# Supporting Information

## Dahotre et al. 10.1073/pnas.1714820115

### SI Materials and Methods

**DNA Gate Orthogonality and Kinetics.** All DNA strands were purchased from Integrated DNA Technologies with either HPLC purification (TP and CP strands) or standard desalting (RP strands) and were reconstituted to 100 μM in 1× PBS. Cy5-labeled TP and Iowa Black-labeled CP strands were annealed by heating an equimolar ratio to 95 °C and cooling to 20 °C over 90 min in a T100 thermal cycler (Bio-Rad). Free TP:CP duplexes or TP:CP duplexes conjugated to antibodies were mixed with RP strands at an equimolar ratio (250 nM final concentration), and fluorescence was monitored using a Cytation 5 plate reader (BioTek).

**Antibodies and pMHC Tetramers for DGS and Staining.** Antibody clones used for kinetic studies were as follows: anti-human CD3 (clone: HIT3a; BD Pharmingen), anti-human CD4 (clone: RPA-T4; BD Pharmingen), and anti-human CD8 (clone: HIT8a; BD Pharmingen). Antibody clones used for cell sorting were as follows: anti-mouse CD8 (clone: 53-6.7; Biolegend), anti-mouse CD4 (clone: RM4-5; Biolegend), and anti-mouse CD19 (clone: 6D5; Biolegend). Antibodies used to check purity were as follows: anti-mouse CD8 (clone: KT15; Bio-Rad), anti-mouse CD4 (clone: RM4-4; Biolegend), and anti-mouse/human B220 (clone: RA3-6B2; Biolegend). PE- or APC-conjugated tetramers were synthesized in house.

**DNA Strand Displacement on Cell Surfaces.** Cell lines were maintained at 37 °C, 5% $CO_2$. Jurkat and CCRF-CEM cells (ATCC) were grown in RPMI 1640 (Gibco) supplemented with 10% FBS (Gibco) and 100 U/mL penicillin-streptomycin (Gibco). TALL-104 cells (ATCC) were grown in Iscove's modified Dulbecco's medium (IMDM) (Gibco) supplemented with 20% FBS, 100 U/mL penicillin-streptomycin, 2.5 μg/mL human albumin (Sigma), 0.5 μg/mL D-mannitol (Sigma), and 100 U/mL hIL-2 (Roche). For studies with Ab-DNA gates, $5 \times 10^5$ cells were stained with quenched Ab-TP:CP complexes (Jurkat plus αCD3-Gate A; CCRF-CEM plus αCD4-Gate B; TALL-104 plus αCD8-Gate C) for 30 min, and baseline Cy5 fluorescence was measured on a BD Accuri C6. RP strands were added to cells at ∼1 μM final concentration for 30 min before remeasuring Cy5 signal. For studies with tet-DNA gates, biotinylated Db-LMCV $GP_{33–41}$ or Db-$GP100_{25–33}$ monomer was mixed with quenched StvC-TP:CP complexes at a 4:1 ratio. Then, $1 \times 10^6$ splenocytes from pmel or P14 mice (The Jackson Laboratory) were stained with 1 μg of appropriate quenched tetramer and anti-mouse CD8 (clone: KT15; Bio-Rad) for 30 min, and baseline Cy5 fluorescence was measured on a BD Accuri C6. RP strands were added to cells at 5 μM final concentration for 30 min before remeasuring Cy5 signal.

**LCMV Armstrong Infection.** Experiments were performed in accordance with approved Emory University Institutional Animal Care and Use Committee protocols. Six- to 8-wk-old female C57BL/6J mice (The Jackson Laboratory) were infected intraperitoneally with $2 \times 10^5$ plaque forming units (pfu) of the Armstrong strain of lymphocytic choriomeningitis virus (LCMV).

**Comparing DGS- and MACS-Sorting Efficiency.** $CD8^+$ T cells were sorted from splenocytes by DGS using the above protocol and by MACS using CD8a (Ly-2) microbeads (Miltenyi Biotec), following the manufacturer's protocol. Cell purity was measured by staining recovered cells with anti-mouse CD8 (clone: KT15; Bio-Rad) and analyzing $CD8^+$ frequency on a BD Accuri C6. Cell viability after sorting was measured by costaining recovered cells with Annexin V (BD Pharmingen) and 7-AAD (BD Pharmingen) and analyzing on a BD Accuri C6. Viable cells were considered to be Annexin $V^-$/7-$AAD^-$. Cell yield was measured by counting recovered cells using a hemocytometer.

**Cellular Functional Assays After DGS and MACS Sorting.** EL4 and EG7-OVA cells (ATCC) were grown in RPMI 1640 supplemented with 10% FBS and 25 mM Hepes (Gibco) or with 10% FBS, 10 mM Hepes, 1 mM sodium pyruvate (Gibco), 0.05 mM 2-mercaptoethanol (Sigma), and 0.4 mg/mL G418 (InvivoGen), respectively. $CD8^+$ T cells were isolated from C57BL/6J (proliferation studies) or OT1 (killing assays; The Jackson Laboratory) splenocytes by DGS using the above protocol and by MACS using CD8a (Ly-2) microbeads (Miltenyi Biotec), following the manufacturer's protocol. Cells were activated by seeding in 96-well plates coated with anti-mouse CD3e (clone: 145–2C11; BD Pharmingen) and anti-mouse CD28 (clone: 37.51; BD Pharmingen) at $9 \times 10^5$ to $1.25 \times 10^6$ cells per mL in RPMI 1640 supplemented with 10% FBS, 100 U/mL penicillin-streptomycin, 1× nonessential amino acids (Gibco), 1 mM sodium pyruvate, 0.05 mM 2-mercaptoethanol, and 30 U/mL hIL-2 (Roche). After 2 d, cells were transferred to non-αCD3e/αCD28–coated plates. On day 5, sorted $CD8^+$ cells from C57BL/6J mice were stained with anti-mouse CD8 (clone: KT15; Bio-Rad), anti-mouse/human CD44 (clone: IM7; Biolegend), and anti–Ki-67 (BD Pharmingen) and then analyzed on a BD FACSAria Fusion. Sorted $CD8^+$ cells from OT1 mice were coincubated with EL4 control or EG7-OVA target cells at a 1:1 ratio for 5 h [Brefeldin A (Invitrogen) was added after 4 h] and then stained with anti-mouse CD8 (clone: 53–6.7; BD Pharmingen), anti-mouse/human CD44 (clone: IM7; Biolegend), and anti-mouse Granzyme B (clone: NGZB; eBioscience), and then analyzed on a BD Accuri C6.

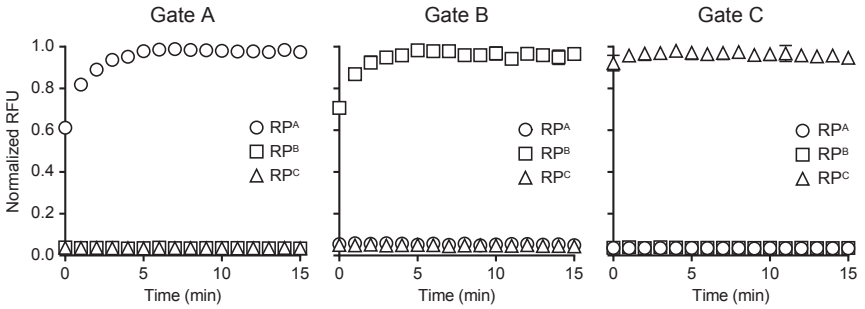


**Fig. S1.** Kinetics of free DNA gates in solution ($n = 3$). Quenched TP:CP duplexes are incubated with RP strands from one of the gates. Strand displacement occurs rapidly, with a significant fraction of strands displacing in the lag time between strand mixing and the first fluorescence reading. Data shown as mean ± SD.

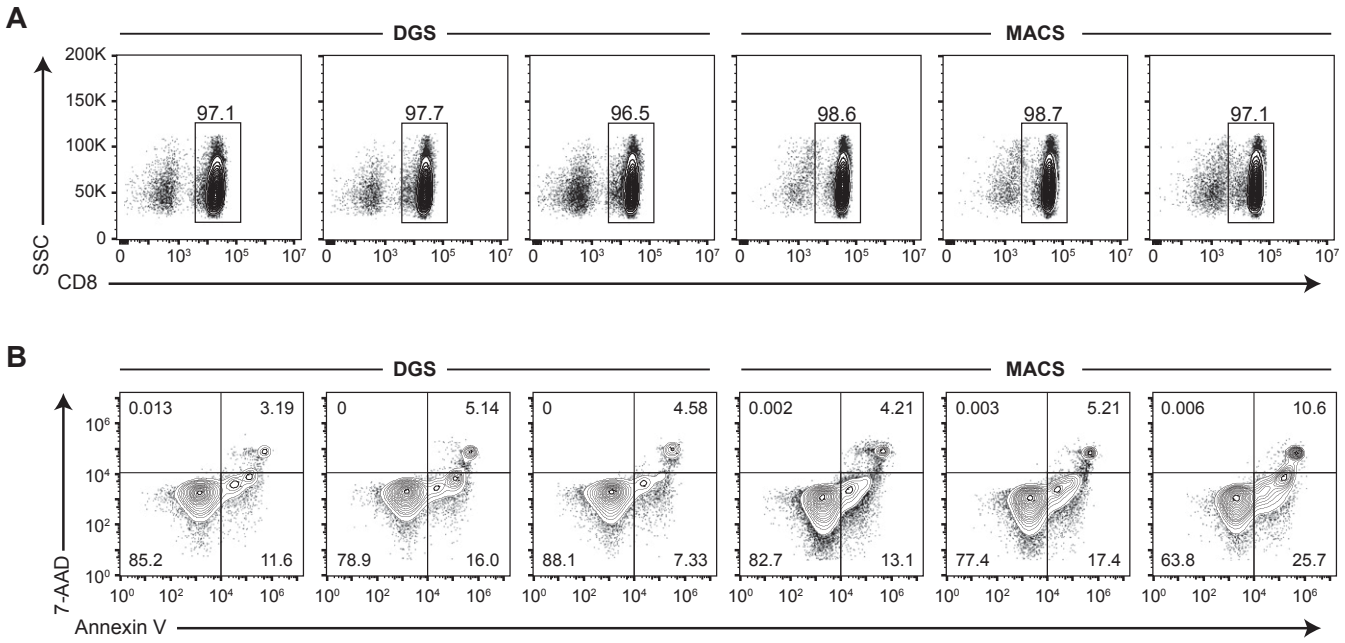


**Fig. S2.** Flow plots for assessing CD8$^+$ purity (*A*) and viability (*B*) after DGS or MACS sorting. Plots in *B* are gated on CD8$^+$ cells. SSC, side scatter.

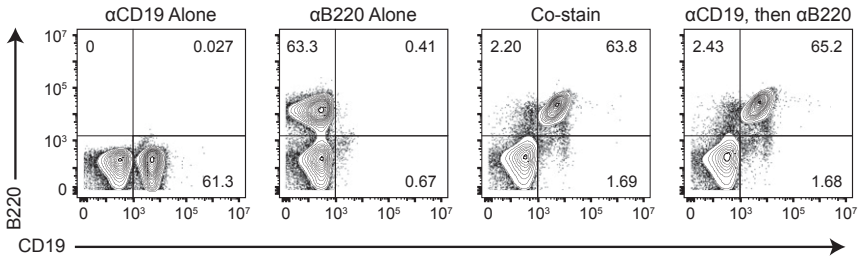


**Fig. S3.** B220 is coexpressed with CD19 and can be used to measure CD19$^+$ frequency.

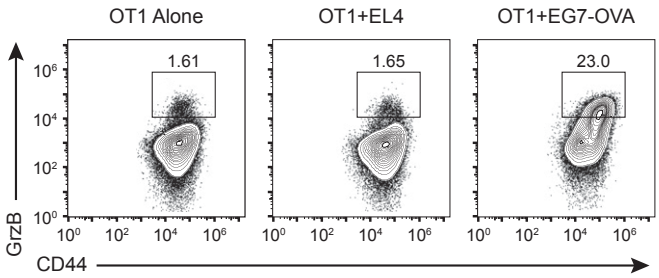


**Fig. S4.** CD8$^+$ cells sorted from OT1 mice by MACS produce elevated Granzyme B when coincubated with EG7-OVA target cells compared with EL4 control cells.

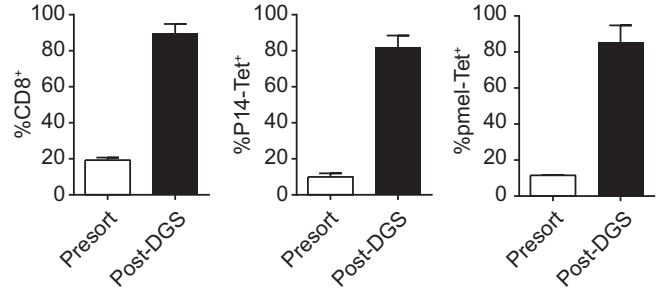


**Fig. S5.** Purity of isolated cells from dual gated DGS using anti-mouse CD8 and pMHC tetramers in combination. Data shown as mean ± SD, $n = 3$.

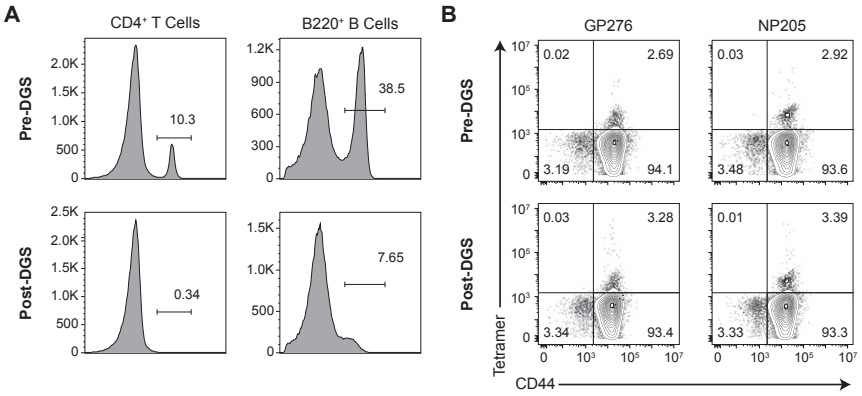


**Fig. S6.** (*A*) Depletion of CD4$^+$ T cells and B220$^+$ B cells by DGS from splenocytes isolated from LCMV-infected mice. (*B*) CD4/CD19 depletion has minimal effect on the frequency of LCMV-specific T cell populations.

**Table S1. DNA gate sequences used in this paper**

Sequences for kinetic studies and testing displacement on cell surfaces

| | |
|---|---|
| $TP^A$ | 5′ NH2-GGA ACT TAA CTG GGC GCA CGA TCT AT-Cy5 3′ |
| $CP^A$ | 5′ IAbRQ-ATA GAT CGT GCG CCC AGT TA 3′ |
| $RP^A$ | 5′ ATA GAT CGT GCG CCC AGT TAA GTT CC 3′ |
| $TP^B$ | 5′ NH2-GTC TCA GTC TCA GTG GCG TAA TAA CC-Cy5 3′ |
| $CP^B$ | 5′ IAbRQ-GGT TAT TAC GCC ACT GAG AC 3′ |
| $RP^B$ | 5′ GGT TAT TAC GCC ACT GAG ACT GAG AC 3′ |
| $TP^C$ | 5′ NH2-GGT CAT GGG GCT ATA ACA ACG TCT CT-Cy5 3′ |
| $CP^C$ | 5′ IAbRQ-AGA GAC GTT GTT ATA GCC CC 3′ |
| $RP^C$ | 5′ AGA GAC GTT GTT ATA GCC CCA TGA CC 3′ |

Sequences for multiplexed cell sorting*

| | |
|---|---|
| $TP^A$ | 5′ NH2-GGA ACT TAA CTG GGC GCA CGA TCT AT 3′ |
| $CP^A$ | 5′ BiotinTEG-AAA AAA AAA AAT AGA TCG TGC GCC CAG TTA 3′ |
| $RP^A$ | 5′ ATA GAT CGT GCG CCC AGT TAA GTT CC 3′ |
| $TP^B$ | 5′ NH2-GTC TCA GTC TCA GTG GCG TAA TAA CC 3′ |
| $CP^B$ | 5′ BiotinTEG-AAA AAA AAA AGG TTA TTA CGC CAC TGA GAC 3′ |
| $RP^B$ | 5′ GGT TAT TAC GCC ACT GAG ACT GAG AC 3′ |
| $TP^C$ | 5′ NH2-GGT CAT GGG GCT ATA ACA ACG TCT CT 3′ |
| $CP^C$ | 5′ BiotinTEG-AAA AAA AAA AAG AGA CGT TGT TAT AGC CCC 3′ |
| $RP^C$ | 5′ AGA GAC GTT GTT ATA GCC CCA TGA CC 3′ |

Toeholds in TP strands are highlighted in color, which correspond with the schematic in Fig. 4*B*.

*Domain sequences are identical to those used in the kinetic studies. However, the fluorophore and quencher are removed from TP and CP strands, respectively, and CP strands are derivatized with biotin and a poly$(A)_{10}$ region.

**Table S2. Additional examples of DNA gate sequences (sequences for multiplexed cell sorting)**

| Gate | Probe | Sequence |
|---|---|---|
| α | TP | 5′ NH2-GAG TTG GAG AGT TGT GAG GGA GTA TG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACA TAC TCC CTC ACA ACT CTC 3′ |
| | RP | 5′ CAT ACT CCC TCA CAA CTC TCC AAC TC 3′ |
| β | TP | 5′ NH2-GTT GAG GTG AGA TGG AAG GAT GTT GG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACC AAC ATC CTT CCA TCT CAC 3′ |
| | RP | 5′ CCA ACA TCC TTC CAT CTC ACC TCA AC 3′ |
| γ | TP | 5′ NH2-GTG TAG GGA GGG TTG TAG TAG GAA TG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACA TTC CTA CTA CAA CCC TCC 3′ |
| | RP | 5′ CAT TCC TAC TAC AAC CCT CCC TAC AC 3′ |
| δ | TP | 5′ NH2-GAT GTG GGT GGT GTA ATG AGT GAG AG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACT CTC ACT CAT TAC ACC ACC 3′ |
| | RP | 5′ CTC TCA CTC ATT ACA CCA CCC ACA TC 3′ |
| ε | TP | 5′ NH2-GGA TAG GTG GAG AAG GTT GAG GTT AG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACT AAC CTC AAC CTT CTC CAC 3′ |
| | RP | 5′ CTA ACC TCA ACC TTC TCC ACC TAT CC 3′ |
| ζ | TP | 5′ NH2-GTA AGG GTG TAG GTG AAT AGG TGG AG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACT CCA CCT ATT CAC CTA CAC 3′ |
| | RP | 5′ CTC CAC CTA TTC ACC TAC ACC CTT AC 3′ |
| η | TP | 5′ NH2-GTG AAG GAG TGA GTG AGG TTA AGT GG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACC ACT TAA CCT CAC TCA CTC 3′ |
| | RP | 5′ CCA CTT AAC CTC ACT CAC TCC TTC AC 3′ |
| θ | TP | 5′ NH2-GAG ATG GGA TAA GTA GGT GTG GGT AG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACT ACC CAC ACC TAC TTA TCC 3′ |
| | RP | 5′ CTA CCC ACA CCT ACT TAT CCC ATC TC 3′ |
| ι | TP | 5′ NH2-GAA GTG GTG GTT AGG AAG TGA GAG TG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACA CTC TCA CTT CCT AAC CAC 3′ |
| | RP | 5′ CAC TCT CAC TTC CTA ACC ACC ACT TC 3′ |
| κ | TP | 5′ NH2-GTA GTG GTG AAA TGG TAT GGG TGG AG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACT CCA CCC ATA CCA TTT CAC 3′ |
| | RP | 5′ CTC CAC CCA TAC CAT TTC ACC ACT AC 3′ |
| λ | TP | 5′ NH2-GTA TGG GTG TGG TGT AGA ATG GAG AG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACT CTC CAT TCT ACA CCA CAC 3′ |
| | RP | 5′ CTC TCC ATT CTA CAC CAC ACC CAT AC 3′ |
| μ | TP | 5′ NH2-GGT AAG GTG AGA GGA GTA GGT ATG TG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACA CAT ACC TAC TCC TCT CAC 3′ |
| | RP | 5′ CAC ATA CCT ACT CCT CTC ACC TTA CC 3′ |
| ν | TP | 5′ NH2-GAG TAG GTG TGG GAA GTA GGT GTA AG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACT TAC ACC TAC TTC CCA CAC 3′ |
| | RP | 5′ CTT ACA CCT ACT TCC CAC ACC TAC TC 3′ |
| ξ | TP | 5′ NH2-GTG ATG GGT AGG GTT GAT TGG GAA AG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACT TTC CCA ATC AAC CCT ACC 3′ |
| | RP | 5′ CTT TCC CAA TCA ACC CTA CCC ATC AC 3′ |
| ο | TP | 5′ NH2-GTA GAG GGA GAG TAT TGT AGA GGT GG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACC ACC TCT ACA ATA CTC TCC 3′ |
| | RP | 5′ CCA CCT CTA CAA TAC TCT CCC TCT AC 3′ |
| π | TP | 5′ NH2-GAT AGG GTA AGA ATG GGA GTT GGT GG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACC ACC AAC TCC CAT TCT TAC 3′ |
| | RP | 5′ CCA CCA ACT CCC ATT CTT ACC CTA TC 3′ |
| ρ | TP | 5′ NH2-GAT TGG GAT GGT AGG GAG TGT AGA TG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACA TCT ACA CTC CCT ACC ATC 3′ |
| | RP | 5′ CAT CTA CAC TCC CTA CCA TCC CAA TC 3′ |
| σ | TP | 5′ NH2-GGT ATG GTG GAG AGT GTG AAA GGT TG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACA ACC TTT CAC ACT CTC CAC 3′ |
| | RP | 5′ CAA CCT TTC ACA CTC TCC ACC ATA CC 3′ |
| τ | TP | 5′ NH2-GAT GAG GAT GGA TGA GGT GAT TGA GG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACC TCA ATC ACC TCA TCC ATC 3′ |
| | RP | 5′ CCT CAA TCA CCT CAT CCA TCC TCA TC 3′ |
| υ | TP | 5′ NH2-GGA ATG GTT GGG TGA GAG TAG AAG TG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACA CTT CTA CTC TCA CCC AAC 3′ |
| | RP | 5′ CAC TTC TAC TCT CAC CCA ACC ATT CC 3′ |
| φ | TP | 5′ NH2-GGT TAG GGT TTA GAT GAG TGG GAA GG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACC TTC CCA CTC ATC TAA ACC 3′ |
| | RP | 5′ CCT TCC CAC TCA TCT AAA CCC TAA CC 3′ |

**Table S2. Cont.**

| Gate | Probe | Sequence |
|---|---|---|
| χ | TP | 5′ NH2-GAA TGG GAT AAG TTG GGA GTG GGT AG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACT ACC CAC TCC CAA CTT ATC 3′ |
| | RP | 5′ CTA CCC ACT CCC AAC TTA TCC CAT TC 3′ |
| ψ | TP | 5′ NH2-GGA TTG GGA TAG TGA AAT GGT GTG GG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACC CAC ACC ATT TCA CTA TCC 3′ |
| | RP | 5′ CCC ACA CCA TTT CAC TAT CCC AAT CC 3′ |
| ω | TP | 5′ NH2-GTT AGG GAT GGA ATG GTT AGG AGG TG 3′ |
| | CP | 5′ BiotinTEG-AAA AAA AAA ACA CCT CCT AAC CAT TCC ATC 3′ |
| | RP | 5′ CAC CTC CTA ACC ATT CCA TCC CTA AC 3′ |