uCoTarget

Single-cell version-2025

Reagents

- STE buffer: 10 mM Tris-HCl pH 8.0, 50 mM NaCl, and 1mM EDTA
- 0.1% (m/v) BSA-PBS
- 36.5% formaldehyde (sigma)
- 2.5 M Glycine (do not adjust pH)
- 100% methanol (pre-chill methanol at -20°C before use)
- Wash buffer: 1 ml 1 M HEPES pH 7.5, 1.5 ml 5 M NaCl, 12.5 µl 2 M spermidine, 10 mM sodium butyrate, and the final volume to 50 ml with ddH_2O.
- Wash-buffer-Dig: Wash buffer adding 0.01% Digitonin.
- Wash-buffer-TX: Wash buffer adding 0.01% Digitonin, 0.05% TX-100
- Wash-buffer-TX-high salt: Wash buffer adding 0.01% Digitonin, 0.05% TX-100 and final 300 mM NaCl
- Reaction buffer: 10 mM TAPS pH 8.3, 10 mM $MgCl_2$, 10 mM sodium butyrate, 0.01% Digitonin.
- NSB (nuclei suspension buffer) buffer: 10mM Tris-HCl, pH 7.5, 10mM NaCl, 3mM MgCl2, 0.01% TX-100
- Lysis buffer: 10 mM Tris-HCl pH 8.5, 0.05% SDS and 0.1 mg/ml Proteinase K.
- 10×T4 ligation buffer (NEB)
- 400 U/µl T4 Ligase (NEB)
- 10 mM PMSF (Sigma): dissolve PSMF powder to 100 mM with isopropanol. Dilute 100 mM PMSF to 10 mM with ddH_2O before use.

Preparing oligonucleotides for ligations

1. Dissolve the round 1 linker with STE buffer to 90 µM. Dissolve the round 1 barcodes with STE buffer to 100 µM. Dissolve the round 2 linker with STE buffer to 110 µM. Dissolve the round 2 barcodes with STE buffer to 120 µM.

2. Mix 20 µl round 1 linker and 20 µl round 1 barcodes and place them at a thermal cycler for 5 min at 95°C followed by programmed temperature decrease at 0.1°C/s to 25°C. The resulting round 2 hybridization adaptor concentration is 45 µM linker and 50 µM barcodes. Store the annealed round 1 hybridization adaptors at 4°C before use.

3. Mix 20 µl round 2 linker and 20 µl round 2 barcodes and place them at a thermal cycler for 5 min at 95°C followed by programmed temperature decrease at 0.1°C/s to 25°C. The resulting round 2 hybridization adaptor concentration is 55 µM linker and 60 µM barcodes. Store the annealed round 2 hybridization adaptors at 4°C before use.

4. Dilute the hybridization adaptors to working solution: take 2 µl annealed round 1 or 2 hybridization adaptor to 38 µl STE buffer to make a 40 µl mix (20-fold dilution). The working solution is suggested to be used within 1 week.

Samples fixation

1. Harvest cells and wash cells once with 0.1% BSA-PBS.

2. Resuspend cells with 1 ml cold 0.1% BSA-PBS and add 7 μl 36.5% formaldehyde to the system (working solution of formaldehyde is 0.25%), quickly turn upside down to mix well. Sit the system on ice for 5 minutes for fixation. Other fixation conditions are also available and practical, such as 0.1%-0.25% FA at room-temperature for 3 min, 1%-4% PFA on ice 3 min, methanol only, etc.

3. Add 14 μl 2.5 M Glycine to the system, quickly turn upside down to mix well. Sit the system on ice for another 5 minutes to quench free formaldehyde.

4. Wash cells twice with 0.1% BSA-PBS.

5. Resuspend cells with 100 μl 0.1% BSA-PBS and add 900 μl pre-chilled -20°C methanol dropwise to the system. The fixed samples can be stored at -80°C for one year.

Antibody-PAT-T7 complex assembly

1. Set up the following assembly system at minimal volume. Incubate the system at room temperature for 1 hour.

Antibody------0.5 μg (3.33 pmol)

37.5 μM PAT-T7------0.22 μl (8.25 pmol)

Wash buffer------5 μl

2. The Antibody-PAT-T7 complex is suggested to be used in uCoTarget experiments within 24 hours of assembly.

Antibody-tethered tagmentation

1. Wash cells twice with 0.1% BSA-PBS.

2. Incubate cells with 1st kind of Antibody-PAT-T7 complex in 100 μl Wash-buffer-TX-high salt plus 2 mM EDTA at room temperature for 1 hour.

3. Wash cells 3 times with Wash-buffer-TX-high salt to remove free unbound complex.

4. Resuspend cells with 50 μl Reaction buffer and incubate the reaction at 37°C for 1 hour at a thermal cycler. Hot lid is set 40°C.

5. Centrifuge the cells and remove the Reaction buffer. Resuspend cells with 180 μl Wash-buffer-TX-high salt with 5 mM EDTA and rotate at room temperature for 5 minutes.

6. Repeat the above 2-5 steps for the other kinds of Antibody-PAT-T7 complex.

Free PAT tagmentation

1. Incubate cells with secondary antibodies corresponding to the species in which the primary antibody conjugates at 1:1000 dilution factor in 100 μl Wash-buffer-TX at 4°C for 30 minutes.

2. Wash cells 3 times with Wash-buffer-Dig.

3. Distribute cells to 8 wells with different PAT-T5 (dilute 37.5 μM PAT-T5 at 450 fold in the system) in each well in 100 μl Wash-buffer-TX-high salt. Incubate the system at 4°C for 1 hour.

4. Wash cells twice with Wash-buffer-TX-high salt.
5. Resuspend cells with 50 μl reaction buffer and incubate the reaction at 30°C for 1 hour at a thermal cycler. Hot lid is set 40°C.
6. Remove the reaction buffer by centrifugation. Resuspend cells with 180 μl Wash-buffer-TX-high salt with 5 mM EDTA and rotate at room temperature for 5 minutes.

2 round Ligation

1. Resuspend cells with ~1 ml NSB and distribute cells to 96 wells at 10 μl each well. The 96 wells have contained 40 μl 1st round ligation mix. Incubate the system at room temperature for 30 minutes with gentle shaking (300 rpm).
10×T4 ligation buffer------5 μl
10% TX-100------0.2 μl
NSB------10 μl
ddH_2O------22.8 μl
Round 1 hybridization adaptor------2 μl
cells in NSB buffer------10 μl
2. Add the following 10 μl round 1 blocking mix to each well. Incubate the system at room temperature for 30 minutes with gentle shaking (300 rpm).
100 μM round 1 blocking------0.11 μl
10×T4 ligation buffer------2 μl
ddH_2O------7.89 μl
3. Combine all wells and distribute cells to a new plate of 96 wells at 50 μl each well. The new 96 wells have contained the following 10 μl round 2 hybridization mix in each well. Incubate the system at room temperature for 30 minutes with gentle shaking (300 rpm).
Round 2 hybridization adaptor------2 μl
ddH_2O------8 μl
4. Add the following 10 μl round 2 blocking mix to each well. Incubate the system at room temperature for 30 minutes with gentle shaking (300 rpm).
100 μM round 2 blocking------0.132 μl
1% TX-100------1 μl
ddH_2O------8.868 μl
5. Combine all wells and wash cells twice with NSB. Resuspend cells with the following 200 μl final ligation system. Incubate the system at room temperature for 30 minutes with gentle shaking (300 rpm).
10×T4 ligation buffer------20 μl
400 U/μl T4 Ligase------10 μl
1% TX-100------10 μl
NSB------40 μl
ddH2O------120 μl
• Note: final ligation system can be scaled down to 20 μl for each sample in pilot experiments.
6. Wash cells twice with NSB. Count cells density and dilute cells to 2,000-5,000/μl with 0.1%

BSA-PBS.

7. Aliquot cells to as much as possible wells at 1 μl/well. The wells have contained 4 μl lysis buffer. Incubate the mix at 55°C for 30 minutes to lyse cells.

8. Add 1 μl 1.8% TX-100 and 1 μl 10 mM PMSF to each well and incubate the system at 37°C for 15 minutes to quench SDS and deactivate proteinase K.

Library preparation

1. Prepare index PCR mix as follows. Perform PCR as: 72°C 5 min; 95°C 2 min; 9 cycles of 98°C 20s, 65°C 30s, 72°C 1 min; 72°C 5 min.

5xHiFi buffer------10 μl
10 mM dNTP------1 μl
Truseq-i5-connector (25 μM) ------1 μl
Truseq-i5-connector (25 μM) ------1 μl
25 mM MgCl2------1 μl
KAPA enzyme------0.5 μl
ddH2O------to 50 μl

2. Digest excess primers by adding 0.5 μl ExoI (NEB) and incubate at 37 °C for 60 min followed by 72 °C for 20 min.

3. Prepare index PCR mix as follows. Perform PCR as: 95°C 2 min; 6 cycles of 98°C 20s, 65°C 30s, 72°C 1 min; 72°C 5 min.

5xHiFi buffer------2 μl
10 mM dNTP------0.2 μl
Truseq P5 (25 μM) ------1 μl
Truseq P7(25 μM) ------1 μl
25 mM MgCl2------0.2 μl
KAPA enzyme------0.1 μl
ddH2O------to 10 μl

4. Purify the PCR product with 0.9× AMPure XP beads followed by 0.45× + 0.45× for size selection. Elute the final library product in 20 μl ddH_2O.

5. Measure the library concentration with Qubit.

6. Libraries can be sequenced with the standard recipe on the Nova-seq platform (Illumina).

Table 1. The sequences of custom oligonucleotides used in uCoTarget experiments (Xiong et al., Science Advances, 2024).

Name	Sequence	Modification
TnExtMErev_p	CTGTCTCTTATACACATCT	5'P
round1-linker	GACGCTGCCGACGATCGGACGATCATGGG	
round1-blocking_oligo	CCCATGATCGTCCGATCGTCGGCAGCGTC	
round2-linker	CAAGTATGCAGCGCGCTCAAGCACGTGGAT	
round2-blocking_oligo	ATCCACGTGCTTGAGCGCGCTGCATACTTG	
Truseq-i5-connector	ACACTCTTTCCCTACACGACGCTCTTCCGATCT	
Truseq-i7-connector	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTCGTGGGCTCGGCTGTCCC	

Table 2. The sequences of PAT-T7 used in uCoTarget experiments (Xiong et al., Science Advances, 2024).

Name	Barcode	Sequence	Modification
PAT-T7-1	AAGTAT	GTCTCGTGGGCTCGGCTGTCCCTGTCCAAGTATAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-2	CAGACT	GTCTCGTGGGCTCGGCTGTCCCTGTCCCAGACTAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-3	GACTCG	GTCTCGTGGGCTCGGCTGTCCCTGTCCGACTCGAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-4	TACCGA	GTCTCGTGGGCTCGGCTGTCCCTGTCCTACCGAAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-5	TAGAGG	GTCTCGTGGGCTCGGCTGTCCCTGTCCTAGAGGAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-6	TATTTC	GTCTCGTGGGCTCGGCTGTCCCTGTCCTATTTCAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-7	CCGTAA	GTCTCGTGGGCTCGGCTGTCCCTGTCCCCGTAAAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-8	CGAAAG	GTCTCGTGGGCTCGGCTGTCCCTGTCCCGAAAGAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-9	TGAATT	GTCTCGTGGGCTCGGCTGTCCCTGTCCTGAATTAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-10	TCAGTG	GTCTCGTGGGCTCGGCTGTCCCTGTCCTCAGTGAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-11	TCATCA	GTCTCGTGGGCTCGGCTGTCCCTGTCCTCATCAAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-12	TCCAAG	GTCTCGTGGGCTCGGCTGTCCCTGTCCTCCAAGAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-13	TGGCAG	GTCTCGTGGGCTCGGCTGTCCCTGTCCTGGCAGAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-14	TGTGTA	GTCTCGTGGGCTCGGCTGTCCCTGTCCTGTGTAAGATGTGTATAAGAGACAG	5'NH2-C12
PAT-T7-15	TGTTCG	GTCTCGTGGGCTCGGCTGTCCCTGTCCTGTTCGAGATGTGTATAAGAGACAG	5'NH2-C12

• The antibody/target protein identities were discerned using corresponding T7 barcodes and were deconvoluted in data processing.

• The 5'NH2-C12 modification was used for antibody-PAT-T7 barcoded adaptor covalent conjugation (refer to as “labeled protocol”). The labeled protocol was not adopted in the final version of uCoTarget protocol since we found that noncovalent conjugation (refer to as “unlabeled protocol”) showed better performance in data quality.

Table 3. The sequences of PAT-T5 used in uCoTarget experiments (Xiong et al., Science Advances, 2024).

Name	Barcode	Sequence	Modification
PAT-T5-1	AAAGAA	TCGTCGGCAGCGTCAAAGAAAGATGTGTATAAGAGACAG	5'P
PAT-T5-2	AACAGC	TCGTCGGCAGCGTCAACAGCAGATGTGTATAAGAGACAG	5'P
PAT-T5-3	AACGTG	TCGTCGGCAGCGTCAACGTGAGATGTGTATAAGAGACAG	5'P
PAT-T5-4	AAGCCA	TCGTCGGCAGCGTCAAGCCAAGATGTGTATAAGAGACAG	5'P
PAT-T5-5	AAGTAT	TCGTCGGCAGCGTCAAGTATAGATGTGTATAAGAGACAG	5'P
PAT-T5-6	AATTGG	TCGTCGGCAGCGTCAATTGGAGATGTGTATAAGAGACAG	5'P
PAT-T5-7	ACAAGG	TCGTCGGCAGCGTCACAAGGAGATGTGTATAAGAGACAG	5'P
PAT-T5-8	ACCCAA	TCGTCGGCAGCGTCACCCAAAGATGTGTATAAGAGACAG	5'P
PAT-T5-9	CAACCG	TCGTCGGCAGCGTCCAACCGAGATGTGTATAAGAGACAG	5'P
PAT-T5-10	CAAGTC	TCGTCGGCAGCGTCCAAGTCAGATGTGTATAAGAGACAG	5'P
PAT-T5-11	CACCAC	TCGTCGGCAGCGTCCACCACAGATGTGTATAAGAGACAG	5'P
PAT-T5-12	CACTGT	TCGTCGGCAGCGTCCACTGTAGATGTGTATAAGAGACAG	5'P
PAT-T5-13	CAGACT	TCGTCGGCAGCGTCCAGACTAGATGTGTATAAGAGACAG	5'P
PAT-T5-14	CAGGAG	TCGTCGGCAGCGTCCAGGAGAGATGTGTATAAGAGACAG	5'P
PAT-T5-15	CATAGA	TCGTCGGCAGCGTCCATAGAAGATGTGTATAAGAGACAG	5'P
PAT-T5-16	CCACGC	TCGTCGGCAGCGTCCCACGCAGATGTGTATAAGAGACAG	5'P
PAT-T5-17	GAAATA	TCGTCGGCAGCGTCGAAATAAGATGTGTATAAGAGACAG	5'P
PAT-T5-18	GAAGGG	TCGTCGGCAGCGTCGAAGGGAGATGTGTATAAGAGACAG	5'P
PAT-T5-19	GACTCG	TCGTCGGCAGCGTCGACTCGAGATGTGTATAAGAGACAG	5'P
PAT-T5-20	GAGCTT	TCGTCGGCAGCGTCGAGCTTAGATGTGTATAAGAGACAG	5'P
PAT-T5-21	GAGGCC	TCGTCGGCAGCGTCGAGGCCAGATGTGTATAAGAGACAG	5'P
PAT-T5-22	GAGTGA	TCGTCGGCAGCGTCGAGTGAAGATGTGTATAAGAGACAG	5'P
PAT-T5-23	GATCAA	TCGTCGGCAGCGTCGATCAAAGATGTGTATAAGAGACAG	5'P
PAT-T5-24	GCCAGA	TCGTCGGCAGCGTCGCCAGAAGATGTGTATAAGAGACAG	5'P
PAT-T5-25	TAAGCT	TCGTCGGCAGCGTCTAAGCTAGATGTGTATAAGAGACAG	5'P
PAT-T5-26	TAATAG	TCGTCGGCAGCGTCTAATAGAGATGTGTATAAGAGACAG	5'P
PAT-T5-27	TACCGA	TCGTCGGCAGCGTCTACCGAAGATGTGTATAAGAGACAG	5'P
PAT-T5-28	TAGAGG	TCGTCGGCAGCGTCTAGAGGAGATGTGTATAAGAGACAG	5'P
PAT-T5-29	TATTTC	TCGTCGGCAGCGTCTATTTCAGATGTGTATAAGAGACAG	5'P
PAT-T5-30	TCAGTG	TCGTCGGCAGCGTCTCAGTGAGATGTGTATAAGAGACAG	5'P
PAT-T5-31	TCATCA	TCGTCGGCAGCGTCTCATCAAGATGTGTATAAGAGACAG	5'P
PAT-T5-32	TCCAAG	TCGTCGGCAGCGTCTCCAAGAGATGTGTATAAGAGACAG	5'P

• The PAT-T5 barcode was used for 1st round barcoding.

• The 5'P modification was essential for ligation.

Table 4. The sequences of round 1 adaptor used in uCoTarget experiments (Xiong et al., Science Advances, 2024).

Name	Barcode	Sequence	Modification
R1_1	AAAGAA	CGCGCTGCATACTTGAAAGAACCCATGATCGTCCGA	5'P
R1_2	AACAGC	CGCGCTGCATACTTGAACAGCCCCATGATCGTCCGA	5'P
R1_3	AACGTG	CGCGCTGCATACTTGAACGTGCCCATGATCGTCCGA	5'P
R1_4	AAGCCA	CGCGCTGCATACTTGAAGCCACCCATGATCGTCCGA	5'P
R1_5	AAGTAT	CGCGCTGCATACTTGAAGTATCCCATGATCGTCCGA	5'P
R1_6	AATTGG	CGCGCTGCATACTTGAATTGGCCCATGATCGTCCGA	5'P
R1_7	ACAAGG	CGCGCTGCATACTTGACAAGGCCCATGATCGTCCGA	5'P
R1_8	ACCCAA	CGCGCTGCATACTTGACCCAACCCATGATCGTCCGA	5'P
R1_9	ACCTTC	CGCGCTGCATACTTGACCTTCCCCATGATCGTCCGA	5'P
R1_10	ACGGAC	CGCGCTGCATACTTGACGGACCCCATGATCGTCCGA	5'P
R1_11	ACTGCA	CGCGCTGCATACTTGACTGCACCCATGATCGTCCGA	5'P
R1_12	AGACCC	CGCGCTGCATACTTGAGACCCCCCATGATCGTCCGA	5'P
R1_13	AGATGT	CGCGCTGCATACTTGAGATGTCCCATGATCGTCCGA	5'P
R1_14	AGCACG	CGCGCTGCATACTTGAGCACGCCCATGATCGTCCGA	5'P
R1_15	AGGTTA	CGCGCTGCATACTTGAGGTTACCCATGATCGTCCGA	5'P
R1_16	AGTAAA	CGCGCTGCATACTTGAGTAAACCCATGATCGTCCGA	5'P
R1_17	AGTCTG	CGCGCTGCATACTTGAGTCTGCCCATGATCGTCCGA	5'P
R1_18	ATACTT	CGCGCTGCATACTTGATACTTCCCATGATCGTCCGA	5'P
R1_19	ATAGCG	CGCGCTGCATACTTGATAGCGCCCATGATCGTCCGA	5'P
R1_20	ATATAC	CGCGCTGCATACTTGATATACCCCATGATCGTCCGA	5'P
R1_21	ATCCGG	CGCGCTGCATACTTGATCCGGCCCATGATCGTCCGA	5'P
R1_22	ATGAAG	CGCGCTGCATACTTGATGAAGCCCATGATCGTCCGA	5'P
R1_23	ATTAGT	CGCGCTGCATACTTGATTAGTCCCATGATCGTCCGA	5'P
R1_24	CAACCG	CGCGCTGCATACTTGCAACCGCCCATGATCGTCCGA	5'P
R1_25	CAAGTC	CGCGCTGCATACTTGCAAGTCCCCATGATCGTCCGA	5'P
R1_26	CACCAC	CGCGCTGCATACTTGCACCACCCCATGATCGTCCGA	5'P
R1_27	CACTGT	CGCGCTGCATACTTGCACTGTCCCATGATCGTCCGA	5'P
R1_28	CAGACT	CGCGCTGCATACTTGCAGACTCCCATGATCGTCCGA	5'P
R1_29	CAGGAG	CGCGCTGCATACTTGCAGGAGCCCATGATCGTCCGA	5'P
R1_30	CATAGA	CGCGCTGCATACTTGCATAGACCCATGATCGTCCGA	5'P
R1_31	CCACGC	CGCGCTGCATACTTGCCACGCCCCATGATCGTCCGA	5'P
R1_32	CCGATG	CGCGCTGCATACTTGCCGATGCCCATGATCGTCCGA	5'P
R1_33	CCGTAA	CGCGCTGCATACTTGCCGTAACCCATGATCGTCCGA	5'P
R1_34	CCTCTA	CGCGCTGCATACTTGCCTCTACCCATGATCGTCCGA	5'P
R1_35	CGAAAG	CGCGCTGCATACTTGCGAAAGCCCATGATCGTCCGA	5'P
R1_36	CGAGCA	CGCGCTGCATACTTGCGAGCACCCATGATCGTCCGA	5'P

R1_37	CGCATA	CGCGCTGCATACTTGCGCATACCCATGATCGTCCGA	5'P
R1_38	CGGCGT	CGCGCTGCATACTTGCGGCGTCCCATGATCGTCCGA	5'P
R1_39	CGGTCC	CGCGCTGCATACTTGCGGTCCCCCATGATCGTCCGA	5'P
R1_40	CGTTAT	CGCGCTGCATACTTGCGTTATCCCATGATCGTCCGA	5'P
R1_41	CTAGGT	CGCGCTGCATACTTGCTAGGTCCCATGATCGTCCGA	5'P
R1_42	CTATTA	CGCGCTGCATACTTGCTATTACCCATGATCGTCCGA	5'P
R1_43	CTCAAT	CGCGCTGCATACTTGCTCAATCCCATGATCGTCCGA	5'P
R1_44	CTGTGG	CGCGCTGCATACTTGCTGTGGCCCATGATCGTCCGA	5'P
R1_45	CTTACG	CGCGCTGCATACTTGCTTACGCCCATGATCGTCCGA	5'P
R1_46	CTTGAA	CGCGCTGCATACTTGCTTGAACCCATGATCGTCCGA	5'P
R1_47	GAAATA	CGCGCTGCATACTTGGAAATACCCATGATCGTCCGA	5'P
R1_48	GAAGGG	CGCGCTGCATACTTGGAAGGGCCCATGATCGTCCGA	5'P
R1_49	GACTCG	CGCGCTGCATACTTGGACTCGCCCATGATCGTCCGA	5'P
R1_50	GAGCTT	CGCGCTGCATACTTGGAGCTTCCCATGATCGTCCGA	5'P
R1_51	GAGGCC	CGCGCTGCATACTTGGAGGCCCCCATGATCGTCCGA	5'P
R1_52	GAGTGA	CGCGCTGCATACTTGGAGTGACCCATGATCGTCCGA	5'P
R1_53	GATCAA	CGCGCTGCATACTTGGATCAACCCATGATCGTCCGA	5'P
R1_54	GCCAGA	CGCGCTGCATACTTGGCCAGACCCATGATCGTCCGA	5'P
R1_55	GCCGTT	CGCGCTGCATACTTGGCCGTTCCCATGATCGTCCGA	5'P
R1_56	GCGAAT	CGCGCTGCATACTTGGCGAATCCCATGATCGTCCGA	5'P
R1_57	GCGCGG	CGCGCTGCATACTTGGCGCGGCCCATGATCGTCCGA	5'P
R1_58	GCTCCC	CGCGCTGCATACTTGGCTCCCCCCATGATCGTCCGA	5'P
R1_59	GCTGAG	CGCGCTGCATACTTGGCTGAGCCCATGATCGTCCGA	5'P
R1_60	GCTTGT	CGCGCTGCATACTTGGCTTGTCCCATGATCGTCCGA	5'P
R1_61	GGACGA	CGCGCTGCATACTTGGGACGACCCATGATCGTCCGA	5'P
R1_62	GGATTG	CGCGCTGCATACTTGGGATTGCCCATGATCGTCCGA	5'P
R1_63	GGCCAT	CGCGCTGCATACTTGGGCCATCCCATGATCGTCCGA	5'P
R1_64	GGGATC	CGCGCTGCATACTTGGGGATCCCCATGATCGTCCGA	5'P
R1_65	GGTAGG	CGCGCTGCATACTTGGGTAGGCCCATGATCGTCCGA	5'P
R1_66	GGTGCT	CGCGCTGCATACTTGGGTGCTCCCATGATCGTCCGA	5'P
R1_67	GTACAG	CGCGCTGCATACTTGGTACAGCCCATGATCGTCCGA	5'P
R1_68	GTCCTA	CGCGCTGCATACTTGGTCCTACCCATGATCGTCCGA	5'P
R1_69	GTCGGC	CGCGCTGCATACTTGGTCGGCCCCATGATCGTCCGA	5'P
R1_70	GTGGTG	CGCGCTGCATACTTGGTGGTGCCCATGATCGTCCGA	5'P
R1_71	GTTAAC	CGCGCTGCATACTTGGTTAACCCCATGATCGTCCGA	5'P
R1_72	GTTTCA	CGCGCTGCATACTTGGTTTCACCCATGATCGTCCGA	5'P
R1_73	TAAGCT	CGCGCTGCATACTTGTAAGCTCCCATGATCGTCCGA	5'P
R1_74	TAATAG	CGCGCTGCATACTTGTAATAGCCCATGATCGTCCGA	5'P
R1_75	TACCGA	CGCGCTGCATACTTGTACCGACCCATGATCGTCCGA	5'P

R1_76	TAGAGG	CGCGCTGCATACTTGTAGAGGCCCATGATCGTCCGA	5'P
R1_77	TATTTC	CGCGCTGCATACTTGTATTTCCCCATGATCGTCCGA	5'P
R1_78	TCAGTG	CGCGCTGCATACTTGTCAGTGCCCATGATCGTCCGA	5'P
R1_79	TCATCA	CGCGCTGCATACTTGTCATCACCCATGATCGTCCGA	5'P
R1_80	TCCAAG	CGCGCTGCATACTTGTCCAAGCCCATGATCGTCCGA	5'P
R1_81	TCGCCT	CGCGCTGCATACTTGTCGCCTCCCATGATCGTCCGA	5'P
R1_82	TCGGGA	CGCGCTGCATACTTGTCGGGACCCATGATCGTCCGA	5'P
R1_83	TCTAGC	CGCGCTGCATACTTGTCTAGCCCCATGATCGTCCGA	5'P
R1_84	TGAATT	CGCGCTGCATACTTGTGAATTCCCATGATCGTCCGA	5'P
R1_85	TGAGAC	CGCGCTGCATACTTGTGAGACCCCATGATCGTCCGA	5'P
R1_86	TGCGGT	CGCGCTGCATACTTGTGCGGTCCCATGATCGTCCGA	5'P
R1_87	TGCTAA	CGCGCTGCATACTTGTGCTAACCCATGATCGTCCGA	5'P
R1_88	TGGCAG	CGCGCTGCATACTTGTGGCAGCCCATGATCGTCCGA	5'P
R1_89	TGTGTA	CGCGCTGCATACTTGTGTGTACCCATGATCGTCCGA	5'P
R1_90	TGTTCG	CGCGCTGCATACTTGTGTTCGCCCATGATCGTCCGA	5'P
R1_91	TTAAGA	CGCGCTGCATACTTGTTAAGACCCATGATCGTCCGA	5'P
R1_92	TTCGCA	CGCGCTGCATACTTGTTCGCACCCATGATCGTCCGA	5'P
R1_93	TTCTTG	CGCGCTGCATACTTGTTCTTGCCCATGATCGTCCGA	5'P
R1_94	TTGCTC	CGCGCTGCATACTTGTTGCTCCCCATGATCGTCCGA	5'P
R1_95	TTGGAT	CGCGCTGCATACTTGTTGGATCCCATGATCGTCCGA	5'P
R1_96	TTTGGG	CGCGCTGCATACTTGTTTGGGCCCATGATCGTCCGA	5'P

• The round 1 adaptor was used for 2nd round barcoding.

• The 5'P modification was essential for ligation.

Table 5. The sequences of round 2 adaptor used in uCoTarget experiments (Xiong et al., Science Advances, 2024).

Name	Barcode	Sequence
R2_1	AACGTGAT	CCTACACGACGCTCTTCCGATCTAACGTGATATCCACGTGCTTGAG
R2_2	AAACATCG	CCTACACGACGCTCTTCCGATCTAAACATCGATCCACGTGCTTGAG
R2_3	ATGCCTAA	CCTACACGACGCTCTTCCGATCTATGCCTAAATCCACGTGCTTGAG
R2_4	AGTGGTCA	CCTACACGACGCTCTTCCGATCTAGTGGTCAATCCACGTGCTTGAG
R2_5	ACCACTGT	CCTACACGACGCTCTTCCGATCTACCACTGTATCCACGTGCTTGAG
R2_6	ACATTGGC	CCTACACGACGCTCTTCCGATCTACATTGGCATCCACGTGCTTGAG
R2_7	CAGATCTG	CCTACACGACGCTCTTCCGATCTCAGATCTGATCCACGTGCTTGAG
R2_8	CATCAAGT	CCTACACGACGCTCTTCCGATCTCATCAAGTATCCACGTGCTTGAG
R2_9	CGCTGATC	CCTACACGACGCTCTTCCGATCTCGCTGATCATCCACGTGCTTGAG
R2_10	ACAAGCTA	CCTACACGACGCTCTTCCGATCTACAAGCTAATCCACGTGCTTGAG
R2_11	CTGTAGCC	CCTACACGACGCTCTTCCGATCTCTGTAGCCATCCACGTGCTTGAG
R2_12	AGTACAAG	CCTACACGACGCTCTTCCGATCTAGTACAAGATCCACGTGCTTGAG
R2_13	AACAACCA	CCTACACGACGCTCTTCCGATCTAACAACCAATCCACGTGCTTGAG
R2_14	AACCGAGA	CCTACACGACGCTCTTCCGATCTAACCGAGAATCCACGTGCTTGAG
R2_15	AACGCTTA	CCTACACGACGCTCTTCCGATCTAACGCTTAATCCACGTGCTTGAG
R2_16	AAGACGGA	CCTACACGACGCTCTTCCGATCTAAGACGGAATCCACGTGCTTGAG
R2_17	AAGGTACA	CCTACACGACGCTCTTCCGATCTAAGGTACAATCCACGTGCTTGAG
R2_18	ACACAGAA	CCTACACGACGCTCTTCCGATCTACACAGAAATCCACGTGCTTGAG
R2_19	ACAGCAGA	CCTACACGACGCTCTTCCGATCTACAGCAGAATCCACGTGCTTGAG
R2_20	ACCTCCAA	CCTACACGACGCTCTTCCGATCTACCTCCAAATCCACGTGCTTGAG
R2_21	ACGCTCGA	CCTACACGACGCTCTTCCGATCTACGCTCGAATCCACGTGCTTGAG
R2_22	ACGTATCA	CCTACACGACGCTCTTCCGATCTACGTATCAATCCACGTGCTTGAG
R2_23	ACTATGCA	CCTACACGACGCTCTTCCGATCTACTATGCAATCCACGTGCTTGAG
R2_24	AGAGTCAA	CCTACACGACGCTCTTCCGATCTAGAGTCAAATCCACGTGCTTGAG
R2_25	AGATCGCA	CCTACACGACGCTCTTCCGATCTAGATCGCAATCCACGTGCTTGAG
R2_26	AGCAGGAA	CCTACACGACGCTCTTCCGATCTAGCAGGAAATCCACGTGCTTGAG
R2_27	AGTCACTA	CCTACACGACGCTCTTCCGATCTAGTCACTAATCCACGTGCTTGAG
R2_28	ATCCTGTA	CCTACACGACGCTCTTCCGATCTATCCTGTAATCCACGTGCTTGAG
R2_29	ATTGAGGA	CCTACACGACGCTCTTCCGATCTATTGAGGAATCCACGTGCTTGAG
R2_30	CAACCACA	CCTACACGACGCTCTTCCGATCTCAACCACAATCCACGTGCTTGAG
R2_31	GACTAGTA	CCTACACGACGCTCTTCCGATCTGACTAGTAATCCACGTGCTTGAG
R2_32	CAATGGAA	CCTACACGACGCTCTTCCGATCTCAATGGAAATCCACGTGCTTGAG
R2_33	CACTTCGA	CCTACACGACGCTCTTCCGATCTCACTTCGAATCCACGTGCTTGAG
R2_34	CAGCGTTA	CCTACACGACGCTCTTCCGATCTCAGCGTTAATCCACGTGCTTGAG
R2_35	CATACCAA	CCTACACGACGCTCTTCCGATCTCATACCAAATCCACGTGCTTGAG
R2_36	CCAGTTCA	CCTACACGACGCTCTTCCGATCTCCAGTTCAATCCACGTGCTTGAG

R2_37	CCGAAGTA	CCTACACGACGCTCTTCCGATCTCCGAAGTAATCCACGTGCTTGAG
R2_38	CCGTGAGA	CCTACACGACGCTCTTCCGATCTCCGTGAGAATCCACGTGCTTGAG
R2_39	CCTCCTGA	CCTACACGACGCTCTTCCGATCTCCTCCTGAATCCACGTGCTTGAG
R2_40	CGAACTTA	CCTACACGACGCTCTTCCGATCTCGAACTTAATCCACGTGCTTGAG
R2_41	CGACTGGA	CCTACACGACGCTCTTCCGATCTCGACTGGAATCCACGTGCTTGAG
R2_42	CGCATACA	CCTACACGACGCTCTTCCGATCTCGCATACAATCCACGTGCTTGAG
R2_43	CTCAATGA	CCTACACGACGCTCTTCCGATCTCTCAATGAATCCACGTGCTTGAG
R2_44	CTGAGCCA	CCTACACGACGCTCTTCCGATCTCTGAGCCAATCCACGTGCTTGAG
R2_45	CTGGCATA	CCTACACGACGCTCTTCCGATCTCTGGCATAATCCACGTGCTTGAG
R2_46	GAATCTGA	CCTACACGACGCTCTTCCGATCTGAATCTGAATCCACGTGCTTGAG
R2_47	CAAGACTA	CCTACACGACGCTCTTCCGATCTCAAGACTAATCCACGTGCTTGAG
R2_48	GAGCTGAA	CCTACACGACGCTCTTCCGATCTGAGCTGAAATCCACGTGCTTGAG
R2_49	GATAGACA	CCTACACGACGCTCTTCCGATCTGATAGACAATCCACGTGCTTGAG
R2_50	GCCACATA	CCTACACGACGCTCTTCCGATCTGCCACATAATCCACGTGCTTGAG
R2_51	GCGAGTAA	CCTACACGACGCTCTTCCGATCTGCGAGTAAATCCACGTGCTTGAG
R2_52	GCTAACGA	CCTACACGACGCTCTTCCGATCTGCTAACGAATCCACGTGCTTGAG
R2_53	GCTCGGTA	CCTACACGACGCTCTTCCGATCTGCTCGGTAATCCACGTGCTTGAG
R2_54	GGAGAACA	CCTACACGACGCTCTTCCGATCTGGAGAACAATCCACGTGCTTGAG
R2_55	GGTGCGAA	CCTACACGACGCTCTTCCGATCTGGTGCGAAATCCACGTGCTTGAG
R2_56	GTACGCAA	CCTACACGACGCTCTTCCGATCTGTACGCAAATCCACGTGCTTGAG
R2_57	GTCGTAGA	CCTACACGACGCTCTTCCGATCTGTCGTAGAATCCACGTGCTTGAG
R2_58	GTCTGTCA	CCTACACGACGCTCTTCCGATCTGTCTGTCAATCCACGTGCTTGAG
R2_59	GTGTTCTA	CCTACACGACGCTCTTCCGATCTGTGTTCTAATCCACGTGCTTGAG
R2_60	TAGGATGA	CCTACACGACGCTCTTCCGATCTTAGGATGAATCCACGTGCTTGAG
R2_61	TATCAGCA	CCTACACGACGCTCTTCCGATCTTATCAGCAATCCACGTGCTTGAG
R2_62	TCCGTCTA	CCTACACGACGCTCTTCCGATCTTCCGTCTAATCCACGTGCTTGAG
R2_63	TCTTCACA	CCTACACGACGCTCTTCCGATCTTCTTCACAATCCACGTGCTTGAG
R2_64	TGAAGAGA	CCTACACGACGCTCTTCCGATCTTGAAGAGAATCCACGTGCTTGAG
R2_65	TGGAACAA	CCTACACGACGCTCTTCCGATCTTGGAACAAATCCACGTGCTTGAG
R2_66	TGGCTTCA	CCTACACGACGCTCTTCCGATCTTGGCTTCAATCCACGTGCTTGAG
R2_67	TGGTGGTA	CCTACACGACGCTCTTCCGATCTTGGTGGTAATCCACGTGCTTGAG
R2_68	TTCACGCA	CCTACACGACGCTCTTCCGATCTTTCACGCAATCCACGTGCTTGAG
R2_69	AACTCACC	CCTACACGACGCTCTTCCGATCTAACTCACCATCCACGTGCTTGAG
R2_70	AAGAGATC	CCTACACGACGCTCTTCCGATCTAAGAGATCATCCACGTGCTTGAG
R2_71	AAGGACAC	CCTACACGACGCTCTTCCGATCTAAGGACACATCCACGTGCTTGAG
R2_72	AATCCGTC	CCTACACGACGCTCTTCCGATCTAATCCGTCATCCACGTGCTTGAG
R2_73	AATGTTGC	CCTACACGACGCTCTTCCGATCTAATGTTGCATCCACGTGCTTGAG
R2_74	ACACGACC	CCTACACGACGCTCTTCCGATCTACACGACCATCCACGTGCTTGAG
R2_75	ACAGATTC	CCTACACGACGCTCTTCCGATCTACAGATTCATCCACGTGCTTGAG

R2_76	AGATGTAC	CCTACACGACGCTCTTCCGATCTAGATGTACATCCACGTGCTTGAG
R2_77	AGCACCTC	CCTACACGACGCTCTTCCGATCTAGCACCTCATCCACGTGCTTGAG
R2_78	AGCCATGC	CCTACACGACGCTCTTCCGATCTAGCCATGCATCCACGTGCTTGAG
R2_79	AGGCTAAC	CCTACACGACGCTCTTCCGATCTAGGCTAACATCCACGTGCTTGAG
R2_80	ATAGCGAC	CCTACACGACGCTCTTCCGATCTATAGCGACATCCACGTGCTTGAG
R2_81	ATCATTCC	CCTACACGACGCTCTTCCGATCTATCATTCCATCCACGTGCTTGAG
R2_82	ATTGGCTC	CCTACACGACGCTCTTCCGATCTATTGGCTCATCCACGTGCTTGAG
R2_83	CAAGGAGC	CCTACACGACGCTCTTCCGATCTCAAGGAGCATCCACGTGCTTGAG
R2_84	CACCTTAC	CCTACACGACGCTCTTCCGATCTCACCTTACATCCACGTGCTTGAG
R2_85	CCATCCTC	CCTACACGACGCTCTTCCGATCTCCATCCTCATCCACGTGCTTGAG
R2_86	CCGACAAC	CCTACACGACGCTCTTCCGATCTCCGACAACATCCACGTGCTTGAG
R2_87	CCTAATCC	CCTACACGACGCTCTTCCGATCTCCTAATCCATCCACGTGCTTGAG
R2_88	CCTCTATC	CCTACACGACGCTCTTCCGATCTCCTCTATCATCCACGTGCTTGAG
R2_89	CGACACAC	CCTACACGACGCTCTTCCGATCTCGACACACATCCACGTGCTTGAG
R2_90	CGGATTGC	CCTACACGACGCTCTTCCGATCTCGGATTGCATCCACGTGCTTGAG
R2_91	CTAAGGTC	CCTACACGACGCTCTTCCGATCTCTAAGGTCATCCACGTGCTTGAG
R2_92	GAACAGGC	CCTACACGACGCTCTTCCGATCTGAACAGGCATCCACGTGCTTGAG
R2_93	GACAGTGC	CCTACACGACGCTCTTCCGATCTGACAGTGCATCCACGTGCTTGAG
R2_94	GAGTTAGC	CCTACACGACGCTCTTCCGATCTGAGTTAGCATCCACGTGCTTGAG
R2_95	GATGAATC	CCTACACGACGCTCTTCCGATCTGATGAATCATCCACGTGCTTGAG
R2_96	GCCAAGAC	CCTACACGACGCTCTTCCGATCTGCCAAGACATCCACGTGCTTGAG

• The round 2 adaptor was used for 3rd round barcoding.

• No modification was required for the round 2 adaptor.

Table 6. The sequences of Truseq P5 and Truseq T7 used in uCoTarget experiments (Xiong et al., Science Advances, 2024).

Name	Barcode	Sequence
Truseq-501	TATAGCCT	AATGATACGGCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-502	ATAGAGGC	AATGATACGGCGACCACCGAGATCTACACATAGAGGCACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-503	CCTATCCT	AATGATACGGCGACCACCGAGATCTACACCCTATCCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-504	GGCTCTGA	AATGATACGGCGACCACCGAGATCTACACGGCTCTGAACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-505	AGGCGAAG	AATGATACGGCGACCACCGAGATCTACACAGGCGAAGACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-506	TAATCTTA	AATGATACGGCGACCACCGAGATCTACACTAATCTTAACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-507	CAGGACGT	AATGATACGGCGACCACCGAGATCTACACCAGGACGTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-508	GTACTGAC	AATGATACGGCGACCACCGAGATCTACACGTACTGACACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-509	TTGCTTGC	AATGATACGGCGACCACCGAGATCTACACTTGCTTGCACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-510	GAGAGGTT	AATGATACGGCGACCACCGAGATCTACACGAGAGGTTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-511	ACCTGGTT	AATGATACGGCGACCACCGAGATCTACACACCTGGTTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-512	AAGCGGAA	AATGATACGGCGACCACCGAGATCTACACAAGCGGAAACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-513	CGGAACAA	AATGATACGGCGACCACCGAGATCTACACCGGAACAAACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-514	GGTAAGCT	AATGATACGGCGACCACCGAGATCTACACGGTAAGCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-515	TGTGGCAT	AATGATACGGCGACCACCGAGATCTACACTGTGGCATACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-516	ACTACGGA	AATGATACGGCGACCACCGAGATCTACACACTACGGAACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-517	AATCTCCA	AATGATACGGCGACCACCGAGATCTACACAATCTCCAACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-518	GTCTAATT	AATGATACGGCGACCACCGAGATCTACACGTCTAATTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-519	CCCAAAGT	AATGATACGGCGACCACCGAGATCTACACCCCAAAGTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-520	TCCGTCCG	AATGATACGGCGACCACCGAGATCTACACTCCGTCCGACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-521	CCTTCAAC	AATGATACGGCGACCACCGAGATCTACACCCTTCAACACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-522	GATCATGC	AATGATACGGCGACCACCGAGATCTACACGATCATGCACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-523	TAGGTCGA	AATGATACGGCGACCACCGAGATCTACACTAGGTCGAACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-524	GTCAGGGT	AATGATACGGCGACCACCGAGATCTACACGTCAGGGTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Truseq-701	CGAGTAAT	CAAGCAGAAGACGGCATACGAGATCGAGTAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-702	TCTCCGGA	CAAGCAGAAGACGGCATACGAGATTCTCCGGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-703	AATGAGCG	CAAGCAGAAGACGGCATACGAGATAATGAGCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-704	GGAATCTC	CAAGCAGAAGACGGCATACGAGATGGAATCTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-705	TTCTGAAT	CAAGCAGAAGACGGCATACGAGATTTCTGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-706	ACGAATTC	CAAGCAGAAGACGGCATACGAGATACGAATTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-707	AGCTTCAG	CAAGCAGAAGACGGCATACGAGATAGCTTCAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-708	GCGCATTA	CAAGCAGAAGACGGCATACGAGATGCGCATTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-709	CATAGCCG	CAAGCAGAAGACGGCATACGAGATCATAGCCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-710	TTCGCGGA	CAAGCAGAAGACGGCATACGAGATTTCGCGGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-711	GCGCGAGA	CAAGCAGAAGACGGCATACGAGATGCGCGAGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Truseq-712	CTATCGCT	CAAGCAGAAGACGGCATACGAGATCTATCGCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-713	AGGAGGAA	CAAGCAGAAGACGGCATACGAGATAGGAGGAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-714	AGCAAGCA	CAAGCAGAAGACGGCATACGAGATAGCAAGCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-715	TCATCACC	CAAGCAGAAGACGGCATACGAGATTCATCACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-716	CGTAGGTT	CAAGCAGAAGACGGCATACGAGATCGTAGGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-717	TCAGATCC	CAAGCAGAAGACGGCATACGAGATTCAGATCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-718	CGTGATCA	CAAGCAGAAGACGGCATACGAGATCGTGATCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-719	AGTCGCTT	CAAGCAGAAGACGGCATACGAGATAGTCGCTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-720	GAACGCTT	CAAGCAGAAGACGGCATACGAGATGAACGCTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-721	TACGCCTT	CAAGCAGAAGACGGCATACGAGATTACGCCTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-722	CTCATCAG	CAAGCAGAAGACGGCATACGAGATCTCATCAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-723	TCTTCTGC	CAAGCAGAAGACGGCATACGAGATTCTTCTGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-724	GCTGGATT	CAAGCAGAAGACGGCATACGAGATGCTGGATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-725	TGTCGTAG	CAAGCAGAAGACGGCATACGAGATTGTCGTAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-726	CAATCATA	CAAGCAGAAGACGGCATACGAGATCAATCATAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-727	GTTCTTAT	CAAGCAGAAGACGGCATACGAGATGTTCTTATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-728	GATGCGAC	CAAGCAGAAGACGGCATACGAGATGATGCGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-729	GAAGAGGG	CAAGCAGAAGACGGCATACGAGATGAAGAGGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-730	TAGTAATC	CAAGCAGAAGACGGCATACGAGATTAGTAATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-731	GTGTGGAG	CAAGCAGAAGACGGCATACGAGATGTGTGGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-732	ACGTTGTA	CAAGCAGAAGACGGCATACGAGATACGTTGTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-733	GCGCTAAT	CAAGCAGAAGACGGCATACGAGATGCGCTAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-734	AGAGCTGC	CAAGCAGAAGACGGCATACGAGATAGAGCTGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-735	CATACTTA	CAAGCAGAAGACGGCATACGAGATCATACTTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
Truseq-736	TTGCACCG	CAAGCAGAAGACGGCATACGAGATTTGCACCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

• The Truseq P5 and T7 adaptor was used for 4th round barcoding.