Double Stranded Biotinylated Bait Sets from Twist Oligo Pools

Introduction

This protocol outlines how to construct double stranded biotinylated bait sets from Twist Oligo Pools for use as fully custom panels in the Single Cell Targeted Gene Expression Assay. Oligo pool sequences for any number of desired set of gene targets can be downloaded from the 10x Genomics Custom Panel Designer and then modified by adding 5' and 3' primer binding sites (PBS). Using the PBS and a set of 5' biotinylated forward and reverse primers, only a single PCR step and cleanup is needed to construct double stranded biotinylated baits that are ready to use with the Single Cell Targeted Gene Expression Assay. Up to eight sets of baits can be constructed from a single oligo pool by multiplexing unique, pre-tested PBS that define each bait set. This protocol was demonstrated using Amplified Biotinylated Twist Oligo Pools designed to target ~1,000 human or mouse genes. Additional optimization may be required when using panel designs that target fewer genes.

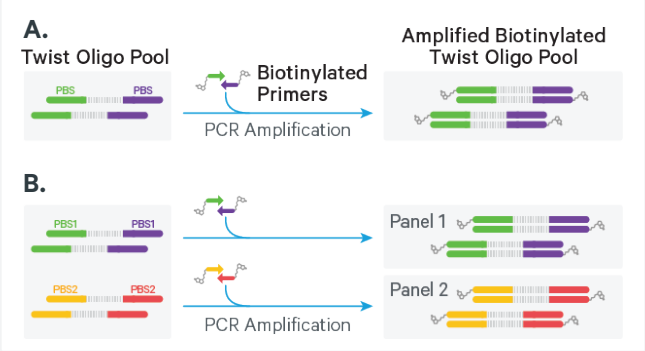

Figure 1. A) Generating a custom panel by PCR-amplifying a Twist Oligo Pool with biotinylated primers. The Twist Oligo Pool contains bait sequences with appended 5' and 3' primer binding sites (PBS). **B)** Multiple custom panels can be generated from a single Twist Oligo Pool with different sets of biotinylated primers.

Additional Guidance

Consult the Targeted Gene Expression - Single Cell User Guide (CG000293) for the complete Targeted Gene Expression workflow.

Specific Reagents & Consumables

Vendor	Item	Part Number
10x Genomics	Amp Mix	2000047*
	10x Magnetic Separator	23003
	Cot DNA	3000478†
	Universal Blockers	2000290†
Beckman Coulter	SPRIselect Reagent	B23318
Thermo Fisher Scientific	Nuclease-free Water	AM9937
IDT	Custom DNA Oligos (see Table 1)	-
	IDTE pH 8.0 (1X TE Solution)	11-05-01-13
Twist Bioscience	Twist Oligo Pools	-
Qiagen	Buffer EB	19086
Millipore Sigma	Ethanol, Pure (200 proof, anhydrous)	E7023-500ML

*Available as part of PN-1000249
†Available as part of PN-1000248

IDT PBS Pair	Forward	Reverse
1	/5Biosg/TGAACGCGCTACTATG	/5Biosg/GCCAATCGACGTTAGT
2	/5Biosg/GAGCAACTATTCGCGT	/5Biosg/TTAACGACCGGTTACG
3	/5Biosg/AGCACGTAATCGTTCG	/5Biosg/ACCTGAATACGCGGTT
4	/5Biosg/AAGGATCGCGCCTATT	/5Biosg/AACGTTCGCGATTGAC
5	/5Biosg/TGCCTTGTAACGCGAA	/5Biosg/TGGCTTAATCGCGACA
6	/5Biosg/ATTGCGCCGAACGTAT	/5Biosg/TCCAATAATGCGCGGT
7	/5Biosg/TTCGCAACGGTCGAAT	/5Biosg/TATGGCCGCGCAATTA
8	/5Biosg/AGTTAGCGTTACGACC	/5Biosg/TTACAGTACTAGCGGC

Table 1. IDT Custom DNA oligo sequences for amplification and biotinylation of Twist Oligo Pools.

Oligo Pool Seq	5' Flanking Sequence Appended to Bait	3' Flanking Sequence Appended to Bait
1	5' TGAACGCGCTACTATG	5' ACTAACGTCGATTGGC
2	5' GAGCAACTATTCGCGT	5' CGTAACCGGTCGTTAA
3	5' AGCACGTAATCGTTCG	5' AACCGCGTATTCAGGT
4	5' AAGGATCGCGCCTATT	5' GTCAATCGCGAACGTT
5	5' TGCCTTGTAACGCGAA	5' TGTCGCGATTAAGCCA
6	5' ATTGCGCCGAACGTAT	5' ACCGCGCATTATTGGA
7	5' TTCGCAACGGTCGAAT	5' TAATTGCGCGGCCATA
8	5' AGTTAGCGTTACGACC	5' GCCGCTAGTACTGTAA

Table 2. 5' and 3' flanking sequences to be appended to Twist Oligo Pool designs to facilitate amplification and, if desired, production of multiple panels in a single Twist Oligo Pool order.

1. Twist Oligo Pool Ordering and Design Considerations

a. Create an account on the Twist Online Portal.

b. Log into the portal and select "Oligo Pools" on the home page.

c. Create a project name and click on "Start New Project".

d. Click on "Step 1, Upload your sequences".

e. Drag and drop desired sequences in .csv format, with one column for sequence name and one column for bait sequence.

Design Considerations

Bait sequences for a desired set of gene targets can be downloaded from the 10x Genomics Custom Panel Designer. Bait sequences are typically between 60 and 120 nt.

Multiple sets of baits for gene enrichment panels can be produced in parallel by appending unique flanking sequences in the bait sequence, and using primers specific to these sequences to amplify a single panel from a multiplexed pligo pool. For each set of baits to be produced from a given oligo pool, add a pair of oligo pool sequences from Table 2 at the 5' and 3' end of each bait. For 120 nt bait sequences, single nucleotides from the 5' and 3' ends may be removed to keep the longest oligo pool sequence ≤ 150 nt and reduce cost.

A corresponding set of biotinylated IDT PBS primers for amplification (Table 1) must be ordered for each Twist Oligo Pool sequence pair.

f. Click on "Continue" and then "Request Quote".

2. Double Stranded Biotinylated Baits from Twist Oligo Pools

a. Resuspend lyophilized Twist Oligo Pool at 1 ng/µl with IDTE pH 8.0 buffer.

b. Dilute IDT PBS primers to 10 µM with IDTE pH 8.0 buffer.

c. For each desired bait set, add 50 µl Amp Mix, 10 µl of each IDT PBS primer, 1 µl Twist Oligo Pool, and 29 µl of nuclease-free water in a tube strip. Pipette mix and centrifuge briefly

d. Transfer tube strip to thermal cycler and start the program in Table 3. *If amplifying a single bait panel from a multiplexed synthesis of up to 4 or 8 bait sets in the same oligo pool, adjust to 15 or 16 cycles respectively.*

Lid Temperature	Reaction Volume	Run Time
105°C	100 µl	~20 min
Step	**Temperature**	**Time**
1	98°C	00:02:00
2	98°C	00:00:10
3	60°C	00:00:20
4	72°C	00:00:20
5	Go to Step 2, repeat 13x for a total of 14 cycles	
6	72°C	00:02:00

Table 3. Thermal cycler program for Step 2d.

e. Add **200 µl** SPRIselect reagent (2.0X) to each sample and pipette mix 15x (pipette set to 200 µl).

f. Incubate **5 min** at **room temperature**.

g. Place on the magnet•**High** until the solution clears.

h. Remove the supernatant.

i. Add **300 µl** 80% ethanol to the pellet. Wait **30 sec**.

j. Remove the ethanol.

k. Add **200 µl** 80% ethanol to the pellet. Wait **30 sec**.

l. Remove the ethanol.

m. Centrifuge briefly and place on the magnet•**Low**.

n. Remove any remaining ethanol. Air dry for **2 min**.

o. Remove from the magnet and add **50.5 µl** Buffer EB. Pipette mix 15x.

p. Incubate **2 min** at **room temperature**.

q. Place the tube strip on the magnet•**High** until the solution clears.

r. Transfer **50 µl** of supernatant to a new tube strip or tube.

s. Run **1 µl** sample at 1:25 or 1:50 dilution on an Agilent LabChip High Sensitivity DNA chip. The expected yield is ~15-25 ng/µl or ~750-1,250 ng of total baits.

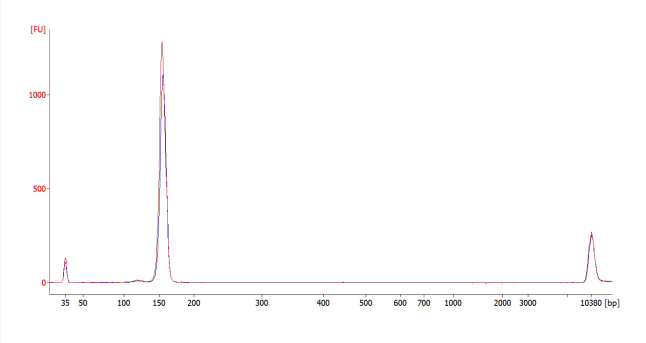

Figure 2. Example LabChip traces of Amplified Biotinylated Twist Oligo Pool baits. Technical replicate 1 (red) and replicate 2 (blue).

3. Using Amplified Biotinylated Baits with the Targeted Gene Expression protocol

a. Add **20 µl** Cot DNA, **2 µl** Universal Blockers, and **300 ng** of amplified biotinylated baits to one 0.2-ml tube in a tube strip for each sample being processed. *Consult Step 1 of the Single Cell Targeted Gene Expression User Guide (CG000293). Baits are added prior to drying due to volume limitations.*

b. Proceed to Step 1.1b of the Single Cell Targeted Gene Expression User Guide (CG000293)

c. Proceed to Step 2.1C of the Single Cell Targeted Gene Expression User Guide (CG000293). *Replace the volume of the fully custom panel working dilution with nuclease-free water.*

d. Proceed to Step 3 of the Single Cell Targeted Gene Expression User Guide (CG000293). *Post-capture PCR cycles should follow the recommendations for Fully Custom Panels, but can be optimized for best performance.*

Results

To demonstrate the application of this protocol, six Chromium Single Cell 3' Gene Expression libraries and six Chromium Single Cell 5' Gene Expression libraries were separately enriched using either IDT NGS Discovery Pool or Amplified Biotinylated Twist Oligo Pool baits, synthesized as described above, for a human custom panel of ~1,100 genes. All samples, regardless of bait type used, had comparable final Targeted Gene Expression library yields. Targeting and complexity metrics for these two bait types were largely similar, where targeting specificity was within sequencing instrument run-to-run variability (Figure 3) and all samples passed performance metrics. The UMI recovery of targeted genes between the IDT NGS Discovery Pool and Amplified Biotinylated Twist Oligo Pools was highly correlated (R^2=0.982) and similar in magnitude (Figure 4). This Demonstrated Protocol showcases a method to generate biotinylated baits from Twist Oligo Pools. When used in the Single Cell Targeted Gene Expression workflow, the performance of these baits is similar to IDT NGS Discovery Pool baits.

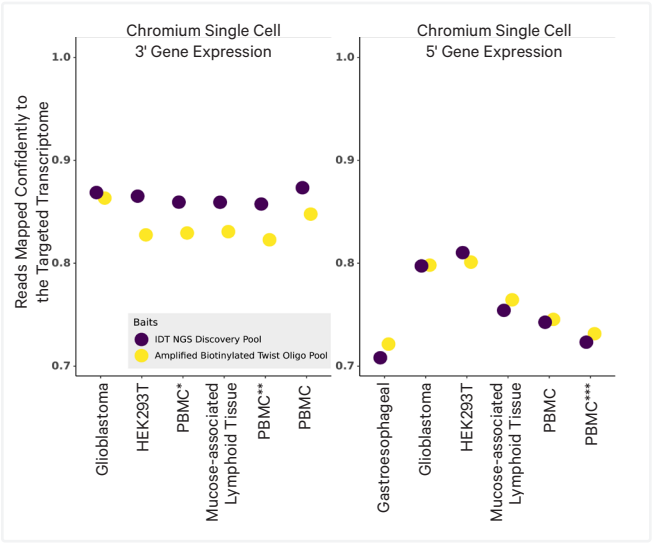

Figure 3. Reads mapped confidently to the targeted transcriptome is similar when using either IDT NGS Discovery Pool or Amplified Biotinylated Twist Oligo Pool baits. Data shown for different sample types across Chromium Single Cell 3' and 5' Targeted Gene Expression datasets. *Lupus Patient, **Multiple Myeloma Patient, ***Rheumatoid Arthritis Patient

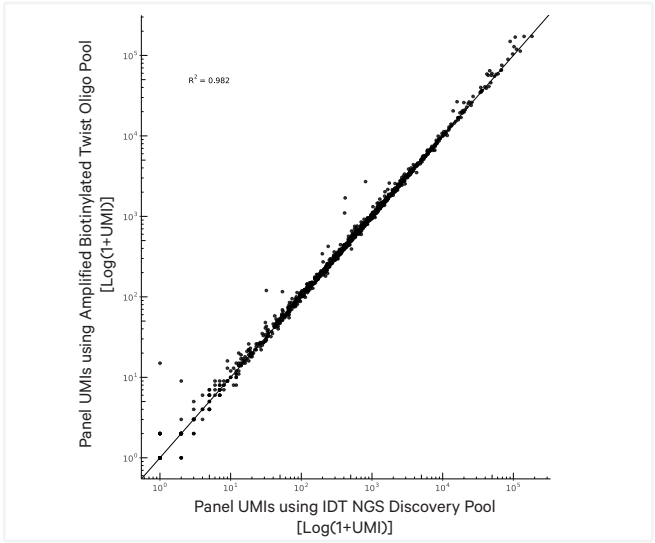

Figure 4. UMI recovery of targeted genes is similar when using either IDT NGS Discovery Pool or Amplified Biotinylated Twist Oligo Pool baits, as shown here for a Chromium Single Cell 5' Gene Expression glioblastoma multiforme sample. The read depth for both targeted datasets was matched.

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