Cloned Oligo Pools

Oligo pool quality is foundational to a successful experiment. Errors during synthesis or cloning can easily skew oligo pool quality, leading to over and underrepresentation of desired sequences. While compensating for errors is possible, it's also expensive and time consuming. Instead, why not avoid the errors altogether?

Twist's industry leading DNA synthesis platform generates oligo pools with unparalleled precision and uniformity. Beyond synthesis, Twist now offers an optimized cloning process that allows you to further avoid the tribulations of testing PCR amplification conditions, selecting the right polymerases and primer pairs, and designing a cloning workflow. Instead, Twist's experts will handle synthesis and cloning for you, enabling you to focus on your experiment.

SPECIFICATIONS

- Yield: Up to 250 µg of plasmid DNA, option to add glycerol stocks
- Length: Up to 300 nt:
  - 250 nts available for your variable region
  - 50 nts conserved for Twist cloning
- Full Length:*
  - Up to 100nt: >95%
  - 101-300nt: >90%
- Turnaround Time:
  - Up to 100 nts in length: 4–6 weeks*
  - Up to 300 nts in length: 6–8 weeks*

KEY BENEFITS

Optimized cloning process that includes

- PCR optimization and primer design
- Cloning strategy using your choice of vector and optimized for your application
- Stringent NGS-verification with no mismatches allowed

Precision editing of target loci

- Chimera rate of <10%
- Highly Uniform pools from synthesis through cloning
- QC performed post amplification and cloning to ensure precision and accuracy
- Add-on services: Sanger Sequencing, Glycerol Stocks

Flexible Pool Sizes to Fit Your Screen

- Design your pool for your assay
- Scale your order to the pool size you need

Leave the tedious molecular biology steps to Twist and go straight from design to experiment

By choosing Twist to clone your Oligo Pools, you get reliable cloning that maintains the uniformity and high quality of the original pool, along with NGS verification data on pool uniformity, and dropouts.

You provide your sequences, let Twist do the rest.

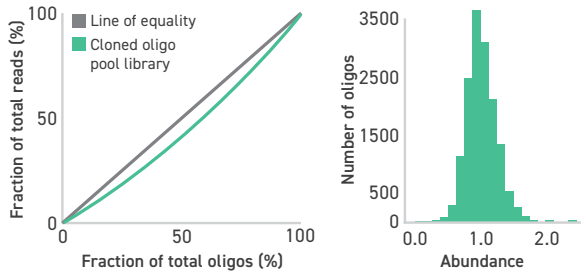
DESIGN OLIGOS	AMPLIFY			CLONE		QUALITY CONTROL	BEGIN
Design and submit sequences	Synthesis and cloning strategy	Engineer vector	Build and amplify Oligo Pool library	Clone Oligo Pool library into the vector	Scale-up and plasmid prep	NGS quality control	Begin your experiment

*Baseline turnaround time for Cloned Oligo Pools, which includes cloning and amplification, of up to 100 and 300 nucleotides is 4-6 weeks and 6-8 weeks, respectively. An additional 2 weeks will be added to this baseline turnaround time for all Cloned Oligo Pools, regardless of length, that require new vector onboarding. For Cloned Oligo Pools that are up to 100 nts, >95% of oligo sequences will be your desired length. For Cloned Oligo Pools that are up to 101-300nt, >90% of oligo sequences will be your desired length.

Uniformity of Twist's cloned oligo pools

Regardless of length, Twist is able to maintain tight uniformity with an high percentage of sequence perfect variants and high perfect of full-length sequences.

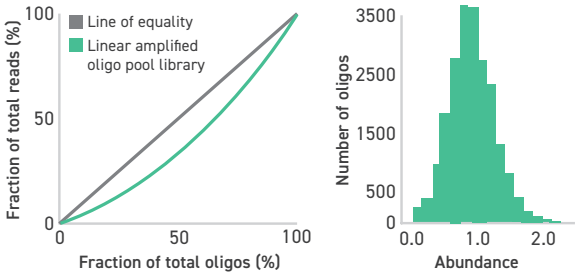
1A. Cloned Oligo Pool Library Uniformity



% Oligos represented without error	96%
Dropouts	0%
Diversity	15154
95th/5th Percentile	2.08
90th/10th Percentile	1.7
Full Length Percentage	99%

Figure 1: Graph of normalized read count of each of the mapped variants (X-axis) for a Cloned Oligo Pool of 141 nucleotides (1A) and a Cloned Oligo Pool of 300 nts. The normalized read counts converging to 1 indicates that the Cloned Oligo Pools are distributed uniformly. This was calculated by dividing each individual variant's read count by the average read count of the pool.

1B. Cloned 300mer Uniformity: Dual Guide CRISPR Library



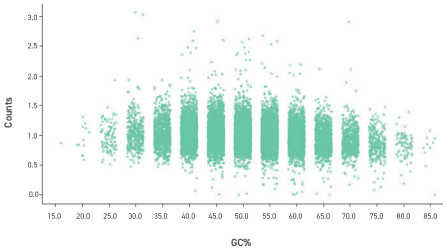
% Oligos represented without error	96%
Dropouts	0.204%
Diversity	21,554
95th/5th Percentile	4.31
90th/10th Percentile	2.92
Full Length Percentage	94.65%
Chimera Rate	3.20%

Key:
- % Oligos represented without error: percent of sequences that are a perfect sequence match to the designed oligo pool
- Dropout: percent of designed sequences not observed in the oligo pool
- Diversity: total number of unique sequences in the designed oligo pool

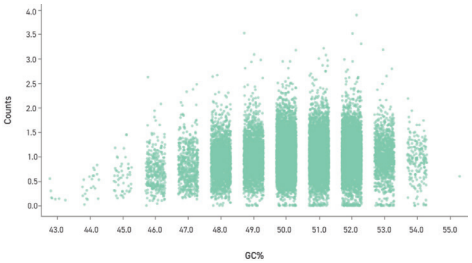
High uniformity even for sequences with high GC content

Regardless of length and GC content, Twist is able to maintain tight uniformity with a high percentage of sequence perfect variants and full-length sequences.

Figure 2: GC plots of the two Oligo Pools from Figure 1 were created and show high GC content of each oligo (x-axis) and the normalized count for that particular oligo (y-axis) after amplification and cloning. Although, both Oligo Pools (2A and 2B) contain high GC content, limited bias with regards to oligo integration in relation to GC content, as well as low dropout rate and high uniformity were achieved.



2A. Uniformity for Cloned Oligo Pools that are comprised of sequences of 141 nucleotides in length with high GC content. Percentile: 2.08, Dropouts: 1



2B. Uniformity for Cloned Oligo Pools that are comprised of sequences of 300 nucleotides in length with high GC content. Percentile: 4.31, Dropouts: 77

QUALITY AND UNIFORMITY COME STANDARD WHEN YOU PARTNER WITH TWIST BIOSCIENCE

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