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A new microcolumn-type microchip for examining the expression of chimeric fusion genes using a nucleic acid sandwich hybridization technique

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ABSTRACT

We report a new type of microcolumn installed in a microchip. The architecture allows use of a nucleic acid sandwich hybridization technique to detect a messenger RNA (mRNA) chain as a target. Data are presented that demonstrate that the expression of a chimeric fusion gene can be detected. The microcolumn was filled with semi-transparent microbeads made of agarose gel that acted as carriers, allowing increased efficiency of the optical detection of fluorescence from the microcolumn. The hybrid between the target trapped on the microbeads and a probe DNA labeled with a fluorescent dye was detected by measuring the intensity of the fluorescence from the microcolumn directly. These results demonstrate an easy and simple method for determining the expression of chimeric fusion genes with no preamplification.
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1. Introduction

Microcolumns for nucleic acid sandwich hybridization techniques have been developed for examining gene expression [1,2]. The technique for detecting a target messenger RNA (mRNA) chain transcribed from the gene is simple and easy, and involves the following steps: (1) preparation of a trap for the target on a carrier, (2) trapping the target on a carrier in the microcolumn by the first hybridization between the target and the trap, where the trap is complementary to a partial sequence of the target, and (3) formation of a sandwich hybrid by a second hybridization with a labeled probe complementary to a specific sequence of the target. This complementary sequence non-overlapping with the sequence of the trap is chosen. If a fluorescent dye is used as a label, the sandwich hybrid can be detected by the direct measurement of the fluorescence intensity of the microcolumn. The first and second hybridization processes can be implemented simultaneously. A diagram illustrating the processes is shown in Fig. 1.

The microcolumn is made by filling a microchannel fabricated in a microchip or a microcapillary with microbeads that act as the carrier. The hybrid is formed in the microscale space of the gaps between the microbeads. Therefore, the hybridization reaction is fast [1–3].

The principle was demonstrated using a poly(A) selection method using a poly(dT) chain as the trap on the carrier. The poly(dT) chain was complementary to a poly(A) tail added to the 3' end of the mRNA chain. This approach is generally used for the recovery and purification of total mRNA chains, because eukaryotic mRNA chains other than histones have a poly(A) tail [4]. In the sandwich hybridization technique, the poly(A) selection method can be used as a first hybridization process. Using this approach, the amount of the mRNA chains of β-actin and glyceraldehyde-3-phosphate dehydrogenase genes have been measured [2].

The transmutation of a gene can cause cancer. For example, in the case of chronic myeloid leukemia (CML), a type of blood cancer, the 9th chromosome is translocated to the 22nd chromosome in tumor cells. The result is a *bcr-abl* chimeric gene produced by the fusion of a *bcr* gene on the 22nd chromosome and an *abl* gene on the 9th. This is followed by transcription [5]. The original *bcr* and *abl*

Abbreviations: A, adenine; T, thymine; G, guanine; C, cytosine; U, uracil; dA, deoxyadenine; dT, deoxythymine; dG, deoxyguanine; dC, deoxycytosine; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; mRNA, messenger RNA; CML, chronic myeloid leukemia; SDS, sodium dodecyl sufate; PEEK, polyetheretherketone.

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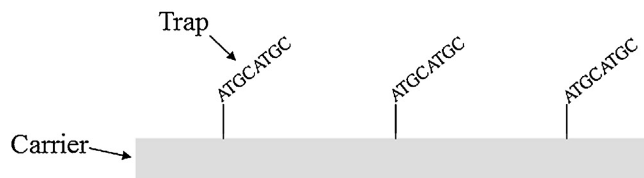
1. Preparation of a trap on a carrier



2 and 3. 1st and 2nd hybridization on the carrier

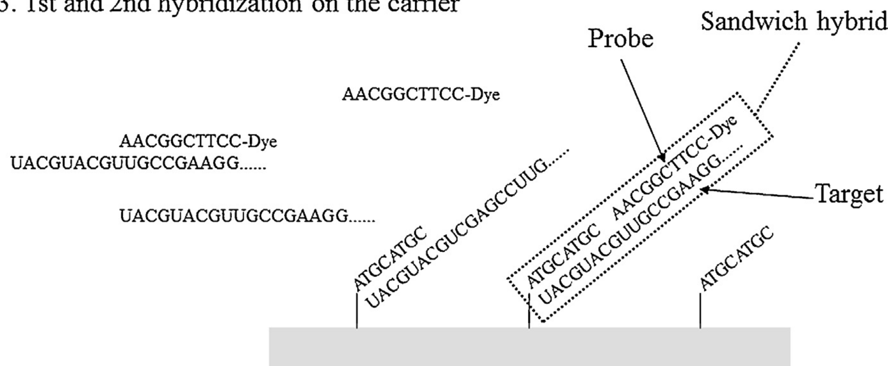


Fig. 1. Diagram of the processes of the sandwich hybridization. Preparation of traps on a carrier is illustrated in (1). Both of 1st hybridization between a target and the trap and 2nd hybridization between a target and a probe are illustrated in (2 and 3).

genes can also be transcribed in the patient's other cells. Therefore, an RNA sample extracted from the patient's tissues should contain both the RNA chains transcribed from the chimeric and from the original genes. Thus, we have attempted to use a trap and a probe complementary to each specific sequence of the original gene and of the chimeric fusion gene in order to examine the expression of the chimeric fusion gene.

2. Materials and methods

2.1. Nucleic acid chains

Long chain RNA was prepared and used as a test target (target 1). The RNA was prepared as follows: (1) A DNA extract was isolated from K562 cells established from the cells of a CML patient (Philadelphia chromosome positive). (2) The sequence of the *bcr-abl* chimeric fusion gene was cloned from the extract using a reagent kit (Prime Script II 1st strand cDNA Synthesis kit, Takara). (3) The DNA sequence from bases 601 to 3752 was selected and verified by sequencing. (4) A plasmid containing the sequence was prepared, and the RNA chain was synthesized *in vitro* using a reagent kit (T7 RiboMAXTM Express Large Scale RNA Production System, Promega). The RNA chain was identified by electrophoresis.

Polydeoxynucleotides complementary to each specific sequence of the *bcr* and *abl* genes in target 1 were synthesized as a trap and as a probe, respectively (C-18 column purification grade, Tsukuba Oligoservice). The 3′ end of the trap was biotinylated (trap 1) and the 5′ end of the probe was labeled with a fluorescent dye (1-[3-(4-monomethoxytrityloxy)propyl]-1′-[3-[(2-cyanoethyl)-(*N*,*N*-diisopropyl) phosphoramidityl]propyl]-3,3,3′,3′-tetramethylindocarbocyanine chloride, Cy3) (probe 1). The peak wavelengths of the excitation and emission of Cy3 are 532 and 568 nm, respectively [6]. Trap 1 labeled at the 5′ end with Cy3 was prepared in order to estimate the ratio of formation of the sandwich hybrid in the microcolumn (trap 1L).

A random polydeoxynucleotide sequence was used as the test target (target 2). Two polydeoxynucleotides with non-overlapping sequences complementary to the specific sequence of target 2 were synthesized as a trap and as a probe (C-18 column purification grade, Tsukuba Oligoservice). The 3' end of the trap was biotinylated (trap 2) and the 5' end of the probe was labeled with Cy3 (probe 2).

The sequences of the targets, traps, and probes are shown in Table 1. The melting points (T_m) of the target–trap and target–probe hybrids were calculated and are shown in Table 2 [7].

2.2. Preparation of the reaction solution

A total of 0.3 M sodium chloride aqueous solution containing 0.2% sodium dodecyl sulfate (SDS) was prepared from a 5 M sodium chloride aqueous solution (#AM9759, Life Technologies) and 10% SDS (#15553-027, Life Technologies) to provide the flow solution. The target and the probe were dissolved in the flow solution to provide the reaction solution. The concentration of target 1 and probe 1 in the reaction solution for target 1 (reaction solution 1) was 17.0 ng/μL and 6.3 pmol/μL, respectively. The concentration of target 2 and probe 2 in the reaction solution for target 2 (reaction solution 2) was 29.8 and 2.0 pmol/μL, respectively.

2.3. Preparation of carriers modified with the trap

Agarose gel microbeads coated with streptavidin (Streptavidin Agarose Resin, a 50% slurry, #20343, Thermo Scientific) and 100 μM trap aqueous solution were prepared. A total of 50 μL of the trap solution was added to 200 μL of the gel microbead suspension in a microtube and vortexed, then 250 μL of MilliQ water was added and the suspension was mixed for over an hour using a microtube mixer (Tomy) to provide a suspension of the carrier modified with the traps via streptavidin–biotin bonding.

2.4. Measurement of light transmission through the carriers

The fluorescence intensity of a label in the microcolumn is dependent on the transmission of the excitation and emission

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