

Claims

- [Claim 1] Improved immuno-PCR method by using cDNA display comprising the steps of:
- immobilizing, to a solid phase, a first antibody capable of binding to a target molecule~~having a binding site to a solid phase:~~
 - contacting a sample fluid to the first antibody so that the first antibody can bind to the~~to bind a target molecule in said sample fluid;~~
 - contacting said target molecule to a cDNA display molecule;~~being composed of 1) a backbone nucleic acid strand in which a DNA sequence to be amplified is included and 2) a covalently bound side chain strand having a polypeptide conjugation site—~~
 - wherein the cDNA display molecule comprises:
 - a nucleic acid being composed of 1) a backbone which is a double stand comprising mRNA and its complementary DNA (cDNA) and 2) a side chain stand having a polypeptide conjugation site, and
 - a polypeptide encoded by the cDNA,
 - wherein the side chain strand is a single-stranded DNA and is bound to the cDNA which forms a double-strand with the mRNA,
 - wherein the polypeptide is bound to the side chain strand at the polypeptide conjugation site, and has activity to bind to the target molecule~~to which a second antibody that binds to said target molecule has been conjugated;~~ and
 - conducting polymerase chain reaction to amplify and detect said DNA quantitatively.
- [Claim 2] The improved immuno-PCR method by using cDNA display according to the claim 1, wherein the first antibody is bound to the solid phase directly.
- [Claim 3] The improved immuno-PCR method by using cDNA display according to the claim 1, wherein the first antibody is bound to the solid phase via biotin-streptavidin interaction.
- [Claim 4] The improved immuno-PCR method by using cDNA display according to any one of the claims 1 to 3, wherein the first antibody is any one selected from the group consisting of IgG, a fragment thereof, a single domain antibody, and an aptamer that binds to an epitope of the target molecule.
- ~~[Claim 5] The improved immuno-PCR method by using cDNA according to any one of the claims 4, wherein the second antibody is a protein being composed of a single polypeptide having~~

~~binding ability to the target molecule.~~

[~~Claim 6~~-5] The improved immuno-PCR method by using cDNA according to any one of the ~~claims~~ 5 claims 1 to 4, wherein the ~~second antibody~~peptide is any one selected from the group consisting of a single chain variable fragment of IgG, a single domain antibody, and a peptide aptamer that binds to a different epitope of the target molecule from the first antibody.

~~[Claim 7]~~ ~~The improved immuno-PCR method by using cDNA according to any one of the claims 1, wherein the said backbone strand comprises a DNA sequence of the second antibody which binds to the target molecule.~~

[~~Claim 8~~-6] The improved immuno-PCR method by using cDNA display according to any one of the ~~claims 1~~ claims 1 to 5, wherein ~~said the~~ polypeptide conjugation site is composed of any one of ~~compound~~ selected from the group consisting of puromycin and derivative thereof.

~~[Claim 9]~~ ~~The improved immune-PCR method by using cDNA according to any one of the claims 1, wherein said PCR is quantitative PCR.~~