Chemical Modified Nucleotide-based Elemental Tags for High-Sensitive Immunoassay

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Reagents

The rare earth elements (REEs): La (NO$_3$)$_3$·6H$_2$O, PrCl$_3$, EuCl$_3$·6H$_2$O, TbCl$_3$·6H$_2$O, HoCl$_3$·6H$_2$O, TmCl$_3$·5H$_2$O and Yb (NO$_3$)$_3$·5H$_2$O were purchased from Aladdin Chemistry Co. Ltd. The azido-mono-amide-DOTA (N$_3$-DOTA) was obtained from Macrocyclics (Dallas TX). The streptavidin coated magnetic microparticles (SA beads) and separation magnets (DynaMag-2) were commercialized from Invitrogen Co. As for biological reagents, the DNA templates (β-g-2 gene carried by pUC57 plasmid), all primers (oligonucleotide DNA chains), SYBR Green dye and paraffin were purchased from Sangon Biotech. (Shanghai, China). The oligonucleotide DNA chains with alkynyl groups ((alkyne)$_n$-ODN) were synthesized by GenScript (Nanjing, China). The 5-ethynyl-dUTP as raw material of polymerase chain reaction was purchased from Mo.Bi.Tec. (Molecular Biotechnology, German). Other normal dNTPs (dATP, dCTP, dGTP, dTTP), PCR buffer and Deep Vent (exo-) polymerase were commercially available from New England Biolab. As for agarose gel electrophoresis, the dry powder of agarose was purchased from Baygene Biotech Company (Shanghai, China). DL2000 (loading buffer and DNA ladder) and the nucleic acid dye Goldview were obtained from BioDee Biotech Co. Ltd (Beijing, China). And the PCR products were purified by Qia-quick PCR purification Kit (Qiagen). All of the antigens, monoclonal antibodies, the immunoassay buffers and 96-well plates were purchased from the Jiangsu Institute of Nuclear Medicine (Wuxi, China). The click reaction ligand BTTAA (4-{Bis-(1-tert-butyl-1H-[1,2,3]triazol-4-ylmethyl)-amino}-methyl)-[1,2,3]triazol-1-yl)-acetic acid) was obtained from MCE (MedChemExpress, Monmouth Junction, NJ, USA). And others inorganic salts were purchased from Sigma Aldrich.

Chemical structure of unnatural nucleotides

![Scheme S1](image-url)  
**Scheme S1.** The A compound was the raw material of chemical synthesized (alkyne)$_n$-ODN and the B compound was the raw material of PCR.
Instruments and methods

In this work, P-2000 laser puller (Sutter Instrument Co., Novato, CA, USA) has been used to make the nanoESI capillary. NanoESI-MS analysis was performed by 1.8 kV voltage. And the molecular weight changing of (alkyne)-ODN and REEs-ODN was monitored by the MOLDI-TOF mass spectrometry (Bruker Daltonics autoflex). And we used the 3-Hydroxypicolinic acid (3-HPA) as matrix solution. The polymerase chain reaction was performed by RT-PCR instruments (Xi’an TianLong Co. Ltd.) and the picture of DNA agarose gel (1.6%) was recorded by Ultra-Violet Products CCD camera (Bio-RAD ChemiDOC™ XRS+ imaging system). The concentrations of PCR products was roughly determined by Nanodrop 2000c (Thermo Fisher Scientific). An iCAP Q ICP-MS (Thermo Fisher Scientific GmnH, Germany) was used for the experiments. The instrument settings was listed in table S1.

**Table S1. ICP-MS operation parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cool gas flow (L/min)</td>
<td>13</td>
</tr>
<tr>
<td>auxiliary gas flow (L/min)</td>
<td>0.7</td>
</tr>
<tr>
<td>nebulizer gas flow (L/min)</td>
<td>0.96</td>
</tr>
<tr>
<td>sample uptake (s)</td>
<td>38</td>
</tr>
<tr>
<td>dwell time (ms)</td>
<td>0.02</td>
</tr>
<tr>
<td>channel</td>
<td>3</td>
</tr>
<tr>
<td>number of repeats per sample</td>
<td>3</td>
</tr>
<tr>
<td>PC detector voltage (V)</td>
<td>1265</td>
</tr>
<tr>
<td>RF power (W)</td>
<td>1578.61</td>
</tr>
<tr>
<td>analogue detector voltage (V)</td>
<td>-1960</td>
</tr>
</tbody>
</table>
Azido-mono-amide-DOTA (N₃-DOTA) chelating rare earth elemental ions (REEs)

Scheme S2. The mechanism of azido-mono-amide-DOTA (N₃-DOTA) chelating rare earth elemental ions.
Nano-ESI MS results of REEs-DOTA chelates

Figure S1. Characterization of REEs-DOTA chelates and DOTA-N₃ by Nano-ESI MS.
Fixed the REEs-oligonucleotides on magnetic microparticles

Scheme S3. Immobile REEs-oligonucleotides/DNA on streptavidin coated magnetic microparticles.

Evaluating the labelling numbers of REEs on (alkyne)$_n$-ODN ($n=1$) and (alkyne)$_n$-DNA (180 bp).

Table S2

<table>
<thead>
<tr>
<th>DNA name</th>
<th>Eu intensity of elemental tag (cps)</th>
<th>RSD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Alkyne)$_1$ – ODN (200 pM)</td>
<td>1559.18</td>
<td>4.48%</td>
</tr>
<tr>
<td>180bp Alkynes - DNA (200 pM)</td>
<td>49893.73</td>
<td>6.43%</td>
</tr>
</tbody>
</table>
BTTAA concentration optimization

In this work, the concentration of BTTAA was optimized for the click reaction. In the procedure of click reaction, a series of BTTAA (0 mM, 0.5 mM, 1 mM, 2 mM, 4 mM, 8 mM) was added in mixture with gently shaking overnight respectively (the alkyne-oligonucleotides carried biotin group on 5'). And then, we fixed the REEs-oligonucleotides on magnetic microparticles. The click reaction products of (alkyne)ₙ-ODN (n=1, 5, 10, 30; 10μL) were added into different centrifuge tube respectively and diluted to 200μL. After shaking 30 minutes at room temperature, the beads were washed 6 times by binding washing buffer (Tris-HCl 5 mM, EDTA 0.5 mM, NaCl 1 M). Adding 1% HNO₃ 200 μL, after 10 minutes, departed the liquid and beads by Magnetic Separator and the solution was introduced into ICP-MS for detection.

Figure S2. The optimization of BTTAA concentrations in click reaction. A series of BTTAA (0, 0.5, 1, 2, 4, 8 mM) was added in system respectively. The beads captured products and the intensity was detected by ICP-MS. 2 mM of BTTAA is the best concentration to catalyse the CC (click chemistry) reaction in this work.
PCR amplification of high-density dsDNA with lots of alkynyl groups

Table S3. PCR reagents

<table>
<thead>
<tr>
<th>Reagents name</th>
<th>Reagents dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep Vent (exo-) polymerase</td>
<td>1 unit / 50 μL</td>
</tr>
<tr>
<td>5-ethynyl-dUTP</td>
<td>200 μM</td>
</tr>
<tr>
<td>Template</td>
<td>400 ng/mL</td>
</tr>
<tr>
<td>Primer(F/R) or 5’-labeled primer(F/R)</td>
<td>1 μM</td>
</tr>
<tr>
<td>PCR buffer</td>
<td>1 ×</td>
</tr>
</tbody>
</table>

PCR procedure was controlled by machine and the reaction program was designed as follows:

Table S4. PCR reaction program designing

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>95°C</td>
<td>5 min</td>
<td></td>
</tr>
<tr>
<td>95°C</td>
<td>30 s</td>
<td></td>
</tr>
<tr>
<td>58°C</td>
<td>30 s</td>
<td>Cycle 35 times</td>
</tr>
<tr>
<td>68°C</td>
<td>2 min</td>
<td></td>
</tr>
<tr>
<td>4°C</td>
<td>storage</td>
<td></td>
</tr>
</tbody>
</table>

The procedure of RT-PCR was same as general PCR amplification, and the different was that the 2 μL of SYBR Green was added in system as fluorescent indicator.

After reaction, the products were purified by Qia-quick PCR purification Kit (Qiagen). The products were characterized by agarose gel electrophoresis and the concentration was roughly determined by Nano-drop.
RT-PCR results

**Figure S3.** RT-PCR of 180 bp, 440 bp 584 bp, 735 bp alkynes-DNA and normal DNA. In legend, the alkyne-dUTP means that the dTTP was replaced by 5-ethynyl-dUTP in RT-PCR. 180 bp, 440 bp, 584 bp and 735 bp means RT-PCR with natural dNTPs.
Sequencing from 180 bp and 440 bp PCR products

The genome sequencing of the amplified products was performed with pair-end sequencing by Sangon Biotech (Shanghai, China).

Figure S4. Pair-end sequencing from 180bp PCR products.

Figure S5. Pair-end sequencing from 440bp PCR products.
The consistency of sequencing results and sequences of target templates

The consistency of sequencing results and sequences of target templates were evaluated by *DNAMAN* V6 software for amplified products. As is shown in Figure S6, the consistency rate of sequencing results and target templates for the 180 bp and 440 bp PCR products was 99.45% and 99.55%, respectively.

![Figure S6](image-url)
Cu(I)-catalysed triazole ligation reaction of N$_3$-DOTA-[Ln$^{III}$] with alkynes-dsDNA

The experiment of click reaction was run by 180 bp and 440 bp alkynes-dsDNA (5-ethynyl-dUTP) and [Eu]-DOTA-N$_3$ and the procedure referred some protocols$^{1-4}$. Briefly, 100 μg of prepared PCR products was dissolved in PBS buffer (Na$_2$HPO$_4$ 10mM, pH=7.4), and the N$_3$-DOTA-[Eu] about 10-fold of the number of alkynes in solution was added. After that, 5mM sodium ascorbate, 1mM CuSO$_4$, and 2 mM BTTAA was added into the mixture. And for avoiding interference of O$_2$, the paraffin was added to block the system. The reaction was incubated under the program of PCR instrument (95 °C: 30 s; 60 °C: 2min; cycle 30 times). The products were analyzed by 1.6% agarose gels electrophoresis and purified by ultrafiltration (30k, Amicon Ultra-0.5 NMWL). Some details needed to be noted. The BTTAA and TBTA as ligand do not only stabilized Cu(I), but also prevent Cu(I) breaking DNA in click reaction$^3$.

Scheme S4. The mechanism of click reaction between alkynes-gene and REEs-DOTA.
Figure S7. Agarose gel of PCR products with different functional group primer. Lane 1 ladder: 100, 250, 500, 750, 1000, 2000 bp (bottom-up); lane 2 normal primer; lane 3 biotin-primer; lane 4 NH$_2$-primer; lane 5 C6-S-S-primer. For all products, the dTTP was displaced by 5-ethynyl-dUTP completely.

**Agarose gel electrophoresis**
As shown in scheme S5, for proving the conjugation of [Eu]-N$_3$ and alkynes-DNA, we used a blade to remove the 180 bp-metalized DNA from the agarose gel after gel electrophoresis. The pieces were soaked in the 1% HNO$_3$ for 15min. And then, the supernatant was introduced into the ICP-MS. The date was recorded and analyzed in Figure S8.

**Figure S8.** ICP-MS assaying region was from 140 to 200 m/z. Re element was as Internal standard.
The results of immunoassays

Figure S9. Scheme displayed application of the elemental tag probe in immunoassay using biotin(b) – streptavidin (SA) system. (b), (c), (d) are the relationship between Eu signal intensities and concentration of CEA antigens. (b) Eu-Tag was the products of 440 bp (blue) and 180 bp (black) alkynes-DNA click reaction. (c) The elemental tag probes were click reaction products of oligonucleotide chains containing 10 alkynyl groups (blue) and 1 alkynyl group (black) with DOTA-[Eu]. (d) Compared the Biotin-T DNA elemental tag and 180 bp DNA elemental tag in CEA ICP-MS immunoassay. The DNA sequences were record in table S7~S13.

In Figure S9, b) At the range of pg/mL ~ ng/mL, 440 bp-tag: \( y=123.38x+4551.06, \ R^2=0.985; \) 180 bp-tag: \( y=47.30x+3125.83, \ R^2=0.995. \) c) At the range of ng/mL ~ \( \mu \)g/mL, Biotin-10T tag: \( y=100.40x+3650.91, \ R^2=0.999; \) Biotin-T tag: \( y=11.69x+849.78, \ R^2=0.993. \)

<table>
<thead>
<tr>
<th>Table S5. Analytical performance of metal-chelated DNA elemental tags with different length</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elemental tags</strong></td>
</tr>
<tr>
<td>(Alkyne)–ODN</td>
</tr>
<tr>
<td>(Alkyne)10–ODN</td>
</tr>
<tr>
<td>180 bp</td>
</tr>
<tr>
<td>440 bp</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table S6. Recoveries of CEA in human serums by elemental tags (intensity mean ± S.D., ( n=3 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Added (pg/mL)</strong></td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>90</td>
</tr>
</tbody>
</table>
DNA sequences and modification

**Table S7.** The sequences of oligonucleotide DNA chains

(T is nucleotide with an alkynyl group)

<table>
<thead>
<tr>
<th>(Alkynyl)_n-ODN name</th>
<th>Sequences</th>
<th>5'-Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>AAAAATCTGTCTGTATCT</td>
<td>5'-biotin</td>
</tr>
<tr>
<td>Biotin-T</td>
<td>AAAAATCTGTATCTATGTATCTCT</td>
<td>5'-biotin</td>
</tr>
<tr>
<td>Biotin-5T</td>
<td>AAAAATCTGTATCTATGTATCT</td>
<td>5'-biotin</td>
</tr>
<tr>
<td>Biotin-10T</td>
<td>AAAAAATCTGTATCTATGTATCTCT</td>
<td>5'-biotin</td>
</tr>
<tr>
<td>Biotin-30T</td>
<td>AAAAAATCTGTATCTATGTATCT</td>
<td>5'-biotin</td>
</tr>
</tbody>
</table>

**Table S8.** 180 bp PCR primers

(F: forward; R: reverse)

<table>
<thead>
<tr>
<th>Primers name</th>
<th>Sequences</th>
<th>Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>F180-Biotin</td>
<td>ACC TTT GCT CAT TGA CGT TAC</td>
<td>5'-Biotin</td>
</tr>
<tr>
<td>F180-NH₂</td>
<td>ACC TTT GCT CAT TGA CGT TAC</td>
<td>5'-NH₂</td>
</tr>
<tr>
<td>F180-SH₂</td>
<td>ACC TTT GCT CAT TGA CGT TAC</td>
<td>5'-C6-S-S</td>
</tr>
<tr>
<td>R180-Biotin</td>
<td>GCC AGT ATC AGA TGC AGT TC</td>
<td>5'-Biotin</td>
</tr>
<tr>
<td>R180-NH₂</td>
<td>GCC AGT ATC AGA TGC AGT TC</td>
<td>5'-NH₂</td>
</tr>
<tr>
<td>R180-SH₂</td>
<td>GCC AGT ATC AGA TGC AGT TC</td>
<td>5'-C6-S-S</td>
</tr>
</tbody>
</table>

**Table S9.** 440 bp PCR primers

(F: forward; R: reverse)

<table>
<thead>
<tr>
<th>Primers name</th>
<th>Sequences</th>
<th>Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>F440-Biotin</td>
<td>GCT CAC GCT GTA GGT ATC TC</td>
<td>5'-Biotin</td>
</tr>
<tr>
<td>F440-NH₂</td>
<td>GCT CAC GCT GTA GGT ATC TC</td>
<td>5'-NH₂</td>
</tr>
<tr>
<td>F440-SH₂</td>
<td>GCT CAC GCT GTA GGT ATC TC</td>
<td>5'-C6-S-S</td>
</tr>
<tr>
<td>R440-Biotin</td>
<td>TTC GTT CCA CTG AGC GTC</td>
<td>5'-Biotin</td>
</tr>
<tr>
<td>R440-NH₂</td>
<td>TTC GTT CCA CTG AGC GTC</td>
<td>5'-NH₂</td>
</tr>
<tr>
<td>R440-SH₂</td>
<td>TTC GTT CCA CTG AGC GTC</td>
<td>5'-C6-S-S</td>
</tr>
</tbody>
</table>

**Table S10.** 580 bp PCR primers

(F: forward; R: reverse)

<table>
<thead>
<tr>
<th>Primers name</th>
<th>Sequences</th>
<th>Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>F580-Biotin</td>
<td>CAA CCC GGT AAG ACA CGA C</td>
<td>5'-Biotin</td>
</tr>
<tr>
<td>F580-NH₂</td>
<td>CAA CCC GGT AAG ACA CGA C</td>
<td>5'-NH₂</td>
</tr>
<tr>
<td>F580-SH₂</td>
<td>CAA CCC GGT AAG ACA CGA C</td>
<td>5'-C6-S-S</td>
</tr>
<tr>
<td>R580-Biotin</td>
<td>GCG GTA TCA TTG CAG CAC</td>
<td>5'-Biotin</td>
</tr>
<tr>
<td>R580-NH₂</td>
<td>GCG GTA TCA TTG CAG CAC</td>
<td>5'-NH₂</td>
</tr>
<tr>
<td>R580-SH₂</td>
<td>GCG GTA TCA TTG CAG CAC</td>
<td>5'-C6-S-S</td>
</tr>
</tbody>
</table>
**Table S11. 735 bp PCR primers**

(F: forward; R: reverse)

<table>
<thead>
<tr>
<th>Primers name</th>
<th>Sequences</th>
<th>Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>F735-Biotin</td>
<td>CGA TTA CTA GCG ATT CCG AC</td>
<td>5'-Biotin</td>
</tr>
<tr>
<td>F735-NH₂</td>
<td>CGA TTA CTA GCG ATT CCG AC</td>
<td>5'-NH₂</td>
</tr>
<tr>
<td>F735-SH₂</td>
<td>CGA TTA CTA GCG ATT CCG AC</td>
<td>5'-C6-S-S</td>
</tr>
<tr>
<td>R735-Biotin</td>
<td>TCA ACC TGG GAA CTC GAT C</td>
<td>5'-Biotin</td>
</tr>
<tr>
<td>R735-NH₂</td>
<td>TCA ACC TGG GAA CTC GAT C</td>
<td>5'-NH₂</td>
</tr>
<tr>
<td>R735-SH₂</td>
<td>TCA ACC TGG GAA CTC GAT C</td>
<td>5'-C6-S-S</td>
</tr>
</tbody>
</table>

**Table S12. DNA templates**

<table>
<thead>
<tr>
<th>pUC57 Plasmid carrier and beta-g2 gene sequence (The yellow marked part is the location of the beta-g2 gene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GACGAAAGGGCGCTGGATACGCTGCACTTATTATATATGGAAGTGGTTTCTCTTAGCTAGCGGACGGCTGCCTTTTTCGGGAAAAGATCGGAAATGAGGCTTTTTATTAATAGGTTAATGTCATGATATATAATGGTTTCTTAGACGTCAGGTCCTTTTCCGGTGTTTTTGCTTACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGAGCTTGGAGCGTGGTTTTGCTCACCATCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGAGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTGCATGCAGGCCTCTGCAGTCGACGGGCCCGACATCGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCACCTGACTCCTGAGGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTAAGGCCCTGGGCACGTTGGTATCAAGGTTCACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGG</td>
</tr>
</tbody>
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ATCTGTCCACTCTGTGATGCTGTATGCGAACCCTAAAGTGAGCTCATGGCAAGAAATGCTCGGTGCCTTTAGTGATGGCGTCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGGTTCGGGATCCGATATCTAGATGCATTCGCGAGGTACCGAGCTCGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAA
CCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCCCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCAC
CGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGCAGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGT
TTTCACCGTCATCACCGAAACGCGCGA

Table S13. Sequences of 180 bp and 440 bp alkynes-DNA

<table>
<thead>
<tr>
<th>alkynes-DNA</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>180 bp</td>
<td>ACCTTTGTCATTGACGTATACCCGCGAGAAGACCCGCGTAACCTCGTGCCA</td>
</tr>
<tr>
<td></td>
<td>GCAGCCCGCTGTTAACCCGAGGGGCTGCAATCGGAAATTACTGGGCGTAA</td>
</tr>
<tr>
<td></td>
<td>AGGCAGCGACGGCGTCTTTTAACTGAGATGGAAATCCCCGGCTCAAACCTG</td>
</tr>
<tr>
<td></td>
<td>GGAACGTGACATCTGATACTGCG</td>
</tr>
<tr>
<td>440 bp</td>
<td>GCTCGACGCTGATAGTACTGATTACCTCGTGATAGTGTCCTGGCTCAAGCTGGGCTCGTGTGCCAGAACCGCGCTTACCGGTAACCTCGTACCTCGGACGGAAGCCGACTG</td>
</tr>
<tr>
<td></td>
<td>GTTGTGACGGAAGCCCGCTTACCGGCGAGCGCTCGCTTACCGGTAACTATC</td>
</tr>
<tr>
<td></td>
<td>GTCTTGGATCGCCAAACCCGAGTAAAGACAGACTTTACCGGCACCTGCGACCAGCAGCCTG</td>
</tr>
<tr>
<td></td>
<td>GTAAAGGATTAGCGAGAGGAGTTATGTCGAGGTGTACACGAGTTCTTTGAAGTGT</td>
</tr>
<tr>
<td></td>
<td>GTGCGCCTAACCTGAGCTCAGCTACGTAAGGAGCAGATTTTTGATCTCGGCTCTGTGAGAAGCCAGTTACCTCGGAAAGAGGTAGGCTAGCTGCTACGGGACGGAAGCCGACTGAAGGGGCCAGACCCGACTG</td>
</tr>
<tr>
<td></td>
<td>GATCTCAAGAAGATCCTTTGATCTTTTTCTACGGGTCGTACGAGTGAACGAA</td>
</tr>
</tbody>
</table>

The T was replaced by alkyne-U in dsDNA completely. According to the Watson and Crick principle, the number of alkynes groups is the total number of A and T in one chain of dsDNA.
LOD of different methods for the ICP-MS immunoassay

Table S14 LOD of different methods for the ICP-MS immunoassay

<table>
<thead>
<tr>
<th>Tags</th>
<th>LOD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>180bp DNA tags (CEA)</td>
<td>0.031 ng/mL</td>
<td>This work</td>
</tr>
<tr>
<td>180bp DNA tags (AFP)</td>
<td>0.053 ng/mL</td>
<td>This work</td>
</tr>
<tr>
<td>AuNPs (AFP)</td>
<td>0.018 ng/mL</td>
<td>5</td>
</tr>
<tr>
<td>Lanthanide-tagged polymer tags (CEA)</td>
<td>0.060 ng/mL</td>
<td>6</td>
</tr>
<tr>
<td>NaYF4 UCNP (AFP)</td>
<td>0.22 ng/mL</td>
<td>7</td>
</tr>
</tbody>
</table>

Reference