Supporting Information

Reversible Oligonucleotide Chain Blocking Enables Bead Capture and Amplification of T-Cell Receptor α and β Chain mRNAs

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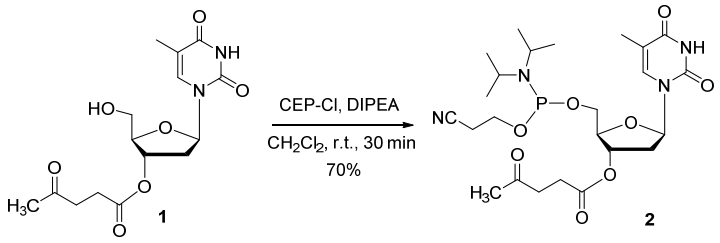
General

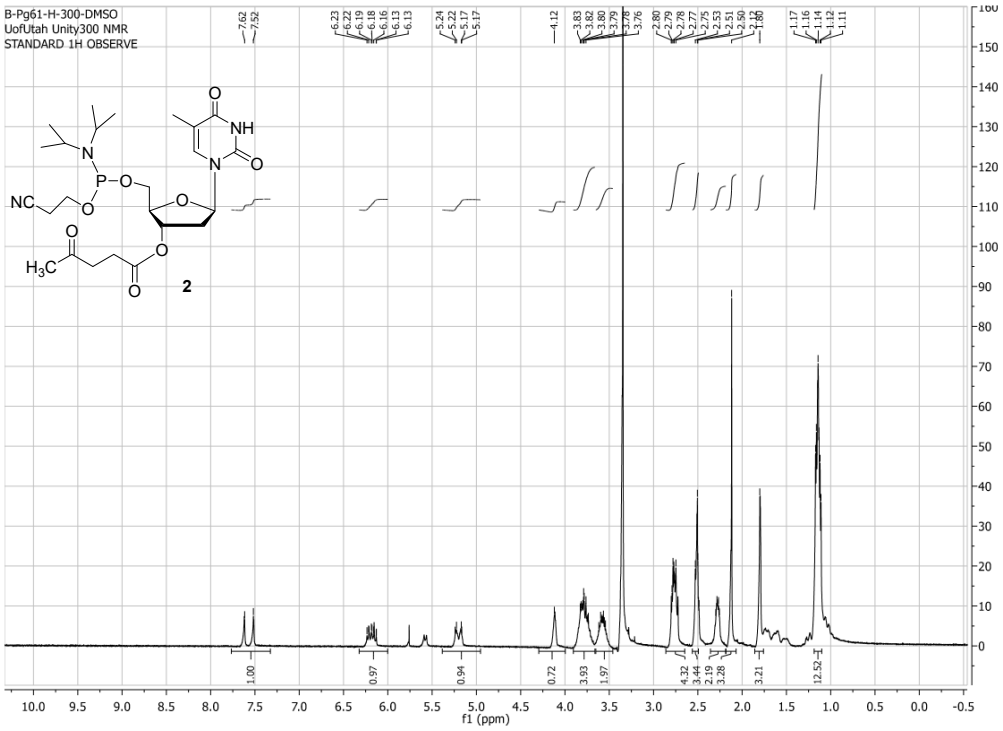
Unless otherwise noted, all starting materials were obtained from commercial suppliers and were used without further purification. Flash column chromatography was carried out using silica gel 60 (230–400 mesh). ^{1}H NMR and ^{31}P NMR chemical shifts are expressed in parts per million (δ). Mass spectra were obtained through the Mass Spectrometry Core Facility, University of Utah.

Synthesis of phosphoramidite 2

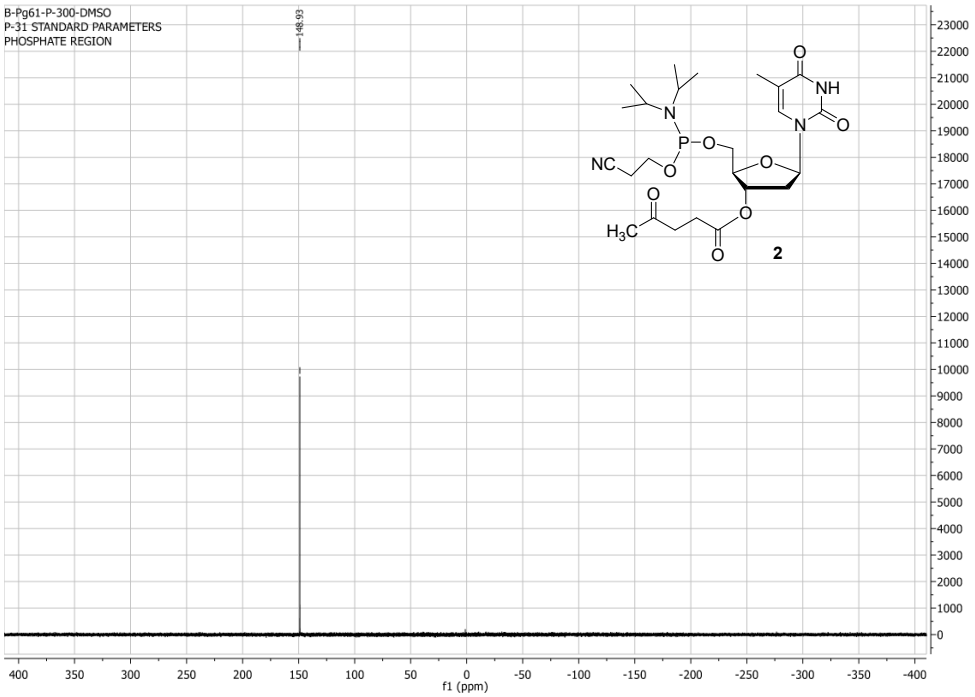


To a solution of compound **1** (225 mg, 0.66 mmol) and DIPEA (0.2 mL, 1.20 mmol) in anhydrous CH_2Cl_2 (4 mL) was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (CEP-Cl, 0.17 mL, 0.73 mmol), and the reaction mixture was stirred under N_2 at room temperature. After 45 min, TLC showed the disappearance of compound **1**, so the reaction mixture was diluted with CH_2Cl_2 (150 mL), washed with 10% $NaHCO_3$ (2 x 30 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (1:1 hexanes:EtOAc, then 99:1 EtOAc:Et_3N) to give **2** as a colorless syrup (250 mg, 70%); R_f = 0.6, (99:1 EtOAc:Et_3N). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.62, 7.52 (s, two single peaks, total 1H), 6.23−6.13 (m, 1H), 5.24−5.17 (m, 1H), 4.12 (s, 1H), 3.83−3.74 (m, 2H), 3.62−3.54 (m, 4H), 2.80−2.73 (m, 4H), 2.53−2.49 (m, 3H), 2.30−2.26 (m, 2H), 2.12 (s, 3H), 1.80 (s, 3H), 1.17−1.11 (m, 12H); ^{31}P NMR (121 MHz, DMSO-d_6) δ 148.9.

^{1}H NMR of compound **2**



^{31}P NMR of compound **2**



Oligonucleotide synthesis using monomers 2 and 3

All DNA synthesis was performed using standard phosphoramidite chemistry and reagents using an ABI 394 synthesizer.

The sequence 3'-TTTT[(3'-ODMT-T)-3'-FAM/(3'-OLev-T)T-5'-DMT] was synthesized using standard 5'-DMT monomers and 0.2 μmole polystyrene supports (Glen research). 3'-ODMT-dT and 3'-OLev-dT monomers were dissolved to 100 mM in anhydrous acetonitrile and coupled to the growing poly-dT chain in a 1:1 mixture for 15 minutes. After standard detritylation, 6-carboxyfluorescein phosphoramidite (Glen Research 10-1964) was then added, followed by DMT-removal and a 5 minute capping reaction using standard reagents (acetic anhydride/N-methylimidazole). Levulinyl deprotection solution (ChemGenes) was then used to remove 3'-levulinyl groups from the remaining thymidine bases, and another 5'-ODMT-dT was coupled to the newly generated 3'-OH groups. The final products were cleaved from the solid support for 1 hour in 30% ammonium hydroxide at room temperature and deprotected for 1 hour at 65 °C. The DNA oligomers were then dried under vacuum and dissolved in 0.1 M triethylamine acetate for HPLC analysis. Reversed-phase HPLC analysis was performed on a Hewlett Packard Series 1100 system with a 5 mm, 250 x 4.6 mm Higgins Proto 200 C_{18} column equilibrated to 40 °C, with 39 min linear gradient from 2% to 80% mobile phase. The stationary phase solution was 5% acetontirile, 20 mM triethylamine acetate and the mobile phase was 80% acetontirile, 20 mM triethylamine acetate. To confirm peak identities, independent syntheses of 3'-TTTT(3'-ODMT-T)-3'-FAM and 3'-TTTT(3'-OLev-T)T-5'-DMT were carried out, and those products analyzed using the same HPLC protocol.

Capture bead synthesis was performed on the 1 μmole scale using Toyopearl HW-65S beads (Tosoh Bioscience) as solid-phase support. Except where noted, 1 min detritylation, 1 min coupling, 30 s capping, and 25 s oxidation times were used, along with 3'-DMT/5'-phosphoramidite monomers (Glen research). 20 mg of HW-65S beads were first derivitized with Spacer18 phosphoramidite (Glen Research 10-1918) using three separate 15 min coupling reactions. Any remaining bead hydroxyl groups were blocked using four 10 min capping reactions with standard acetic anhydride/N-methylimidazole synthesis reagents. The following sequence was then synthesized after removal of the ethylene glycol DMT groups:

$^{5'}$TTTTTTTAAGCAGTGGTATCAACGCAGAGTACJJJJJJJJJJJJNNNNNNNNT(TCRα/ TCRβ)$^{3'}$

where TCRα= 5'-GGT GAA TAG GCA GAC AGA CTT GTC-3', TCRβ= 5'-GAG ACC CTC AGG CGG CTG CT-3', degenerate N positions comprise the molecular identifier region, and J residues are bar code positions (either dA, dG, dC, or dT).

First, the bar code region was synthesized according to the split-pool protocol described by Macosko, et al,[1] using 5mg of beads for each monomer in each synthesis cycle. After bar code synthesis, all 20 mg of beads were recombined, and 10 mg of the bar coded bead pool were used for synthesis of the molecular identifier and TCR sequences. After the addition of the molecular identifier sequence, a single coupling step was used to incorporate a 1:1 mixture of 3'-ODMT-dT and 3'-OLev-dT (15 min coupling). Upon trityl deprotection, TCRβ synthesis was performed and finalized with a 2 min detritylation and 5 min capping step. Finally, the 3'-levulinyly groups were removed, and the TCRα sequence was synthesized, followed by removal

of the ultimate 3'-trityl groups. The completed beads were deprotected in 30% ammonium hydroxide for 20 h at room temperature, washed with acetonitrile, and dried under vacuum.

RNA capture, reverse transcription, and PCR[2]

Capture beads (beads derivatized with T-Cell-specific capture oligos) and raw beads (beads with no capture oligo) were prepared by washing with ethanol and TE-TW (TE buffer with 0.01% Tween 20). Prepared beads were counted and washed in water. For RNA capture and reverse transcription, 2,100 beads of each type were mixed in parallel with 20 ng of total RNA isolated from T cell clones, and subjected to a reverse transcription reaction. Specifically, solutions containing beads, RNA and 1 mM dNTP (6 μL total volume) were heated to 65 °C for 5 min and then chilled on ice to denature any RNA secondary structure. A Maxima H-Reverse transcriptase mixture (1x Maxima RT Buffer, 1 mM dNTPs, 4% Ficoll PM-400, 1 U RNase Inhibitor (Lucigen), 2.5 μM Template Switch Oligo[2] and 10 U of Maxima H Minus Reverse Transcriptase (Thermo Scientific)) was added to the denatured bead/RNA/dNTP solutions, bringing the mixtures to a total volume of 100 μL. Bead/RNA/reverse transcriptase mixtures were incubated for 30 min at room temperature followed by 90 min at 42°C. Subsequently, beads were washed in TE-SDS (TE buffer with 0.5% SDS) and twice with TE-TW and then with water prior to PCR amplification of cDNA.

PCR amplification was used to establish capture of TCRα and TCRβ RNA transcripts by the beads. Each set of treated beads was divided equally into four tubes to allow for multiple reactions, and each aliquot of beads was washed in water. Approximately 500 beads were used per PCR reaction. Thermo Scientific Phusion High-Fidelity PCR master mix was added to each set of beads along with 0.5 μM of each primer. PCR was performed using 52 cycles and an annealing temperature of 61°C. TCRα cDNA was PCR amplified using alpha and capture_a_nested primers and TCRβ cDNA was PCR amplified using beta and capture_b_nested primers. Equal volumes of PCR samples were separated using 1% agarose gels and visualized by ethidium bromide staining. 1 kb-Plus DNA ladder (Thermo Fisher Scientific) was used to determine PCR product size. PCR bands were cut out, purified, and the identity of TCRα and TCRβ PCR bands were confirmed by Sanger sequencing.

Primer sequences (5'-3'):

alpha	CARTGTTCCAGAGGGAGC (where R = A or G)
capture_a_nested	AGAGTCTCTCAGCTGGTACACG
beta	CCAACAATCGATTCTTAGCTG
capture_b_nested	AGGCAGTATCTGGAGTCATTGAG

Sequence of PCR products from capture

(A) TCR-α

GTCCTTCTTCTGGTACAGACAATATTCTGGGAAAAGCCCTGAGTTGATAATGTTCATATACTCCAATGGTGACAA
AGAAGATGGAAGGTTTACAGCACAGCTCAATAAAGCCAGCCAGTATGTTTCTCTGCTCATCAGAGACTCCCAGC
CCAGTGATTCAGCCACCTACCTCTGTGCCGTGAGTAGCAGCTATAAATTGATCTTCGGGAGTGGGACCAGACTGC
TGGTCAGGCCTGATATCCAGAACCCTGACCCTGCCGTGTAC

(B) TCR-β

GCCTGCAGAACTGGAGGATTCTGGAGTTTATTTCTGTGCCAGCAGCGCCGGAGGAGCCCTCACCGGGGAGCTGT
TTTTTGGAGAAGGCTCTAGGCTGACCGTACTGGAGGACCTGAAAAACGTGTTCCCACCCGAGGTCGCTGTGTTTG
AGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACC
ACGTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAA
GGAGCAGCCCGCCCTCAATGACTCCAGATACT

Figure S1. Sequencing results for PCR products amplified from mRNA captured from clone GDB4. (A) TCR-α composed of TRAV12.2 variable and TRAJ12 joining genes. (B) TCR-β composed of TRBV14 variable and TRBJ2.2 joining genes. Sequences corresponding to variable (green), joining regions (blue), CDR3 regions (pink) and constant regions (black) are shown. The sequences of the CDR3 regions were exact matches for cDNA from the expected clone: GDB4.

References

1. Macosko, E. Z.; Basu, A.; Satija, R.; Nemesh, J.; Shekhar, K.; Goldman, M.; Tirosh, I.; Bialas, A. R.; Kamitaki, N.; Martersteck, E. M.; Trombetta, J. J.; Weitz, D. A.; Sanes, J. R.; Shalek, A. K.; Regev, A.; McCarroll, S. A. *Cell* **2015**, *161*, 1202.

2. Drop-Seq Laboratory Protocol, version 3.1 (12/28/15), Evan Macosko and Melissa Goldman, Steve McCarrol Lab, Harvard Medical School, http://mccarrolllab.com/dropseq/.