

Supplementary Figures

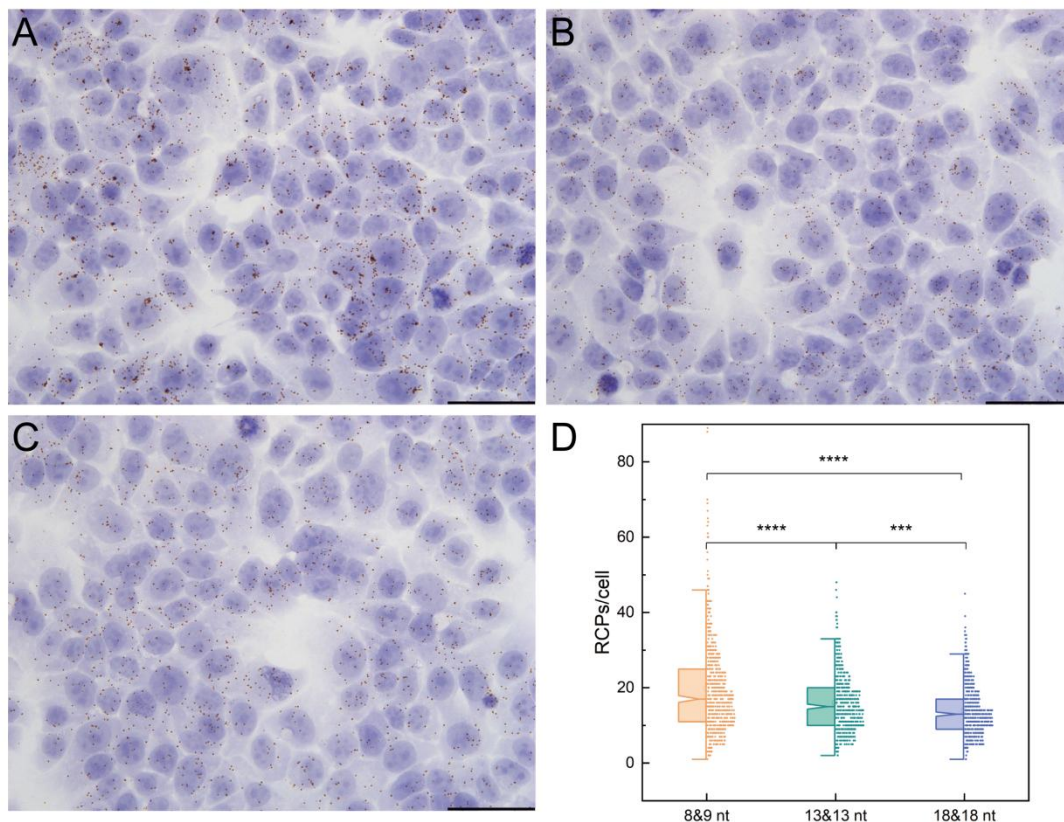


FIGURE S1. Comparison of the length of base pairing between V probes and circle precursors. The 8 to 9 base pairings group (A) shows better detection efficiency than the other two groups (B, C). Scale bar, 50 μ m. (D) Statistics plot of the detected RCPs per cell for ESR1 in MCF-7 cells. (***) $p < 0.001$, (****) $p < 0.0001$)

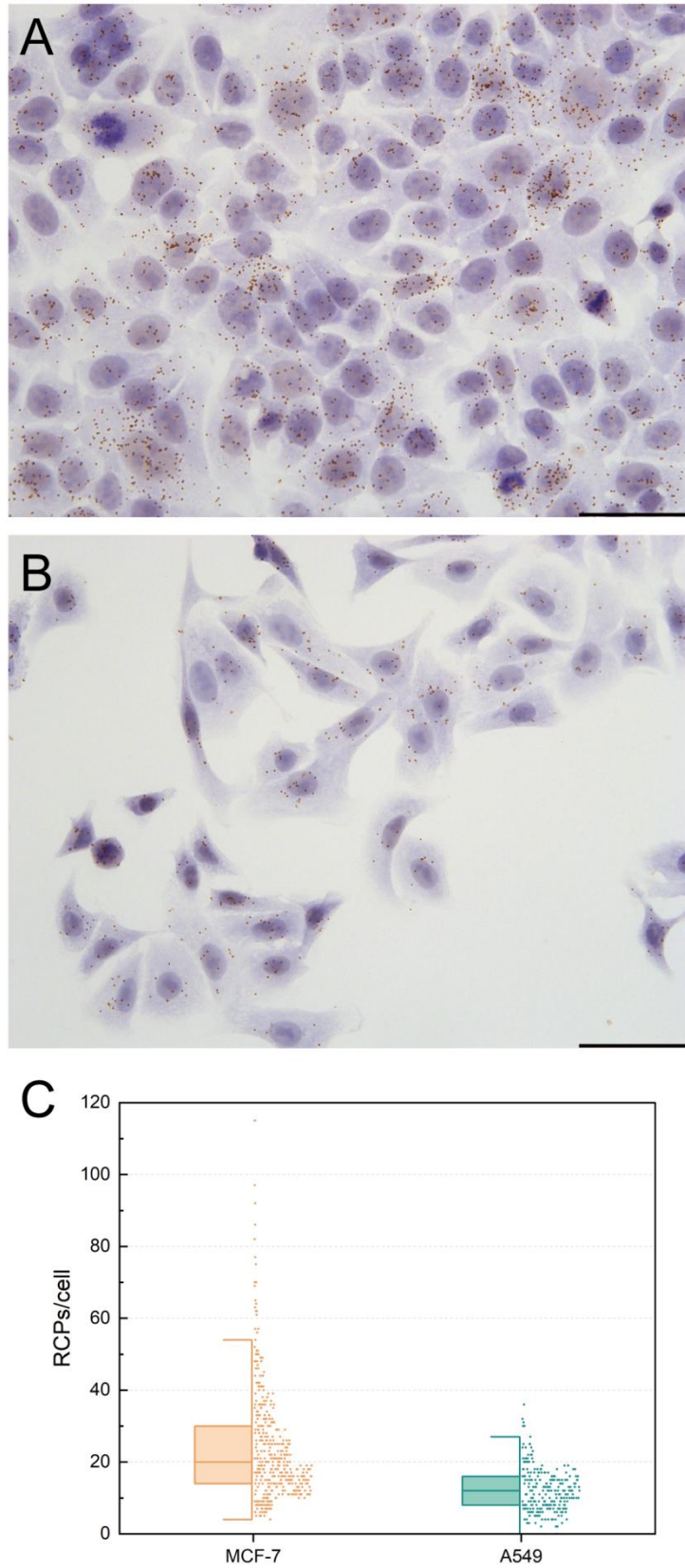


FIGURE S2. Detection of *HER2* mRNA expression in MCF-7 (A) and A549 (B) cells. Scale bar, 50 μ m. (C) Quantification of the expression level of *HER2*.

Table S1 Sequences of oligonucleotide

Oligonucleotide name	Sequence	Vendor
HPV-18 E6 - V probe-L1	5'- GACGCTAATATGTATAGAAAAAAAAAAATG <u>CAGCATGCGGTAT</u>	General Biol (Chuzhou, China)
HPV-18 E6 - V probe-R1	5'- <u>TCTATACATTTATGGCAAAAAAAAAAGGCCT</u> CTCGACACTCTT	General Biol (Chuzhou, China)
HPV-18 E6 - V probe-L2	5'- GACGCTAATATGTATAGAAAAAAAAAAATCTA <u>TAGTGCCCAGCT</u>	General Biol (Chuzhou, China)
HPV-18 E6 - V probe-R2	5'- <u>ACGAATGGCACTGGCCAAAAAAAAAAGGC</u> CTCTCGACACTCTT	General Biol (Chuzhou, China)
HPV-18 E6 - V probe-L3	5'- GACGCTAATATGTATAGAAAAAAAAAAATCC <u>GTGCACAGATCAGG</u>	General Biol (Chuzhou, China)
HPV-18 E6 - V probe-R3	5'- <u>GCAGTGAAGTGTTTCAGTAAAAAAAAAAGGC</u> CTCTCGACACTCTT	General Biol (Chuzhou, China)
HPV-18 E6 - V probe-L4	5'- GACGCTAATATGTATAGAAAAAAAAAAACC <u>GCAGGCACCTTAT</u>	General Biol (Chuzhou, China)
HPV-18 E6 - V probe-R4	5'- <u>TTCAACGGTTTCTGGCAAAAAAAAAAAGGCC</u> TCTCGACACTCTT	General Biol (Chuzhou, China)
HPV-18 E7 - V probe-L1	5'- GACGCTAATATGTATAGAAAAAAAAAAATTGT <u>GTTTCTCTGCGTC</u>	General Biol (Chuzhou, China)
HPV-18 E7 - V probe-R1	5'- <u>GGTCCATGCATTTATACAAAAAAAAAAGGC</u> CTCTCGACACTCTT	General Biol (Chuzhou, China)
HPV-18 E7 - V probe-L2	5'- GACGCTAATATGTATAGAAAAAAAAAACTG <u>GGATGCACACCAC</u>	General Biol (Chuzhou, China)
HPV-18 E7 - V probe-R2	5'- <u>ACAGGCACATTTACTGAAAAAAAAAAGGCC</u> TCTCGACACTCTT	General Biol (Chuzhou, China)
PGR - V probe-L1	5'- GACGCTAATAGCGATTAAAAAAAAAAATA <u>GGTGGAGTGGTACA</u>	Sangon Bitotech (Shanghai, China)
PGR - V	5'-	Sangon

probe-R1	<u>CATGCAAAGGTAGGGATAAAAAAAAAAAAAAG</u> CGCGAGACACTCTT	Bitotech (Shanghai, China)
<i>PGR</i> - V probe-L2	5'- GACGCTAATAGCGATTAAAAAAAAAAAAATTAC <u>TACCAACACCCCC</u>	Sangon Bitotech (Shanghai, China)
<i>PGR</i> - V probe-R2	5'- <u>CAGCTGCTGTGTTTAACAAAAAAAAAAAAAG</u> CGCGAGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>PGR</i> - V probe-L3	5'- GACGCTAATAGCGATTAAAAAAAAAAAAAAT <u>CTAGGCCCAGTTCA</u>	Sangon Bitotech (Shanghai, China)
<i>PGR</i> - V probe-R3	5'- <u>TCCAACCTGAACTCAATAAAAAAAAAAAAAAG</u> CGCGAGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>PGR</i> - V probe-L4	5'- GACGCTAATAGCGATTAAAAAAAAAAAAATGT <u>ATTGTGGGCTCTGG</u>	Sangon Bitotech (Shanghai, China)
<i>PGR</i> - V probe-R4	5'- <u>AGGTAATGACTCGAAGCAAAAAAAAAAAAAAG</u> CGCGAGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>PGR</i> - V probe-L5	5'- GACGCTAATAGCGATTAAAAAAAAAAAAATTG <u>GGTAATTGTGCAGC</u>	Sangon Bitotech (Shanghai, China)
<i>PGR</i> - V probe-R5	5'- <u>CCATCCCTGCCAATATCAAAAAAAAAAAAAAG</u> CGCGAGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-L1	5'- GACGCTAATATGTATAGAAAAAAAAAAAAATGT <u>GTACACTCCAGAAT</u>	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-R1	5'- <u>AGGGTGCTGGACAGAAAAAAAAAAAAAGG</u> CCTCTCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V	5'-	Sangon

probe-L2	GACGCTAATATGTATAGAAAAAAAAAATGAA <u>GCATAGTCATTGC</u>	Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-R2	5'- <u>ACTCCATAATGGTAGCC</u> AAAAAAAAAAGGC CTCTCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-L3	5'- GACGCTAATATGTATAGAAAAAAAAAAGCG <u>GAACCGAGATGATG</u>	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-R3	5'- <u>CCCTGCAGATTCATCATA</u> AAAAAAAAAAGGC CTCTCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-L4	5'- GACGCTAATATGTATAGAAAAAAAAAACTG <u>AGTTCAGCTGTGAA</u>	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-R4	5'- <u>GAGCCCCAACCCATAG</u> AAAAAAAAAAGGC CTCTCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-L5	5'- GACGCTAATATGTATAGAAAAAAAAAAAGG <u>GTATGAGTCGGCAC</u>	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-R5	5'- <u>AATCCAGCCAACCCCTA</u> AAAAAAAAAAGGC CTCTCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> - V probe-L1-53nt	5'- ACTGGACGCTAATATGTATAGAGTCGAAAA AAAAAAT <u>GTGTACACTCCAGAAT</u>	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-R1-53nt	5'- <u>AGGGTGCTGGACAG</u> AAAAAAAAAAAACTA CTGGCCTCTCGACACTCTTGACG	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-L2-53nt	5'- ACTGGACGCTAATATGTATAGAGTCGAAAA AAAAAAT <u>GAAGCATAGTCATTGC</u>	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-R2-53nt	5'- <u>ACTCCATAATGGTAGCC</u> AAAAAAAAAACTA CTGGCCTCTCGACACTCTTGACG	General Biol (Chuzhou, China)
<i>ESR1</i> - V	5'-	General Biol

probe-L3-53nt	ACTGGACGCTAATATGTATAGAGTCGAAAA AAAAAA <u>GCGGAACCGAGATGATG</u>	(Chuzhou, China)
<i>ESR1</i> - V probe-R3-53nt	5'- <u>CCCTGCAGATTCATCATAAAAAAAAAAACTAC</u> TGGCCTCTCGACACTCTTGACG	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-L4-53nt	5'- ACTGGACGCTAATATGTATAGAGTCGAAAA AAAAAA <u>CTGAGTTCAGCTGTGAA</u>	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-R4-53nt	5'- <u>GAGCCCCAACCCATAGAAAAAAAAAACTA</u> CTGGCCTCTCGACACTCTTGACG	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-L5-53nt	5'- ACTGGACGCTAATATGTATAGAGTCGAAAA AAAAAA <u>AGGGTATGAGTCGGCAC</u>	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-R5-53nt	5'- <u>AATCCAGCCAACCCCTAAAAAAAAAACTA</u> CTGGCCTCTCGACACTCTTGACG	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-L1-63nt	CATTCACTGGACGCTAATATGTATAGAGTC GATAGCAAAAAAAAAAAT <u>GTGTACACTCCAG</u> <u>AAT</u>	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-R1-63nt	<u>AGGGTGCTGGACAGAAAAAAAAAAAAATAC</u> GGCTACTGGCCTCTCGACACTCTTGACGG CGTA	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-L2-63nt	CATTCACTGGACGCTAATATGTATAGAGTC GATAGCAAAAAAAAAAAT <u>GAAGCATAGTCAT</u> <u>TGC</u>	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-R2-63nt	<u>ACTCCATAATGGTAGCCAAAAAAAAAAATAC</u> GGCTACTGGCCTCTCGACACTCTTGACGG CGTA	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-L3-63nt	CATTCACTGGACGCTAATATGTATAGAGTC GATAGCAAAAAAAAAAAG <u>GCGGAACCGAGATG</u> <u>ATG</u>	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-R3-63nt	<u>CCCTGCAGATTCATCATAAAAAAAAAAAATAC</u> GGCTACTGGCCTCTCGACACTCTTGACGG CGTA	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-L4-63nt	CATTCACTGGACGCTAATATGTATAGAGTC GATAGCAAAAAAAAAA <u>CTGAGTTCAGCTGT</u> <u>GAA</u>	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-R4-63nt	<u>GAGCCCCAACCCATAGAAAAAAAAAAATAC</u> GGCTACTGGCCTCTCGACACTCTTGACGG CGTA	General Biol (Chuzhou, China)
<i>ESR1</i> - V probe-L5-63nt	CATTCACTGGACGCTAATATGTATAGAGTC GATAGCAAAAAAAAAAAG <u>GGGTATGAGTCGG</u> <u>CAC</u>	General Biol (Chuzhou, China)

<i>ESR1</i> - V probe-R5-63nt	<u>AATCCAGCCAACCCCTAAAAAAAATAC</u> GGCTACTGGCCTCTCGACACTCTTGACGG CGTA	General Biol (Chuzhou, China) Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-L1	5'- GACGCTAATATAGCACCAAAAAAAAAA <u>ATTA</u> <u>CTTGCAGGTTCTG</u>	Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-R1	5'- <u>GCAGAATTCGTCCCCGGAAAAAAAAAATAA</u> GGCCCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-L2	5'- GACGCTAATATAGCACCAAAAAAAAAA <u>AGA</u> <u>TCTCTGTGAGGCTT</u>	Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-R2	5'- <u>CAAGACCCCTCCTTTCAAAAAAAAAAATAA</u> GGCCCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-L3	5'- GACGCTAATATAGCACCAAAAAAAAAA <u>CGT</u> <u>CGCTTGATGAGGAT</u>	Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-R3	5'- <u>TCCGGATCTTCTGCTGCAAAAAAAAAAATAA</u> GGCCCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-L4	5'- GACGCTAATATAGCACCAAAAAAAAAA <u>CCA</u> <u>AAGATCTTCTTGCA</u>	Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-R4	5'- <u>GCAGAAATGCCAGGCTCAAAAAAAAAAATAA</u> GGCCCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-L5	5'- GACGCTAATATAGCACCAAAAAAAAAA <u>CAG</u> <u>ATCCAAGCACCTTC</u>	Sangon Bitotech (Shanghai, China)
<i>HER2</i> - V probe-R5	5'- <u>GACTGTGCCAAAAGCGCAAAAAAAAAAATAA</u> GGCCCGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>dapB</i> - V	5'-	General Biol

probe-L1	GACGCTAATGGCTCCACAAAAAAAAAAAGCT <u>GTAAATTGGCAGA</u>	(Chuzhou, China)
<i>dapB</i> - V probe-R1	5'- <u>GAAGAATGGGGCAGGAAAAAAAAAAAAATAG</u> ACGCAGACACTCTT	General Biol (Chuzhou, China)
<i>dapB</i> - V probe-L2	5'- GACGCTAATGGCTCCACAAAAAAAAAAAGAA <u>CAACCGGTTTCTCA</u>	General Biol (Chuzhou, China)
<i>dapB</i> - V probe-R2	5'- <u>AGTCCGTCCAGTTGTGCGAAAAAAAAAATAG</u> ACGCAGACACTCTT	General Biol (Chuzhou, China)
<i>dapB</i> - V probe-L3	5'- GACGCTAATGGCTCCACAAAAAAAAAAAGA <u>GGAGCGGAGCAAAA</u>	General Biol (Chuzhou, China)
<i>dapB</i> - V probe-R3	5'- <u>AAATTCTCCCAGGAGCAAAAAAAAAAATAG</u> ACGCAGACACTCTT	General Biol (Chuzhou, China)
<i>PPIB</i> - V probe-L1	5'- GACGCTAATGGCTCCACAAAAAAAAAAACCG <u>CCCTGGATCATGAA</u>	Sangon Bitotech (Shanghai, China)
<i>PPIB</i> - V probe-R1	5'- <u>CTCCCCTGGTGAAGTCTAAAAAAAAAATAG</u> ACGCAGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>PPIB</i> - V probe-L2	5'- GACGCTAATGGCTCCACAAAAAAAAAAAAA <u>ATACACCTTGACGG</u>	Sangon Bitotech (Shanghai, China)
<i>PPIB</i> - V probe-R2	5'- <u>TCTCCAATTCGTAGGTCAAAAAAAAAAATAGA</u> CGCAGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>PPIB</i> - V probe-L3	5'- GACGCTAATGGCTCCACAAAAAAAAAAAAAG ATGTCCCTGTGCCC	Sangon Bitotech (Shanghai, China)
<i>PPIB</i> - V probe-R3	5'- <u>AGACGGTCACTCAAAGAAAAAAAAAATAG</u> ACGCAGACACTCTT	Sangon Bitotech (Shanghai, China)
<i>PPIB</i> - V probe-L4	5'- GACGCTAATGGCTCCACAAAAAAAAAAATG <u>AAGAACTGGGAGCC</u>	Sangon Bitotech (Shanghai,

		China)
		Sangon
<i>PPIB</i> - V	5'-	Bitotech
probe-R4	<u>CTGTCTTGACTGTCGTG</u> AAAAAAAAAATAG	(Shanghai,
	ACGCAGACACTCTT	China)
	5'-	Sangon
<i>PPIB</i> - V	5'-	Bitotech
probe-L5	GACGCTAATGGCTCCACAAAAAAAAAAT <u>GT</u>	(Shanghai,
	<u>TGCGTTCGGAGAG</u>	China)
	5'-	Sangon
<i>PPIB</i> - V	<u>GCAAGGAGCACCTTCA</u> AAAAAAAAAATAGA	Bitotech
probe-R5	CGCAGACACTCTT	(Shanghai,
		China)
<i>TPT1</i> - V	5'-	General Biol
probe-L1	GACGCTAATGGCTCCACAAAAAAAAAAT <u>GA</u>	(Chuzhou,
	<u>GCGAGTCATCAATG</u>	China)
<i>TPT1</i> - V	5'-	General Biol
probe-R1	<u>GGAGGCATTTCCACCA</u> AAAAAAAAAATAG	(Chuzhou,
	ACGCAGACACTCTT	China)
<i>TPT1</i> - V	5'-	General Biol
probe-L2	GACGCTAATGGCTCCACAAAAAAAAAAT <u>T</u>	(Chuzhou,
	<u>TGTTCTGCAGCCCC</u>	China)
<i>TPT1</i> - V	5'-	General Biol
probe-R2	<u>TAGCAAGGATGTGCTT</u> AAAAAAAAAATAG	(Chuzhou,
	ACGCAGACACTCTT	China)
<i>TPT1</i> - V	5'-	General Biol
probe-L3	GACGCTAATGGCTCCACAAAAAAAAAAC <u>CT</u>	(Chuzhou,
	<u>CACGGTAGTCCAAT</u>	China)
<i>TPT1</i> - V	5'-	General Biol
probe-R3	<u>ATATGGGGTCACACCATA</u> AAAAAAAAAATAG	(Chuzhou,
	ACGCAGACACTCTT	China)
<i>TPT1</i> - V	5'-	General Biol
probe-L4	GACGCTAATGGCTCCACAAAAAAAAAAC <u>AA</u>	(Chuzhou,
	<u>ACTTGCAGGCTCTA</u>	China)
<i>TPT1</i> - V	5'-	General Biol
probe-R4	<u>TTTAAATCAGGGGCTGG</u> AAAAAAAAAATAG	(Chuzhou,
	ACGCAGACACTCTT	China)
<i>TPT1</i> - V	5'-	General Biol
probe-L5	GACGCTAATGGCTCCACAAAAAAAAAAT <u>CA</u>	(Chuzhou,
	<u>CTGGCCTGCTTTTA</u>	China)
<i>TPT1</i> - V	5'-	General Biol
probe-R5	<u>GTTCAAGCAGACCAGAG</u> AAAAAAAAAATAG	(Chuzhou,
	ACGCAGACACTCTT	China)
<i>GAPDH</i> - V	5'-	General Biol

probe-L1	GACGCTAATGGCTCCACAAAAAAAAAAATGA <u>GGCTGTTGTCATAC</u>	(Chuzhou, China)
<i>GAPDH</i> - V probe-R1	5'- <u>GGCATTGCTGATGATCT</u> AAAAAAAAAATAG ACGCAGACACTCTT	General Biol (Chuzhou, China)
<i>GAPDH</i> - V probe-L2	5'- GACGCTAATGGCTCCACAAAAAAAAAAGGT <u>GGCAGTGATGGCAT</u>	General Biol (Chuzhou, China)
<i>GAPDH</i> - V probe-R2	5'- <u>CCATCCACAGTCTTCTG</u> AAAAAAAAAATAG ACGCAGACACTCTT	General Biol (Chuzhou, China)
<i>GAPDH</i> - V probe-L3	5'- GACGCTAATGGCTCCACAAAAAAAAAAGC <u>TCAGGGATGACCTT</u>	General Biol (Chuzhou, China)
<i>GAPDH</i> - V probe-R3	5'- <u>CAGTGAGCTTCCCGTTCA</u> AAAAAAAAAATAG ACGCAGACACTCTT	General Biol (Chuzhou, China)
<i>GAPDH</i> - V probe-L4	5'- GACGCTAATGGCTCCACAAAAAAAAAACC <u>TGGTGCTCAGTGTA</u>	General Biol (Chuzhou, China)
<i>GAPDH</i> - V probe-R4	5'- <u>TGAAGTCAGAGGAGACC</u> AAAAAAAAAATAG ACGCAGACACTCTT	General Biol (Chuzhou, China)
<i>GAPDH</i> - V probe-L5	5'- GACGCTAATGGCTCCACAAAAAAAAAATG <u>AGCTTGACAAAGTG</u>	General Biol (Chuzhou, China)
<i>GAPDH</i> - V probe-R5	5'- <u>CGTTGTCATACCAGG</u> AAAAAAAAAATAG ACGCAGACACTCTT	General Biol (Chuzhou, China)
<i>ALB</i> - V probe-L1	5'- GACGCTAATATGTATAGAAAAAAAAAATCTC <u>CATGGCAGCATTC</u>	General Biol (Chuzhou, China)
<i>ALB</i> - V probe-R1	5'- <u>CAGCACATTCAAGCAG</u> AAAAAAAAAAGGC CTCTCGACACTCTT	General Biol (Chuzhou, China)
<i>ALB</i> - V probe-L2	5'- GACGCTAATATGTATAGAAAAAAAAAATAGT <u>CTTCTGCACAGGG</u>	General Biol (Chuzhou, China)
<i>ALB</i> - V probe-R2	5'- <u>TCAGGACCACGGATAG</u> AAAAAAAAAAGGC CTCTCGACACTCTT	General Biol (Chuzhou, China)
<i>ALB</i> - V probe-L3	5'- GACGCTAATATGTATAGAAAAAAAAAAGT <u>GCAGTTTGTCTTCT</u>	General Biol (Chuzhou, China)

<i>ALB</i> - V probe-R3	5'- <u>TGTTTCACGAGCTCAAC</u> AAAAAAAAAAGGC CTCTCGACACTCTT	General Biol (Chuzhou, China)
<i>ALB</i> - V probe-L4	5'- GACGCTAATATGTATAGAAAAAAAAAAGCA <u>GCTTGGCAACATTC</u>	General Biol (Chuzhou, China)
<i>ALB</i> - V probe-R4	5'- <u>GGCAGGCAGCTTTATCA</u> AAAAAAAAAAGGC CTCTCGACACTCTT	General Biol (Chuzhou, China)
<i>ALB</i> - V probe-L5	5'- GACGCTAATATGTATAGAAAAAAAAAAGC <u>AGCACGACAGAGTA</u>	General Biol (Chuzhou, China)
<i>ALB</i> - V probe-R5	5'- <u>TCTTGGCAAGTCTCAGC</u> AAAAAAAAAAGGC CTCTCGACACTCTT	General Biol (Chuzhou, China)
circle probe precursor-L	5'- ATTAGCGTCCAGTGAATGCGAGTCCTCAAT GCTGCTGCTGTACTACGCCGTCAAGAGTGT C	Sangon Bitotech (Shanghai, China)
circle probe precursor-S1	5'-GAGAGGCCGTGCTATACAT	Sangon Bitotech (Shanghai, China)
circle probe precursor-S1-30 nt	5'- GAGAGGCCAGTAGGTCGCGACTCTATACA T	General Biol (Chuzhou, China)
circle probe precursor-S1-40 nt	5'- GAGAGGCCAGTAGCCGTAGTCGGCTATCG ACTCTATACAT	General Biol (Chuzhou, China)
circle probe precursor-S2	5'-TCGCGCTTGGTATAATCGCT	Sangon Bitotech (Shanghai, China)
circle probe precursor-S3	5'-GGGCCTTATTCCGGTGCTAT	Sangon Bitotech (Shanghai, China)
circle probe precursor-S4	5'-TGCGTCTATTTAGTGGAGCC	Sangon Bitotech (Shanghai, China)
<i>ESR1</i> -padlock probe 1	5'- <u>TGTGTACACTCCAGAATTT</u> CCTTTTACGACC TCAATGCTGCTGCTGTACTACTCTTAGGGT	General Biol (Chuzhou, China)

	<u>GCTGGACAGAAA</u>	
<i>ESR1</i> -padlock probe 2	5'- <u>TGAAGCATAGTCATTGCTTCCTTTTACGAC</u> <u>CTCAATGCTGCTGCTGTACTACTCTTACTC</u> <u>CATAATGGTAGCC</u>	General Biol (Chuzhou, China)
<i>ESR1</i> -padlock probe 3	5'- <u>GCGGAACCGAGATGATGTTCCCTTTTACGAC</u> <u>CTCAATGCTGCTGCTGTACTACTCTTCCCT</u> <u>GCAGATTCATCAT</u>	General Biol (Chuzhou, China)
<i>ESR1</i> -padlock probe 4	5'- <u>CTGAGTTCAGCTGTGAATTCCTTTTACGAC</u> <u>CTCAATGCTGCTGCTGTACTACTCTTGAGC</u> <u>CCCAACCCATAGA</u>	General Biol (Chuzhou, China)
<i>ESR1</i> -padlock probe 5	5'- <u>AGGGTATGAGTCGGCACTTCCTTTTACGAC</u> <u>CTCAATGCTGCTGCTGTACTACTCTTAATC</u> <u>CAGCCAACCCCTA</u>	General Biol (Chuzhou, China)
<i>HER2</i> -padlock probe 1	5'- <u>ATTACTTGCAGGTTCTGTTCCCTTTTACGACC</u> <u>TCAATGCTGCTGCTGTACTACTCTTGCAGA</u> <u>ATTCGTCCCCGG</u>	General Biol (Chuzhou, China)
<i>HER2</i> -padlock probe 2	5'- <u>AGATCTCTGTGAGGCTTTTCCTTTTACGAC</u> <u>CTCAATGCTGCTGCTGTACTACTCTTCAAG</u> <u>ACCCCTCCTTTCA</u>	General Biol (Chuzhou, China)
<i>HER2</i> -padlock probe 3	5'- <u>CGTCGCTTGATGAGGATTTTCCTTTTACGAC</u> <u>CTCAATGCTGCTGCTGTACTACTCTTCCG</u> <u>GATCTTCTGCTGC</u>	General Biol (Chuzhou, China)
<i>HER2</i> -padlock probe 4	5'- <u>CCAAAGATCTTCTTGCAATTCCTTTTACGACC</u> <u>TCAATGCTGCTGCTGTACTACTCTTGCAGA</u> <u>AATGCCAGGCTC</u>	General Biol (Chuzhou, China)
<i>HER2</i> -padlock probe 5	5'- <u>CAGATCCAAGCACCTTCTTCCTTTTACGAC</u> <u>CTCAATGCTGCTGCTGTACTACTCTTGACT</u> <u>GTGCCAAAAGCGC</u>	General Biol (Chuzhou, China)
HRP labeled detection probe	5'-CCTCAATGCTGCTGCTGTACTAC	TaKaRa

Underline marks the target hybridization sequences. All circle probe precursors and padlock probes were phosphorylated prior to use. Mixture containing 2 μM probes, 1 $\times$ PNK buffer A, 1 mM ATP, 0.2 U/ μL T4 polynucleotide kinase (Cat No. EK0032; Thermo Scientific) for 45 min at 37 °C, incubate at 65 °C for 10 min. Phosphorylated

probes can be stored at -20°C until use.