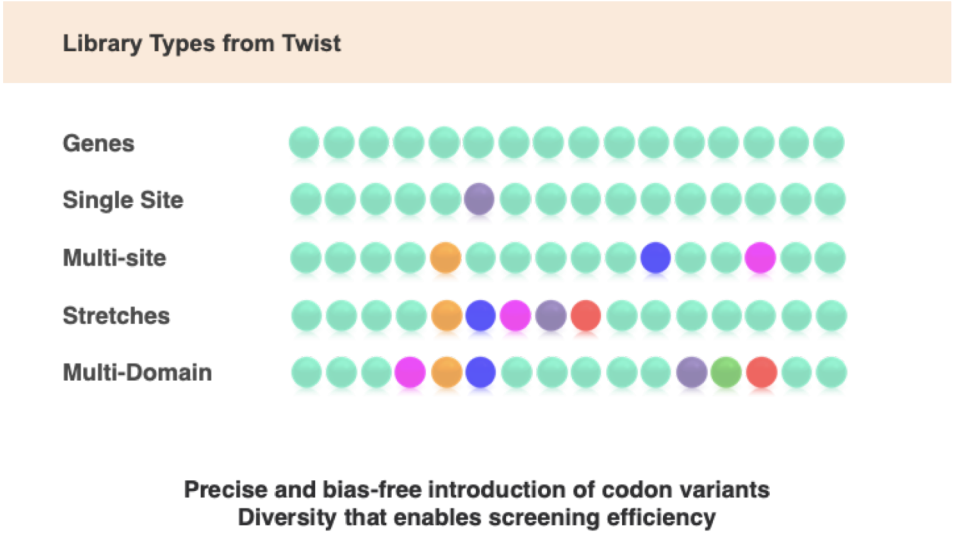
Potential Pitfall: Current methods like error prone PCR lead to unknown variants or large fraction of empty vectors.

DNA Input: Use Twist's Combinatorial Variant Libraries (CVL)

Precisely Controlled Library Fabrication

Twist Combinatorial Libraries eliminates compromises.

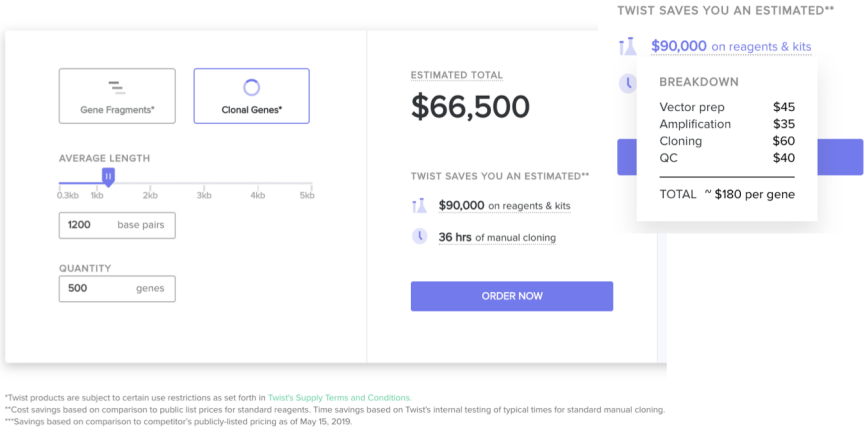




Verified library composition: Negative data yields useful information

Step 3: Validate Your Hits

Twist's clonal genes saves time and costs



*Twist products are subject to certain use restrictions as set forth in Twist's Supply Terms and Conditions.
**Cost savings based on comparison to public list prices for standard reagents. Time savings based on Twist's internal testing of typical times for standard manual cloning.
***Savings based on comparison to competitor's publicly-listed pricing as of May 15, 2019.

Potential Pitfall: Due to high cost and time, scientists often only pick a subset of their antibody candidates to produce.

Twist Handy Tip: Codon optimization can be used to enhance expression in final host cell.

DNA Input: Use Twist's Clonal Genes

Friends don't let friends clone.

Validating hits with clonal genes

Gene Fragments

Fragments with industry leading error rate

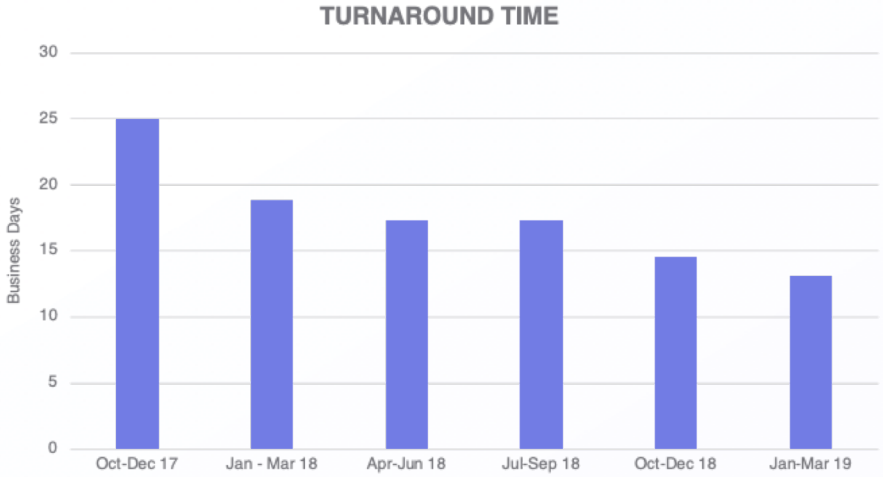
Clonal Genes

Sequence perfect genes cloned into a Twist stock vector or a vector of your choice

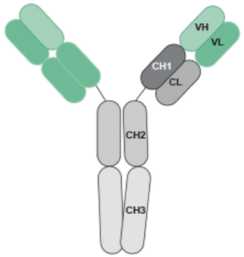
Perfect Sequence for 1 or 10,000 Genes, or More

- **YOUR GENES, YOUR WAY**
- **INDUSTRY LEADIING PRICE AND TURN AROUND TIME**
- **SCALABLE SYNTHESIS**

Twist is Constantly Iterating



Challenges with Therapeutic Protein Screens



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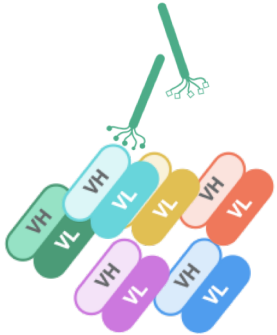
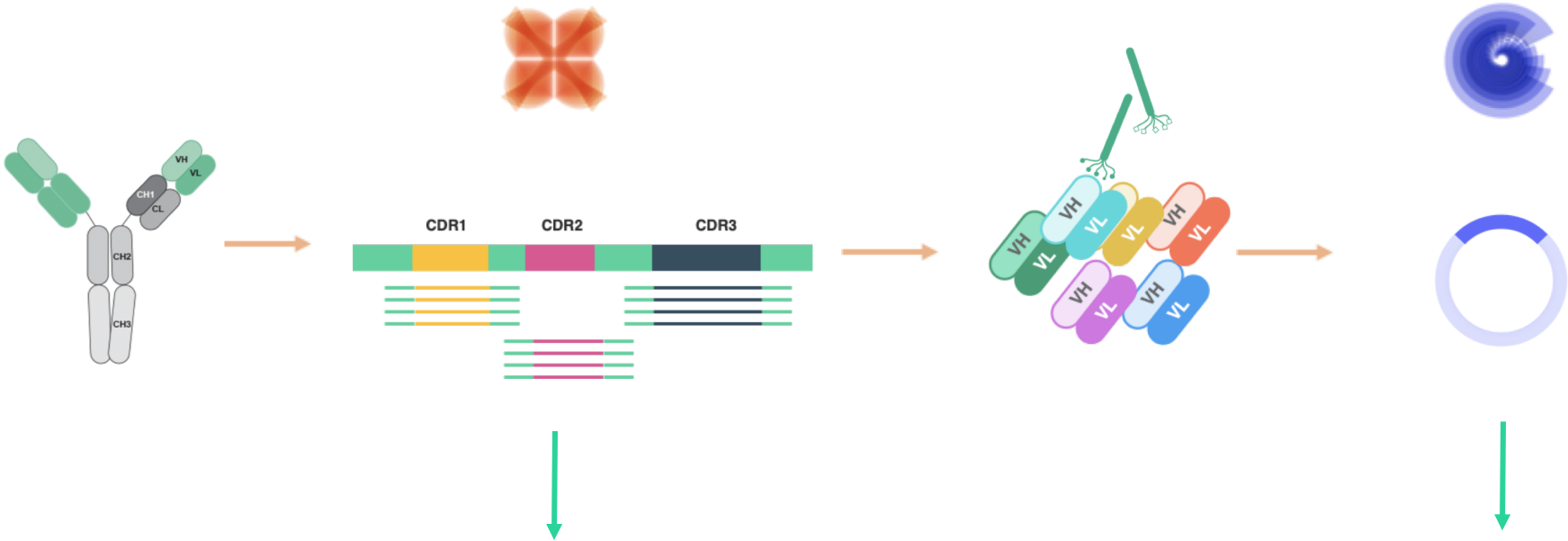


Gene synthesis is often too costly to screen many hits

DNA Solutions for Therapeutic Protein Screens



Twist Combinatorial Variant Libraries (CVLs)

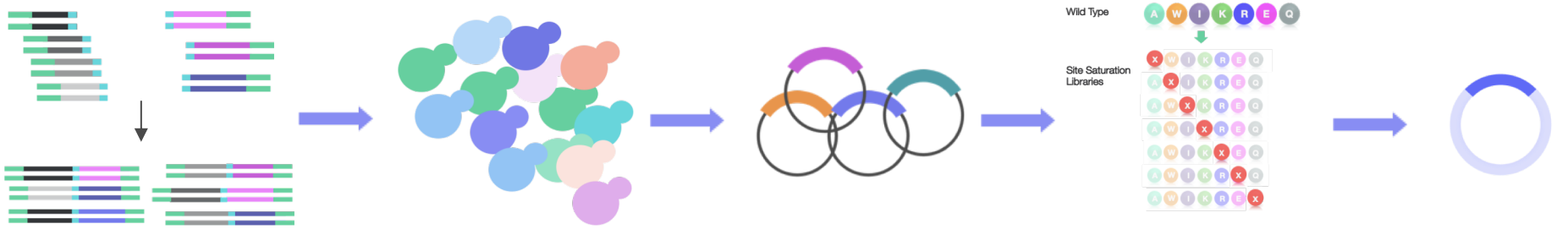
Twist Clonal Genes

Pathway Engineering

Pathway Engineering

Pathway Engineering turns cells into living factories for sustainable manufacturing.

Pathway Engineering



Promoter and Gene Shuffling; Combinatorial Assembly

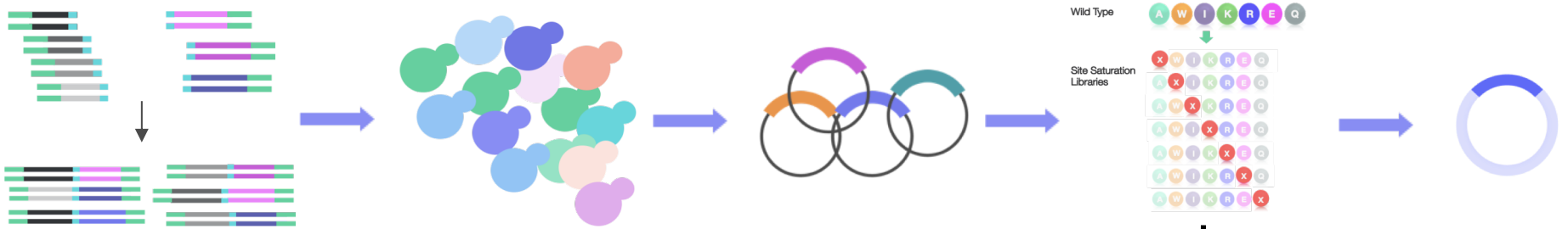
Screen for desired phenotype

Identify Intermediate hits

Further optimize hit: site saturation library

Validate hits through clonal gene

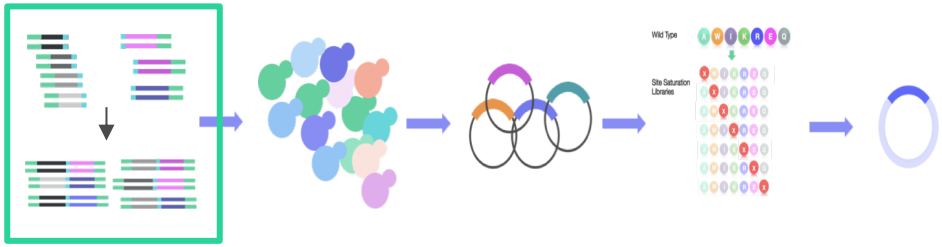
Challenges with a Pathway Engineering

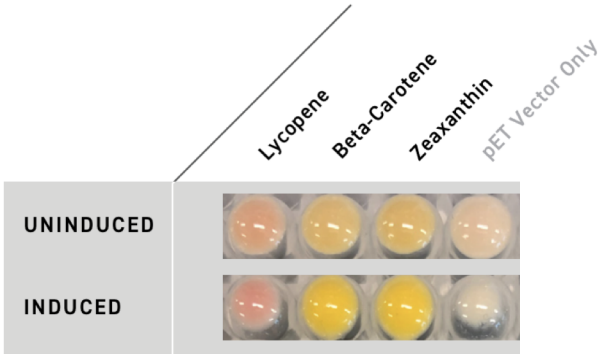


Creating highly diverse, cloned combinatorial assemblies of genes are expensive, time consuming and challenging to construct and QC.

Creating defined Site Saturation Libraries requires many different inputs. Random site saturation libraries lose the ability to learn from negative results.

Step 1: Generating Promoter/Gene Libraries





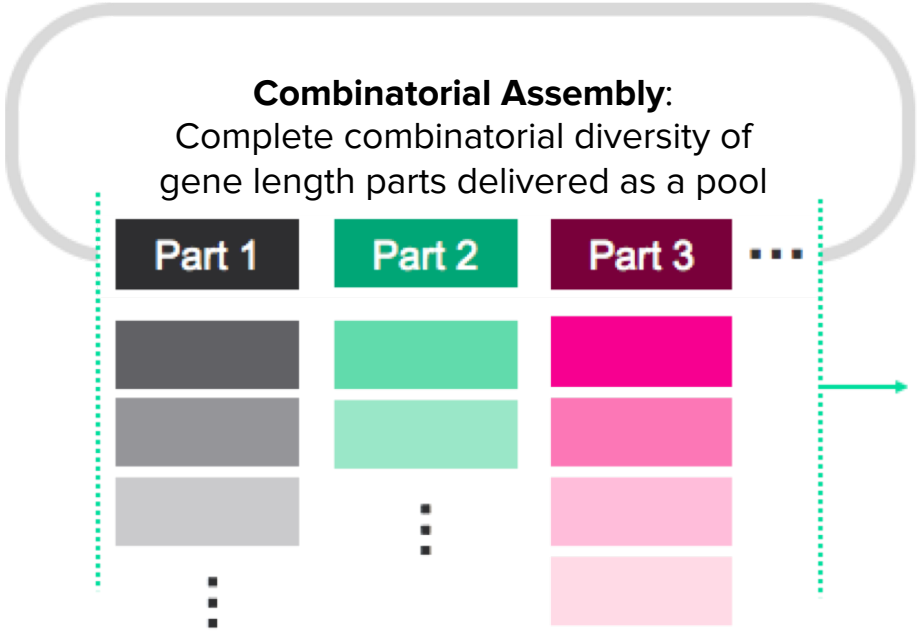
Engineering ß-caratenoid pathways in *E.coli*

Potential Pitfall: Not all orthologous genes or promoters with defined functions perform well in all cell types. Methods for constructing combinatorial assemblies can be laborious.

Twist Handy Tip: KEGG pathway database is a great resource to identify pathways and enzymes

DNA Input: Use Twist's Combinatorial Assembly

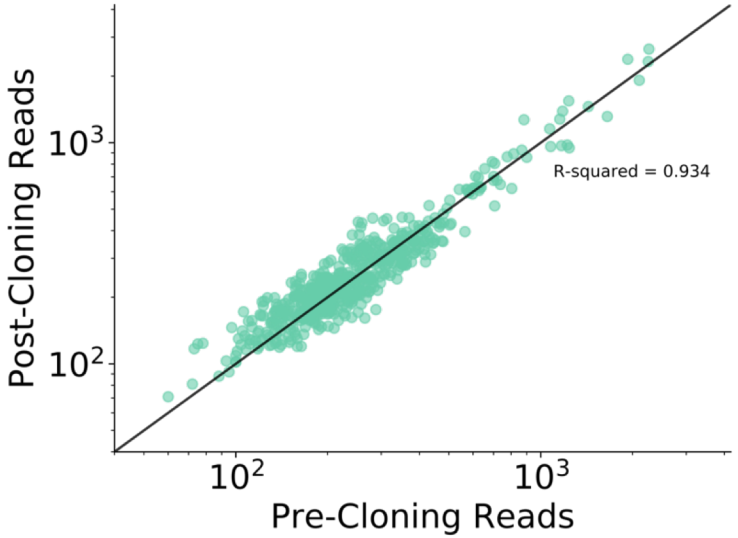
Combinatorial Assembly vs Combinatorial Libraries



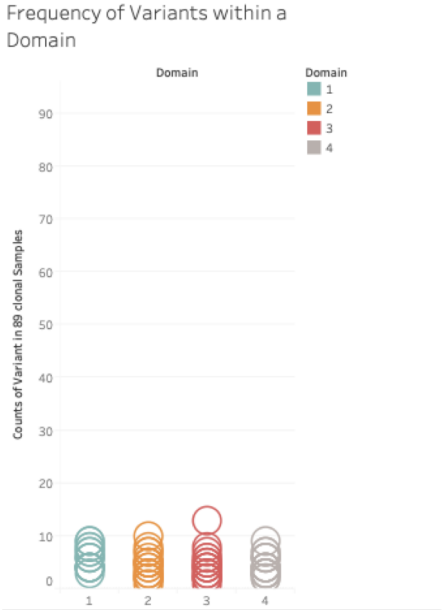
- Synthetic pathways parts
- Protein subunits
- Inputs are genes >300bp

Combinatorial Assembly

Combinatorial assembly with 4 input pools and a diversity of ~150,000 combinations



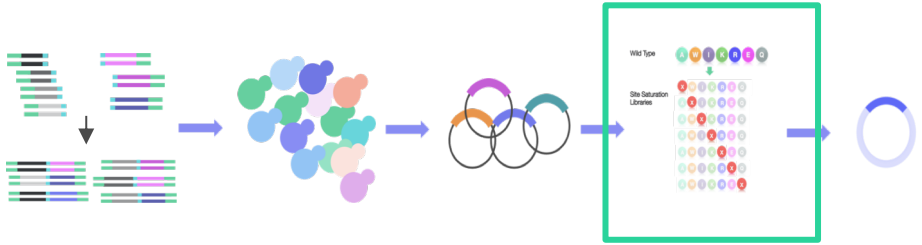
Uniformity of full length sequences are seen before and after cloning



Uniform Variant Frequency

Twist's experience in combinatorial DNA Assembly results in little bias

Step 4: Further Optimize Hits



From a needle in a haystack

Random Diversity

To a stack of needles

Explicit Diversity

Potential Pitfall: Not all orthologous genes function at top capacity in host organisms. Scientists often re-optimize hits to increase function.

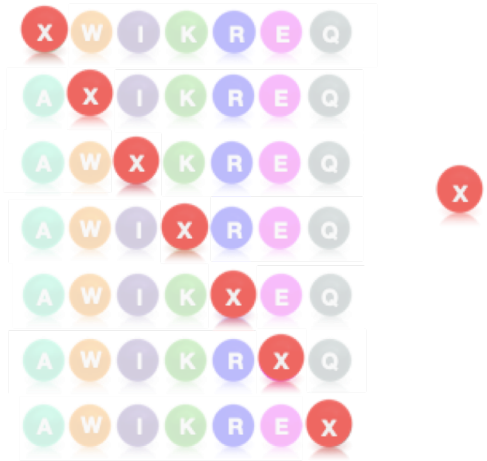
Twist Handy Tip: Site Saturation Libraries can be used as a rapid way to generate diversity at key sites within genes or explore explicit codon optimization.

DNA Input: Use Twist's Site Saturation Libraries.

Further Optimizing Your Hits

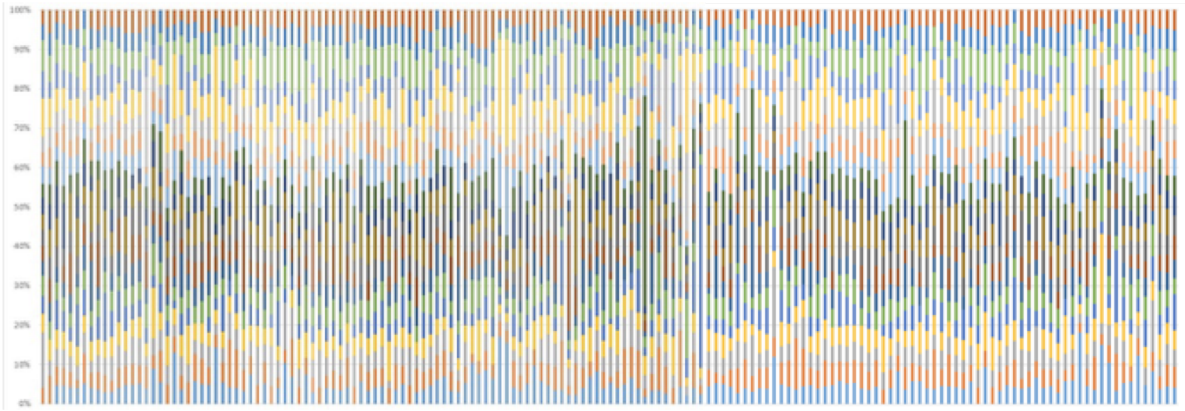
Twist offers Site Saturation Libraries to refine pathway components

Wild-Type A W I K R E Q



X

Any codon in any combination



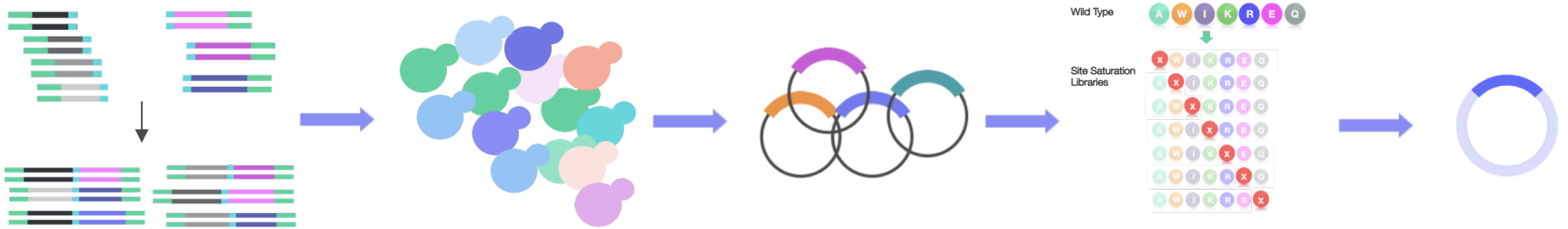
Amino Acids, represented by color, at uniform ratios in every single site as denoted by user

Twist's Site Saturation libraries show highly uniform representation of every mutant across all 161 positions.

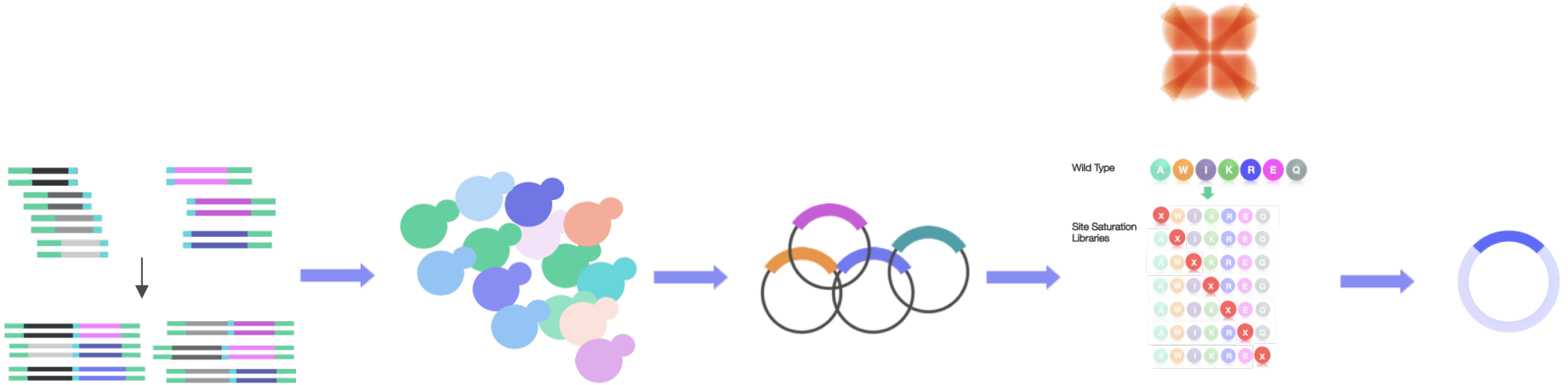
Challenges with Pathway Engineering

Creating highly diverse, cloned combinatorial assemblies of genes are expensive, time consuming and challenging to construct and QC.

Creating defined Site Saturation Libraries requires many different inputs. Random site saturation libraries lose the ability to learn from negative results.



DNA Solutions for Pathway Engineering

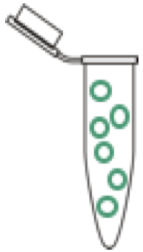


Twist Innovation Lab!

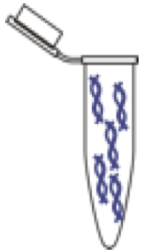
Twist Combinatorial Assembly

Twist Single Site Variant Libraries (SVL)

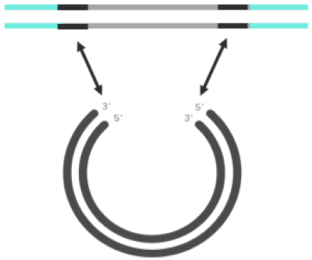
Build a Better Workflow



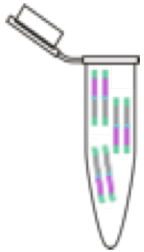
Cloned Oligo Pools



Gene Pools



NUGE



Combinatorial Assembly

What's Your Workflow?

Twist Innovation Lab

A program designed to translate customer needs into new, disruptive products facilitated by synthetic DNA at a scale previously unavailable.

Bring us your needs.

www.twistbioscience.com/innovation-lab

Come talk to us at Booth #225

Questions?