

3C Based DNA Hybridization Method for Chromosomal Translocation Screening

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Research Article

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Abstract

Background: DNA probes have been widely used as diagnostic tools for chromosomal translocations in malignancies. PCR-based methods often fail to detect translocations such as MYC/TRD in chronic lymphocytic leukemia. In addition, microscopic techniques cannot be helpful due to size detection limitations. This study sought to design a screening tool using immobilized ssDNA probes on a nitrocellulose membrane followed by 3C library fragments hybridization.

Results: Hence, we focused on developing a suitable 27 bp specific probe for the juxtaposed region of MYC and TRD. Colloidal gold nanoparticles (AuNP) functionalized translocation fragments of the MYC gene with a thiol group (MYC-AuNP-probe). Then TRD-probes were immobilized on nitrocellulose surface to detect TRD/MYC translocation in the SKW3 cells. Hybridization between DNA probes and 3C-library fragments of SKW3 cells was determined by color intensity. Optimal hybridization of the 3C library sample of the cell line to TRD-probe and MYC-AuNP-probe showed higher color intensity due to their convenient proximity to the juxtaposed region compared with normal cells.

Conclusions: Our results demonstrated that DNA hybridization colorimetric assay could be a helpful technique in chromosomal rearrangements screening. Accordingly, the combination of 3C based techniques and DNA-DNA hybridization can identify cancer cells with high specificity and sensitivity.

Background

Colorimetric diagnostic techniques have attracted much attention due to their simplicity, high speed, and low cost. Gold nanoparticles (AuNps) are widely used in colorimetric methods regarding their high extinction coefficient and size-dependent optical properties [1, 2]. Numerous studies have been performed to identify analytes using DNA probes covalently attached to the surface of AuNps [3–6]. Gold nanoparticle detection methods based on inter-particle cross-linking aggregation were first introduced by Mirkin et al. [7]. They used AuNps conjugated to thiolated oligonucleotides as detector probes (AuNPs-probe) to identify target DNA.

In nucleic acid biosensor research, nucleic acids are immobilized on the sensor surface by different methods such as adsorption, biotin-avidin interaction, covalent bonding, polymer matrix entrapment, self-assembly, and ionic bonding [8–10]. Moreover, single-stranded DNA (ssDNA) and RNA sequences can be attached to immobilized complementary components on the sensor surface. The binding of DNA and RNA to complementary molecules occurs according to Shargoff's law. Accordingly, mutations can be investigated by binding probes with complementary strands immobilized on biosensor surface to sample containing the mutation.

The chromosome conformation capture (3C) approach introduced by Dekker et al. This method includes chromatin fixation using fixative reagents such as formaldehyde to create cross-linking in nuclear proteins and cutting the chromatin with six cut restriction enzymes. During this process, the sticky ends of the enzymatic cleavages are ligated to each other by reassembly, and a library of chromatin fragments

of different sizes is obtained [11, 12]. DNA fragments with linear space in chromatin nucleation re-ligate close to each other during cross-linking, cleavage, and re-ligation. In our previous study, the SKW3 cell line was used for 3C library preparation, and the fragment of interest we detected was called the viewpoint [13].

Depending on the location of the viewpoint fragment, primers were designed at both ends, and reverse PCR was performed. The obtained fragments were analyzed by Sanger sequencing and demonstrated the existence of (8;14) (q24; q11) MYC/TRD. Our method was successfully used to evaluate chromosomal translocation existence in SKW3 cells. This 184 juxtaposed fragment was used to design a specific screening probe in the SKW3 cell line. The developed biosensor revealed a high specificity for the selected complementary sequences from all the other translocated sequences in the juxtaposed region of Chr-8 and Chr-14. Hence, this study aimed to perform a sensitive colorimetric assay for chromosomal translocation screening. For this purpose, a thiol modified DNA probe was functionalized with AuNps. The MYC-AuNP-probe served as a complementary sequence to hybridize with the TRD-probe and 3C libraries prepared from the SKW3 and normal cells.

Materials And Methods

Chromosome conformation capture

The juxtaposed MYC/TRD fragment in the SKW3 cell line was analyzed based on our recent study to design suitable detection and capture probes. As we mentioned in our previous study, 3C Library preparation was operated on SKW3 and HUVEC cells. These cells were suspended in the 3C buffer [25] and fixative reagent (Formaldehyde 37%). DNA digestion was done by EcoRI (Jena Bioscience, Germany) according to the manufacturer's protocol. Proteinase K and its buffer were added to the 3C library to reverse the cross-linked DNA. After phenol-chloroform purification, the SKW3 cell line 3C library was analyzed through agarose gel electrophoresis [26]. For inverse PCR, two regions were selected upstream and downstream of the viewpoint locus on the MYC gene based on EcoRI restriction sites, as shown in Figure 6a. PCR products were sequenced using standard Sanger sequencing service (Bioneer Company, South Korea). The juxtaposed fragment sequence was used for confirming translocation PCR using two specific primers.

DNA-DNA hybridization assay

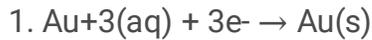
As described above, 184 bp sequence from translocation PCR was selected for screening probe synthesis. These probes contain 27 bp fragments on the MYC gene (MYC-AuNP-probe) and the TRD gene (TRD-probe).

AuNps synthesis

The most common method for preparing colloidal gold involves reducing gold (III) ions by citrate ions in a dilute aqueous solution (10 mM). Gold (III) ions are usually added to water in hydrogen tetrachloride

(HAuCl₄). Citrate ions (C₆H₅O₇⁻³) are more likely to be oxidized to acetone d-carboxylate ions (C₅H₄O₄⁻²), acting as a two-electron reducing agent (Figure 6b).

The reaction for the synthesis of colloidal gold is given in Equations 1 and 2 [27]:



Spectral information

Colloidal gold contains AuNps that measure about 5 to 50 nm. An absorption peak at 520 nm indicates the formation of AuNps with a 15-40 nm diameter. This optical property of AuNps is unique in their commercial products such as medical diagnostic kits, biosensors, DNA analysis, lasers, and optical filters. To prepare a concentration of 10 mM, dissolve one g of gold powder in 250 ml of distilled water containing a dilute solution of 10 mM citrate. After observing the purple color and taking an absorption peak at 520 nm, we brought it to a concentration of 0.5 mM. Also, the size distribution diagram of synthesized NPs with dynamic light scattering (DLS) was investigated.

Conjugation of AuNps and MYC-*thiol*-modified -probe

To conjugate a thiol-modified lyophilized MYC-probe and a colloidal AuNps, it is necessary to reduce the thiol unit to connect with the AuNps. For this purpose, a 0.1 M TCEP solution was prepared fresh as required and was added directly to the lyophilized probes. To reduction of the thiol group, the tube was incubated at room temperature for one hour. During the incubation time, the sample vortexed and spined several times. Then three M sodium acetate with pH = 5.2 was added, and the tube was vortexed.

Subsequently, 1.5 ml of absolute ethanol was added and stored at -20 ° C for 30 minutes, and the tube was centrifuged at 12,000 rpm for 10 minutes.

The precipitated probe was dissolved in sterile DNase-free water after drying (at concentration 1 μg/μl). In a sterile tube, nine μl of the reduced probe and five ml of AuNps were mixed and kept at room temperature for 16 hours without shaking. Then solvent buffer No. one, including 0.15 M NaCl, tween 20 0.025%, Tris-HCl 10 mM (PH = 7), and EDTA 2.5 mM (PH = 8) up to a volume of 50 ml was added to them. After incubation at room temperature for 40 hours, the mixture was centrifuged (10,000 rpm, 10 min).

Subsequently, the pellet was dissolved in solvent buffer No. two containing 0.15 M NaCl, tween 20 0.025%, Tris-HCl 10 mM (PH = 7) and EDTA 2 mM (PH = 8). The concentration of this oligo attached to the NPs was measured and used for the colorimetric assay. We named this detection probe MYC-AuNP-probe. Also, to ensure a proper functionalization of AuNps with oligo, their absorption was measured at a 400-600 nm wavelength.

Hybridization assay

Hybridization was performed in 6 groups with the same concentrations of TRD-probe, different concentrations of MYC-AuNp-probe, and 3C-libraries. All steps are the same for different groups as follows:

The nitrocellulose membrane was cut by punching into circular pieces with a diameter of 6.4 mm. These circles are the attachment surface for capture probes and the 3C libraries. These membranes were soaked in SSC (saline-sodium citrate) 20X buffer for 30 minutes before TRD-probes immobilization. Next, the desired values of TRD-probes were placed at 100 °C for 10 minutes and then five minutes on ice to ensure that all probe parts were denatured and single-stranded. Then two µg of TRD-probe was dotted without contact of the tip on the membrane. Then, membranes were placed at 80 °C for two hours and transferred to a 96 well plate to immobilize the probe. Next, the pre-hybridization step was performed by pouring 200 µl of a pre-hybridization buffer. This buffer contains 6X SSC, 4X Denhardt's [9], 10 µl Salmon Sperm DNA, and 0.2% SDS for 4 hours at 65 °C. Buffer was removed, and a 200 µl hybridization buffer containing the same materials and values from the 3C library samples of SKW3 and HUVEC cells was replaced in separate wells. The 3C libraries must be denatured (100 °C for 10 minutes and then freeze for 5 minutes) before adding to the hybridization buffer. For the hybridization step, a library sample and a probe attached to the membrane were incubated for 24-28 hours at 65 °C. Washing was performed by pouring 100 µl of SSC 4X (wash buffer) and then 100 µl of SSC 0.4X at 65 °C for 15 minutes to remove non-hybridized DNA fragments from the reaction.

After removing the wash buffer, different concentrations of conjugated AuNps of 100 ng / µl MYC-AuNp-probe (one µg of MYC-AuNp-probe) and 100 µl SSC 4X were added to each well. For hybridization of the conjugated probe with AuNps, a temperature of 65 degrees and six to 18 hours were applied. After the hybridization step, the remaining nanoparticle solution was removed without contact of the tip with the membrane. Washing consisted of three steps pouring 100 µl of SSC 4X, then 100 µl of SSC 0.4X at 65 °C for 15 minutes, and finally PBS at room temperature, respectively. The colored dots were photographed immediately after drying, and color intensity was analyzed by Image J software version 1.52.

Quantification of the colorimetric assay

Image J software was used to calculate and compare the color intensity in normal (HUVEC) and cancerous (SKW3) samples. A histogram tool was used to calculate the color intensity. The higher color intensity is related to a smaller number in a histogram, and the lower color intensity is related to a larger number and the closer it is to 255 (Figure 4a).

Statistical analysis

The paired t-test was used to compare groups. Data analysis was carried out by GraphPad Prism version 8. Results are reported as the mean ± SD and P < 0.05 were considered to be significant.

Results

Probe design based on Chromosome conformation capture

In our previous study, the 517 bp fragment from inverse PCR on the 3C Library of SKW3 cells represented the Juxtaposed fragment from MYC and TRD genes. This fragment was used for translocation PCR [13]. Our study confirmed that a 184 bp fragment (Table 1) represented MYC/TRD translocation. Based on translocation PCR product with juxtaposing sequence of TRD gene (Chr 14: 22423917 to 22423944), following by MYC gene, two 27 nucleotide probes were synthesized. The probe for the MYC gene was a 27 bp fragment (Chr 8:127741798 to 127741825) contains thiol-modification at the 3' end of the probe to bind to AuNPs during the colorimetric step (Table 2, Fig. 1a).

Table 1

Selected sequence in MYC /TRD juxtaposed fragment was used for specific probe design [5].

>tra4-tra-F
CCGAAGGTAGCAGGAGAACAGAGGTCAAGGTAGCAGTTAAGTACACAAAGAGGCATAAGG ACTGGGGAGTTGGGAGGAAGGTGAGGAAAAAACTCCTGTTACTTTAGTTAACCAGTGCCA GTCCCCTGCTCACTCCAAACCCAGCCTGATTTAAAAAAACCTTCATGAGACGGGGTCTTGCTGTGTCA

Table 2

Probe sequences used in the hybridization and colorimetric Assay

Title	sequence
TRD-probe	5'-TGACACAGCAAGACCCCGTCTCATGAAG-3'
MYC-AuNP-probe	5'-CTGGGTTTGGAGTGAGCAGGGGACTGG-3'-Thiol

DLS and evaluation of NPs adsorption before and after conjugation with MYC probe

We synthesized AuNps with sizes ranging from 7-120 nm, and the highest intensity of absorbance was observed in the size of 15–20 nm (Fig. 1b,2a). The conjugated probe at a concentration of 100 ng/μl was used for the colorimetric assay. The binding of the oligo probe to AuNps changed its color from pink to purple (Fig. 2b). Also, to confirm conjugation between the DNA probe and AuNps, the absorption of NPs at a 400–600 nm wavelength showed appropriate change. Considering that the absorption peak of non-conjugated AuNps is at 520 nm, the binding of NPs to oligo leads to the absorption peak shift to the right (Fig. 2c,2d).

Color intensity quantification

The results were analyzed in six groups from A to F (Fig. 3a, 3b), with different concentrations of 3C libraries and Myc-AuNP-probe. The color intensity difference between HUVEC and SKW3 cells on nitrocellulose dots is shown in Figs. 3b and 4a. Also, as shown in Fig. 4b, the color intensity between two groups A and B, is significantly different. In group A, two μg of 3C-library were hybridized with two μg of

MYC-AuNp-probe and group B with one μg of MYC-AuNp-probe. Furthermore, in C, D, E, and F groups, the difference in color intensity between SKW3 and HUVEC cells was not insignificant. The significance level in group A is equal to 0.1 ($p\text{-value} = 0.1$), and the significance level in group B is equal to 0.01 ($p\text{-value} = 0.01$) Fig. 4b. It can be concluded that the detection limit in this method is two micrograms of the sample. On the other hand, if the probe attached to the AuNps is used at a higher concentration, the resolution will decrease. In other words, the desired result will be achieved with the hybridization of two μg 3C libraries and one μg of MYC-AuNp-probe.

Discussion

Biosensors have been used successfully in various fields of medicine, including cancer research [14]. Biosensors-based techniques have received considerable attention due to their low cost and high sensitivity [15]. One of the different types of biosensors is nucleic acid-based (NAB) biosensors, including DNA probe molecules, RNA, Peptide Nucleic Acid, and Aptamers. With the increasing number of cancer patients worldwide, the development of early diagnostic methods seems essential. Also, biosensors in monitoring response to treatment have raised new hopes in precision medicine [16]. Therefore, designing a simple, high-sensitivity, and low-cost method that provides comprehensive disease-based information to specialists seems necessary. Hence, in this study, we sought to design a screening tool using the immobilized ssDNA probe on the nitrocellulose membrane followed by 3C library fragments hybridization.

We designed specific probes to identify MYC/TRD translocation based on the 3C technique in our present study. The probes were then conjugated to gold nanoparticles and placed on a nitrocellulose membrane. Finally, we used it as a biosensor to detect the MYC/TRD translocation in SKW3 cells using the colorimetric assay. In a study conducted in 2017 by Benidi et al., a DNA biosensor for BRCA-1 mutation diagnosis has been designed. The basis of the detection method is the binding of a complementary component with appropriate hybridization power and competition with non-complementary components (P1nC1, P1nC2, and P1nC3), which were used to evaluate the specificity of the biosensor. This biosensor with eight years of stability can be used as a practical method in detecting sequences related to breast cancer [17].

In another study conducted in 2018 by Hong et al., the Surface Plasmon Resonance (SPR) method was used to identify mir-200b [18]. In their study, AuNPs compete with miRNA for binding to probes attached to the surface of a gold-coated membrane. In another related study, a hybridization-based method to identify the HBV genotype was performed by Bao et al. DNA hybridization on the surface of nitrocellulose membranes using the avidin-biotin system leads to increased DNA binding. The hybridization method introduced in this study was a fast method with low contamination risk and is very reliable. Therefore, it can be an efficient tool for accurate clinical diagnosis [19]. We used a biosensor consisting of functionalized AuNPs by a ssDNA probe extracted from our recent study. Then, conjugated probes with AuNps were hybridized to the 3C library and TRD-probe sandwich nitrocellulose membrane. The color intensity was prepared by covering MYC-AuNp-probe on the surface of the nitrocellulose membrane

consisting of three different fragments. Under optimal conditions, the color intensity between the 3C library of translocation cells and the 3C library of normal cells showed a significant difference ($p = 0.1$). We performed a colorimetric assay on SKW3 cells with MYC/TRD translocation. Chromosomal rearrangements with the involvement of the MYC oncogene located at locus 8q24 can occur in each patient with a different nature [20]. In many cases, lymphatic malignancies occur due to errors in class recombination or recombination in the V (D) J (V (D) J recombination) chains.

The various mechanisms that occur in V (D) J recombination have led to variation in the type and sequence of rearranged segments. Therefore, PCR-based methods are not able to identify these breakpoints [21]. Based on the location of these clusters and the displaced fragments, and the length and sequence of the fragments, the expression of MYC oncogene varied from patient to patient. This event could limit the ability of conventional molecular methods to detect these rearrangements. Gene proximity is one of the most critical issues in the field of epigenetics [22]. In our previous study, rearrangement of MYC/TRD in SKW3 cells was detected using the chromosome conformation capture method combined with DNA hybridization colorimetric assay. In the construction of the 3C library, by chromatin fixation and create cross-links, the fragments related to MYC and TRD gene are fixed close to each other. Also, during the enzymatic digestion and ligation process, smaller and more reproducible chimeric fragments with juxtaposed fragments of MYC/TRD sequences [22].

Studies have shown that the spatial conformation of chromatin in different cells of a cell population in healthy or cancerous tissue can lead to different products in terms of size and layout in the 3C library [23]. Our recent study demonstrated that a 184 bp fragment contains the MYC gene locus on chromosome 8 (Chr 8: 127741687 to 127741825), attached to a 45 bp fragment of TRD gene locus on chromosome 14 (Chr 14: 22423917 to 22423962). The cytogenetic method was used to evaluate this rearrangement in the SKW3 cell line. However, the microscopic method was not able to detect this rearrangement. The amplified fragment with sequences related to both MYC and TRD gene loci was employed to design specific probes for the colorimetric assay.

We first synthesized AuNps with a diameter of about 15–20 nm. The MYC locus probe was synthesized with 3' end thiol modification and conjugated to AuNPs (Fig. 5a). The hybridization results from the colorimetric assay of 3C library samples in SKW3 and normal cells with chromosomal rearrangement are significantly different between the two groups (Fig. 5b). We applied different concentrations of colorimetric assay components considering the color intensity difference between control cells and chromosomal rearrangement cells. Increasing and decreasing the concentration of AuNps attached to probe eight caused the same color intensity in control and test groups. Optimize the concentration of detection probe conjugate to AuNps is a requisite step in DNA hybridization colorimetric assay. The use of more than one μg of Myc-AuNps-probe leads to dye deposition on the nitrocellulose membrane. Therefore, the expected color difference between the normal and translocated cells was not observed after washing. Other factors that can affect the quality of the hybridization reaction include the amount of immobilized probe on the nitrocellulose paper, the temperature of the hybridization reaction, reaction time, pre-hybridization buffer composition, wash buffer composition, washing temperature and duration,

the amount of AuNP, and 3C library concentration and quality. Our results indicate that low salt concentration and high hybridization temperatures can be used to prevent non-specific bindings.

To minimize non-specific hybridizations, we used blocking agents such as PVP and BSA in the hybridization and pre-hybridization buffer. Also, to remove the background color resulting from the pink color of AuNPs, the washing step has been performed in conditions with high stringency and high temperature, which leads to the removal of non-hybridized components with weak attachments. Creating a three-dimensional network with high attachment capacity, optimizing probe immobilization without chemical methods, and cheap cost are advantages of using nitrocellulose membrane [24]. Indeed, we detected MYC/TRD chromosomal rearrangements using 3C based molecular and colorimetric method without cytogenetic and complex NGS.

Conclusion

This study is the first report of t (8; 14) MYC/TRD screening using a 3C-based colorimetric method. A small chromosomal rearrangement was screened using the DNA-DNA hybridization method without the need for specific primers and FISH probes. As we indicated in our recent study, TRD fragment juxtapositioning to MYC locus could enhance MYC oncogene effects with different clinical phenotypes due to the discrete nature of gene clustering in this translocation. 3C library creates a genomic map of gene proximity and interactions. Based on this library, we performed a colorimetric DNA-DNA hybridization assay for screening a rare chromosomal rearrangement. We used only one of the probable translocated fragments as a detection probe. Therefore, the complete understanding of the mechanism of chromosomal rearrangement at this gene locus requires further studies. According to this method's ability to screen small chromosomal rearrangements, designing other screening studies for chromosomal translocation in clinical samples recommended.

Abbreviations

AuNPs	Gold Nanoparticles
HAuCl ₄	Tetrachloroauric acid
SPR	<i>Surface Plasmon Resonance</i>
PBS	<i>Phosphate Buffered Saline</i>
SN	Supernatant
SDS	Sodium dodecyl Sulfate
Rpm	Revolutions per minute
MYC	<i>MYC</i> proto-Oncogene
TRD	T-cell Receptor Delta
TBE	Tris Boric EDTA
Chr	Chromosome
DLS	Dynamic light scattering
TCEP	Tris (2-carboxyethyl) phosphine hydrochloride
SSC	saline-sodium citrate Buffer
der(8)	Derived from chromosome 8
t(8;14)	Translocation between chromosome 8 and 14
ATCC	American Type Culture Collection
TCR	T-cell Receptor
3C	Chromosome Conformation Capture
4C	Chromosome Conformation Capture on Chip
AML	Acute Myeloid Leukemia
CDS	Chronocoulometric DNA Sensor
ssDNA	Single-Stranded DNA

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Gholamreza Tavoosidana and Moloud Absalan contributed to conception and design and conducted Molecular experiments. Roya karimi and Zahra Jabbarpour conducted cellular experiments. Mohammad hossein Ghahremani and Elaheh Motevaseli responsible for overall supervision. Fatemeh Mahmoudian was responsible for nanomaterial production and characterization. Moloud Absalan wrote the manuscript, which was revised and rewrite by Zahra Jabbarpour. All authors read and approved the final manuscript.

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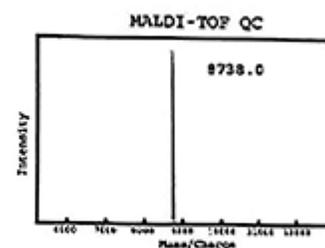
Figures

a

probe.8

CTG GGT TTG GAG TGA GCA GGG GAC TGG

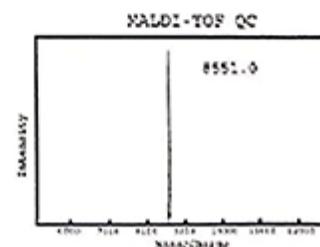
Optical Density	11.0	Purification	BIO-RP
Total nmole	41.7	Modification	3' Thiol
Scale (umoles)	0.2	GC ratio (%)	63.0
Base count	27	Molecular weight	8736.32
Tm (C)	69.2	Volume for	417.1 ul
		100 pmoles/ul	



probe.14

TGA CAC AGC AAG ACC CCG TCT CAT GAA G

Optical Density	11.0	Purification	BIO-RP
Total nmole	40.3	Modification	None
Scale (umoles)	0.2	GC ratio (%)	53.6
Base count	28	Molecular weight	8551.42
Tm (C)	67.2	Volume for	403.2 ul
		100 pmoles/ul	



b

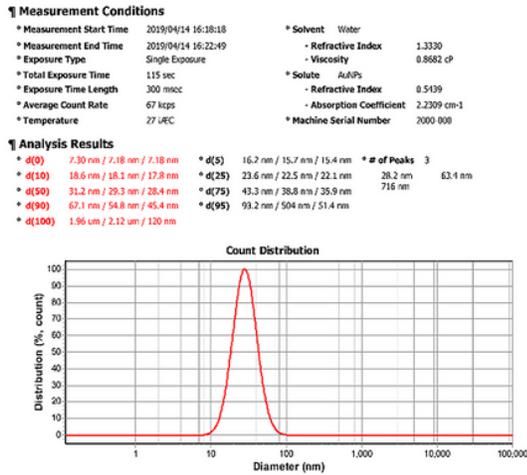
φ Intensity Distribution Table

size	In(%)	Cum(%)									
0.10 nm	0.00	0.00	2.26 nm	0.00	0.00	51.4 nm	6.80	82.77	1.17 um	0.26	99.76
0.12 nm	0.00	0.00	2.64 nm	0.00	0.00	60.1 nm	4.79	87.56	1.37 um	0.14	99.90
0.14 nm	0.00	0.00	3.09 nm	0.00	0.00	70.3 nm	3.49	91.05	1.60 um	0.07	99.97
0.16 nm	0.00	0.00	3.61 nm	0.00	0.00	82.2 nm	2.53	93.58	1.87 um	0.02	99.99
0.19 nm	0.00	0.00	4.22 nm	0.00	0.00	96.1 nm	1.70	95.28	2.19 um	0.01	100.00
0.22 nm	0.00	0.00	4.94 nm	0.00	0.00	112 nm	1.00	96.28	2.56 um	0.00	100.00
0.25 nm	0.00	0.00	5.77 nm	0.00	0.00	131 nm	0.50	96.78	2.99 um	0.00	100.00
0.30 nm	0.00	0.00	6.75 nm	0.00	0.00	154 nm	0.21	96.99	3.50 um	0.00	100.00
0.35 nm	0.00	0.00	7.89 nm	0.01	0.02	180 nm	0.07	97.06	4.09 um	0.00	100.00
0.41 nm	0.00	0.00	9.22 nm	0.07	0.09	210 nm	0.02	97.09	4.78 um	0.00	100.00
0.47 nm	0.00	0.00	10.8 nm	0.26	0.34	245 nm	0.01	97.09	5.59 um	0.00	100.00
0.55 nm	0.00	0.00	12.6 nm	0.79	1.13	287 nm	0.01	97.11	6.53 um	0.00	100.00
0.65 nm	0.00	0.00	14.7 nm	2.01	3.14	335 nm	0.04	97.14	7.64 um	0.00	100.00
0.76 nm	0.00	0.00	17.2 nm	4.27	7.41	392 nm	0.09	97.23	8.93 um	0.00	100.00
0.89 nm	0.00	0.00	20.1 nm	7.54	14.95	459 nm	0.18	97.42	10.4 um	0.00	100.00
1.03 nm	0.00	0.00	23.6 nm	11.11	26.06	536 nm	0.31	97.72	12.2 um	0.00	100.00
1.21 nm	0.00	0.00	27.5 nm	13.69	39.75	627 nm	0.43	98.15	14.3 um	0.00	100.00
1.41 nm	0.00	0.00	32.2 nm	14.17	53.92	733 nm	0.49	98.65	16.7 um	0.00	100.00
1.65 nm	0.00	0.00	37.6 nm	12.46	66.38	857 nm	0.48	99.12	19.5 um	0.00	100.00

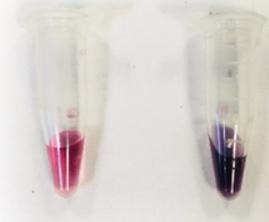
Figure 1

a) Specific screening probe for MYC locus Chr-8 and TRD locus Chr-14 characteristics. b) Intensity distribution table of colloidal AuNPs synthesized.

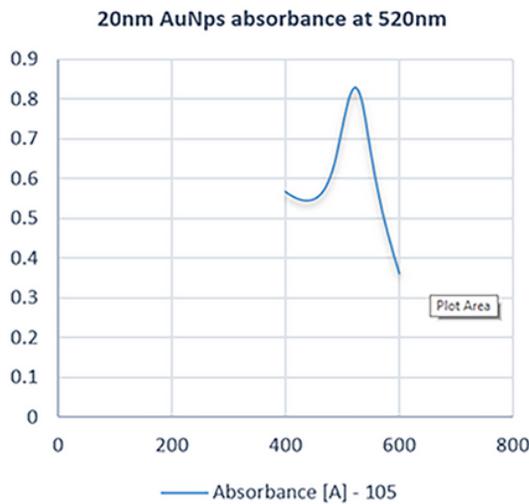
a



b



c



d

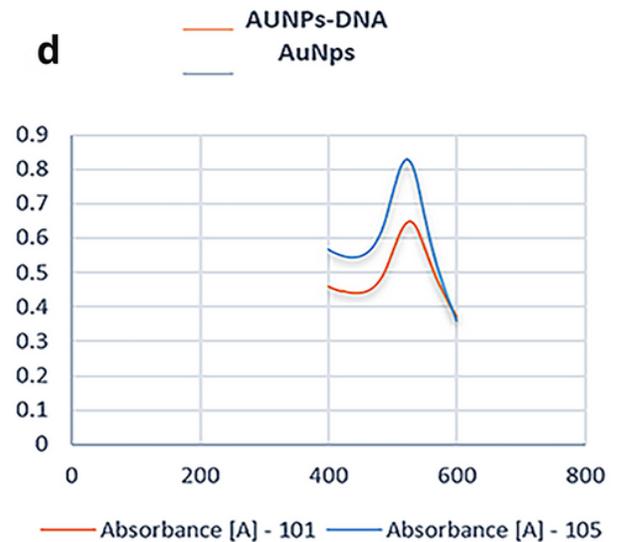


Figure 2

Comparison and characteristics of colloidal NPs before and after conjugation with DNA probe. a) Particle size analysis by DLS, b) colloidal NPs (pink), DNA conjugated NP (Violet), c) AuNps UV absorbance at 520 nm, d) AuNps & AuNps-DNA probe UV absorbance at 520 nm.

a

A		B		C		D		E		F	
Lib 2000ng+ Myc-pr 2000ng		Lib 2000ng+ Myc-pr 1000ng		Lib 2000ng+ Myc-pr 500ng		Lib 800ng+ Myc-pr 1000ng		Lib 1000ng+ Myc-pr 500ng		Lib 500ng+Myc-pr 500ng	
HUVEC	SKW3	HUVEC	SKW3	HUVEC	SKW3	HUVEC	SKW3	HUVEC	SKW3	HUVEC	SKW3
163.057	145.356	203.6	181.397	175.696	171.772	200.824	199.583	175.27	176.454	174.154	177.86
164.431	155.367	192.029	172.463	172.331	176.31	201.902	201.599	156.576	164.726	158.108	169.084
155.983	148.744	197.814	176.93	174.013	174.041	206.922	205.121	165.923	170.58	166.131	173.472

b

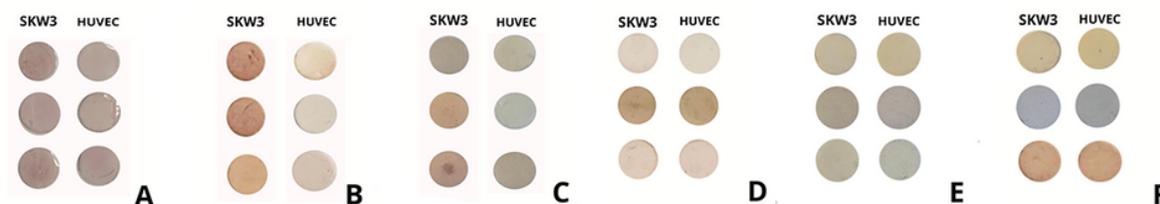
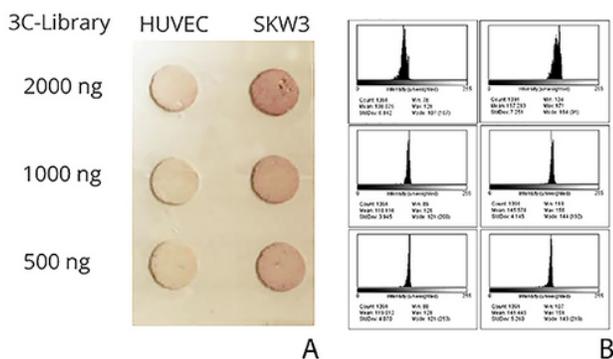


Figure 3

a) Colorimetric assay was performed in 6 groups (A-F). In all groups, TRD-probe concentration was the same. Different test groups consisted of different concentrations of 3C libraries and MYC-AuNp-probe, as we showed. Differences in color intensity were shown according to histogram results in Image J software. Lib: 3C-Library, Myc-Pr: MYC-AuNp-probe. b) Colorimetric assay results in 6 different groups showed different color intensities from different DNA-DNA hybridization efficiency in each concentration group.

a



b

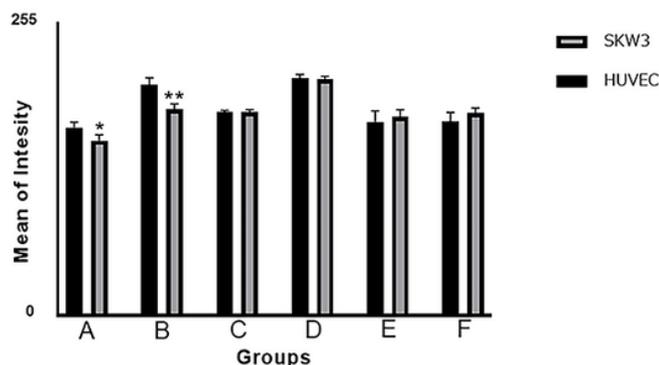


Figure 4

a) Color intensity was analyzed using a histogram in Image J software. A-A) Nitrocellulose dots with different concentrations of 3C libraries. A-B) Histogram color intensity on Image J software. b) Analysis

of colorimetric assay results by Paired-t test using Graphpad Prism version.8 software *P<0.05, **P<0.01, ***P<0.001, ****P <0.0001.

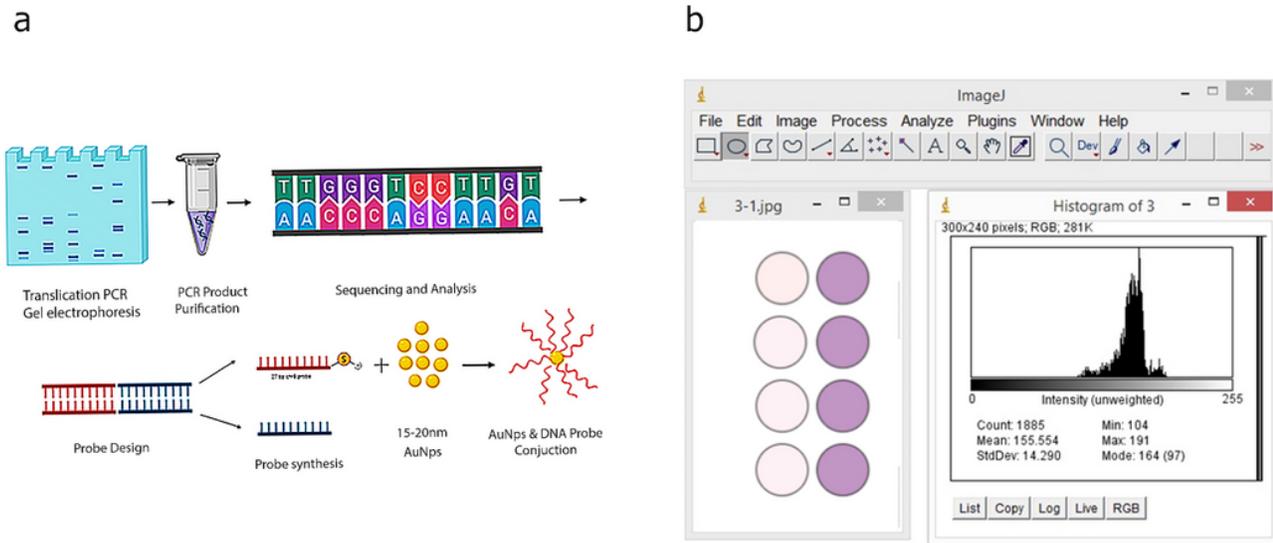
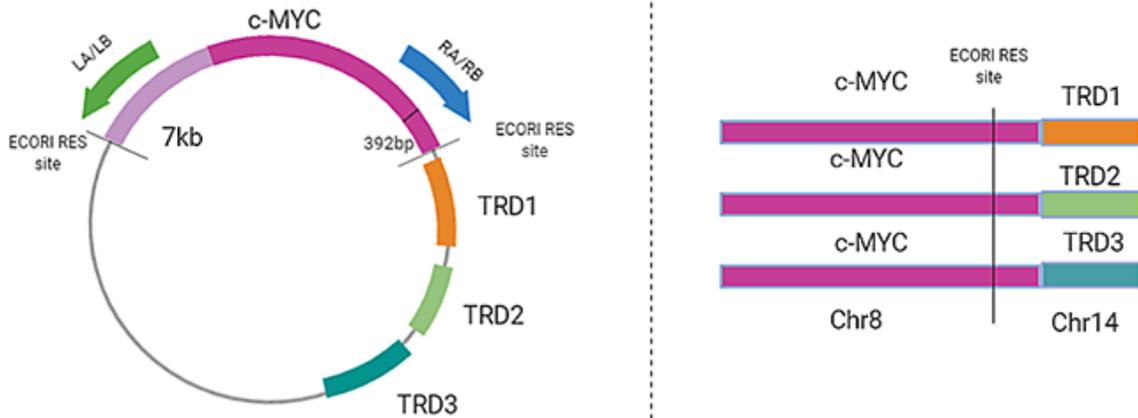


Figure 5

a) Translocation PCR product fragment was selected for specific colorimetric probe design. b) Image J software histogram was used for color intensity analysis.

a



b

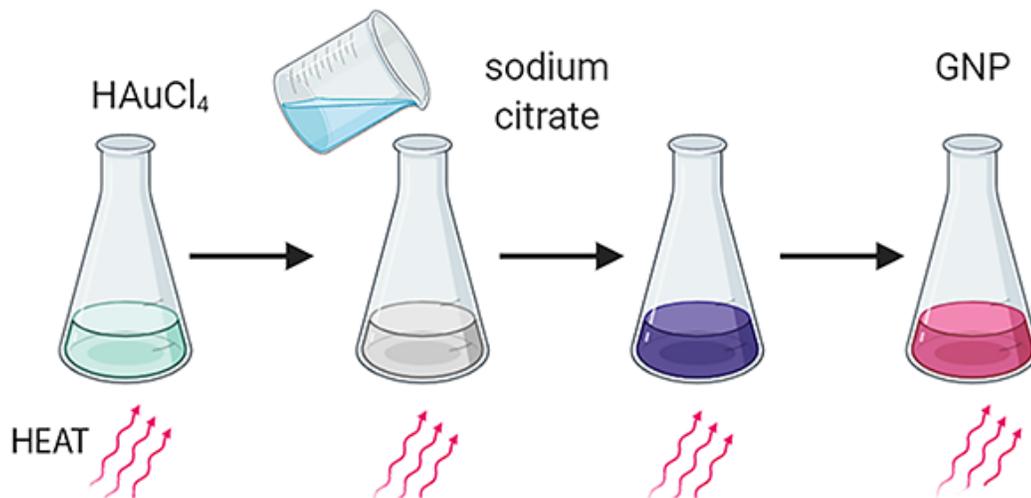


Figure 6

a) Juxtaposed TRD sequences in MYC/TRD translocation. b) Synthesis of gold nanoparticles (AuNPs).

Supplementary Files

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- Graphicalabstract.tif