Cycling of Rational Hybridization Chain Reaction to Enable Enzyme-Free DNA-Based Clinical Diagnosis

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Supplementary Information

Table S1. Sequence of oligonucleotides employed in this work. The colors show related sequences (through complementarity or similarity). The single mismatch position is highlighted with a lowercase letter.

Name	Sequence (5'-3')
U1-1	5'-CTAGCTCATACATC ATCCTATCTATCCAGAC TCTCACACGTACTC
U1-2	5'-CTAGCTCATACATC GTCTGGATAGATAGGAT TCTCACACGTACTC
U2-1	5'-GATGTATGAGCTAG GAGATGCAATCGACTGT GAGTACGTGTGAGA
U2-2	5'-GATGTATGAGCTAG ACAGTCGATTGCATCTC GAGTACGTGTGAGA
Target DNA	5'-TGCCTTGTAAGAGCGACGTAGGTGAATGAG
Capture probe	5'-Biotin-TTTTTTTTTT-CTCATTCACCTACG
U1-1'	5'-TCGCTCTTACAAGGCACTAGCTCATACATC ATCCTATCTATCCAGAC TCTCACACGTACTC
U1-1c	5'-GATGTATGAGCTAG GTCTGGATAGATAGGATGAGTACGTGTGAGA
1-mismatch DNA	5'-TGCCTTGTAAGAGCGACGTAGGTtAATGA
1-mismatch DNA-5'	5'-TGCCTTGTAAGAGCGACtTAGGTGAATGA
1-mismatch DNA-3'	5'-TGCCTTGTAAGAGCGACGTAGGTGAATtA
Non-cognate DNA	5'-TCGACCTGGGCAGGGTTCGCAGATCCTGCGACGTA

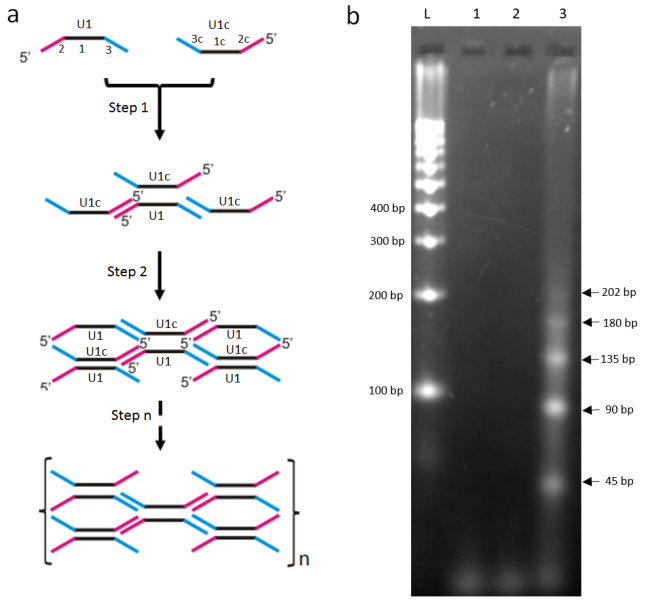

Fig. S1 Reaction principle of a single db DNA-mediated HCR. (**a**) Schematic illustration of synthesis and inter-reaction of a typical db DNA unit. Two starting oligonucleotides (U1-1 or U1-1c) have complementary middle segments (1 and 1c), while the 5′ (2 and 2c) and 3′ (3 and 3c) ends of U1-1 and U1-1c are complementary to each other. In this way, one strand U1-1 can bind to 3 single strands of U1-1c (step 1). The single db DNA-mediated HCR starts by mixing these two starting oligonucleotides together. Here U1-1 is the core, but the reaction can be initiated with U1-1c as well in a symmetric process. Each starting oligonucleotide has three exposed sticky ends, each complementary to the other one. They hybridize and form a large DNA structure (step n), which increases in size exponentially as the reaction progresses; (**b**) Evaluation of the product using gel electrophoresis. Lane L: 100 bp ladder ; lanes 1 and 2:U1 and U1c only respectively ; lane 3 products of U1 and U1c with stoichiometry ratio at 1:1, with concentration at 0.3 µM. Bands appeared in lane 3 from 45 to 202 bp indicative of the expected product formation process. Some bands with larger sizes can also be formed during the reaction (smear).

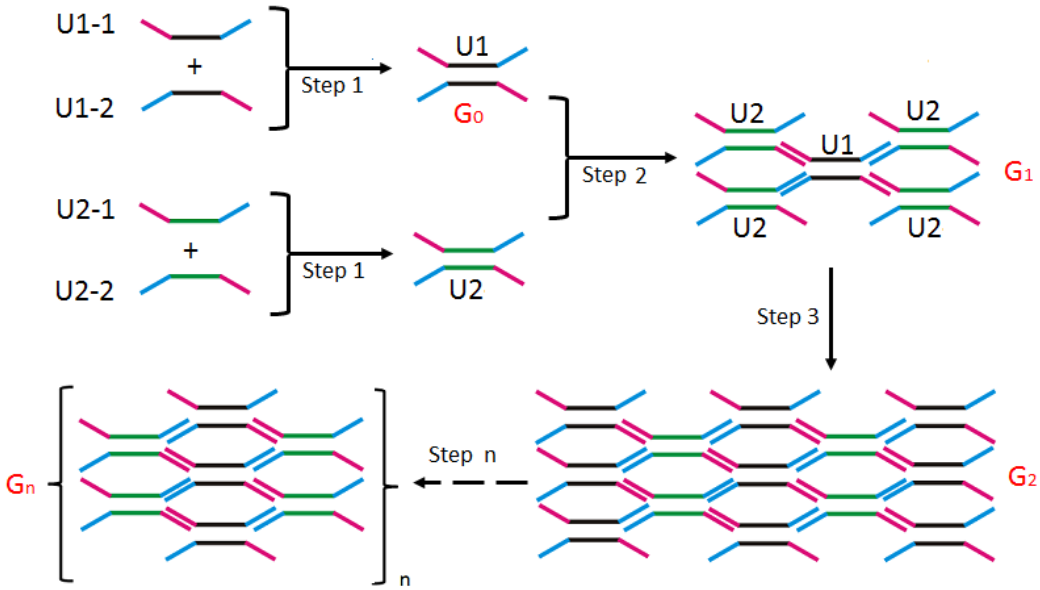

Fig. S2 Formation of the final product of rational HCR. The final product, with a large molecular size, was assembled by adding U1 and U2 successively (in cycles) and repeatedly. U1 here is used as the reaction core (G_0) which has 4 exposed sticky ends. Theoretically 4 U2 molecules can bind at the exposed sticky ends of U1. This hybridization forms the first generation product (G_1) which has 12 exposed sticky ends (each U2 has 3 exposed sticky ends and 1 bound to U1 already). These 12 new sticky ends then bind with the exposed sticky ends of U1 (in a new cycle). Higher generation rational products can be assembled in a similar hybridization chain reaction (Fig. 1).

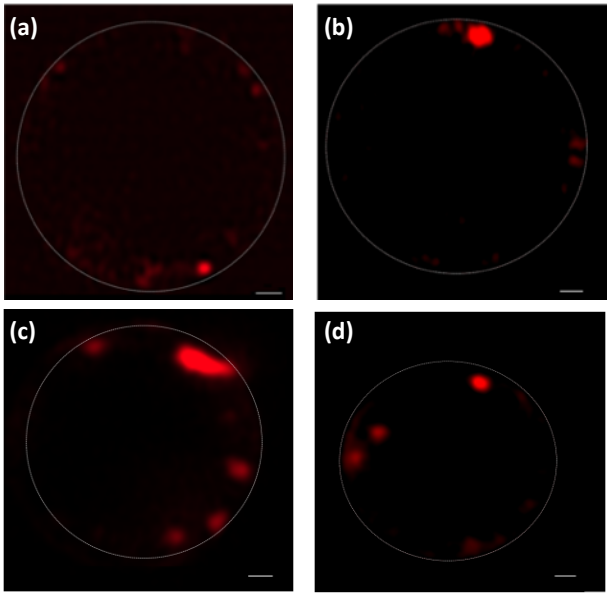

Fig. S3 Fluorescent spots on the surface of an individual microbead as the target DNA concentration increased from 10^3 copies/reaction (a-b) to 10^5 copies/reaction (c-d). (a) and (c) are obtained after 5 cycles, while (b) and (d) are obtained after 7 cycles. All scale bars, 0.5 µm; white dotted lines indicate the edge of the microbead.

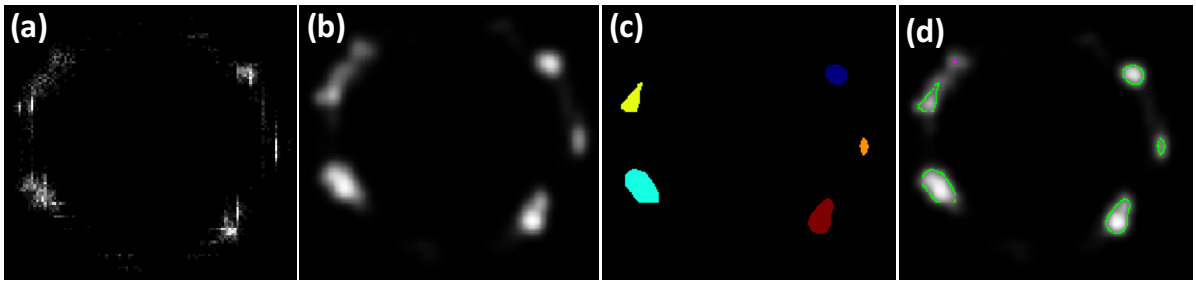

Fig. S4. Illustration of the process of segmentation from a super-resolution image (here from a bead having been processed through 5 cycles). The intensity of the image (containing multiple beads) is normalised between [0, 1] (**a**). It is then smoothed to connect pixels from an area together (**b**). The area is thresholded and segmented into objects (**c**) which were analysed for their shape and intensity (**d**).