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Amplification of DNA for Detection of Ultrasensitive Antigen

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Editorial

Antibody-based detection methods for particular Ags are flexible and powerful tools for molecular and cellular studies, as well as clinical diagnostics. The ability of such systems arises from the high specificity of Abs towards certain antigenic epitopes. However, there are multiple instances where essential biological markers for cancer, infectious illness, or biochemical processes are present in bodily fluids or tissues at too low a concentration to be identified by standard immunoassays.

The development of stronger fluorochromes and chemiluminescent substrates for use in ELISAs, immunofluorescence-based labelling and immunoblotting, and the use of signal amplification techniques such as tyramide deposition are all recent breakthroughs in the field of low-level Ag detection. Although these approaches are quite strong, they typically require more sensitivity and specificity, especially when working with little quantities of sample material or when the Ag density is extremely low.

We developed the newly reported Rolling Circle Amplification (RCA) reporter technology for the detection of protein Ags to meet these objectives. Previous research has shown that DNA amplification may be used to identify Abs bound to Ag. Using streptavidin-biotin interactions, alternative bridging moieties, or covalent linkage, immuno-PCR associates a unique DNA sequence tag with a particular Ab.

The accompanying DNA tag is subsequently amplified by PCR to identify Abs coupled to Ag. Various Abs and DNA tags were employed to evaluate multiple Ags at the same time. Despite the fact that immuno-PCR was shown to be much more sensitive than ELISA, gel electrophoresis was necessary following DNA amplification in solution in order to separate and/or quantify the many amplified DNA tags. Immuno-general PCR's use as an alternative to ELISA has been limited by the need for temperature cycling and product separation by gel electrophoresis, as well as its inability to be used in immunohistochemical or array formats.

Under isothermal settings, RCA powered by DNA polymerase may replicate circularised oligonucleotide probes with either linear or geometric kinetics. RCA makes hundreds of tandemly connected copies of the circular template from a single primer in a matter of minutes. The 59 end of this primer is linked to an Ab in ImmunoRCA. The rolling circle reaction produces a DNA molecule with multiple copies of the circle DNA sequence that remains linked to the Ab in the presence of circular DNA, DNA polymerase, and nucleotides.

Direct incorporation of hapten-tagged or fluorescently labelled nucleotides, or hybridization of fluor-labeled or enzymatically labelled complementary oligonucleotide probes, can all be used to detect amplified DNA. As a result, ImmunoRCA is a unique technique to signal amplification in Ab–Ag recognition events.

Although RCA reactions can employ either linear or geometric kinetics , the signal-generation paradigm we adopted in this investigation is solely based on the linear RCA model. The production of Ab–DNA conjugates is described here, as well as the practicality of employing these conjugates to detect Ags in a variety of immunoRCA forms.

The kinetics of fluorescence accumulation during thermocycling is proportional to the quantity of DNA copies present at the start. The bigger the number of target sequences, the fewer cycles are required to achieve observable fluorescence. The results show that a kinetic method to PCR analysis can detect DNA with high sensitivity, selectivity, and throughout a wide dynamic range [1-5].

Conflict of Interest

None.

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