Construction of highly efficient RNA recovery technology using photo-cross-linkable artificial nucleic acid probe-modified magnetic particles

([1] University of Fukui, [2] NICCA CHEMICAL CO, LTD.) ○ Shuto Yajima,[1] Ayako Koto,[2] Hiroaki Sakamoto,[1] Eiichiro Takamura,[1] Shin-ichiro Suye[1]

Keywords: Photo-cross-linkable artificial nucleic acid; Magnetic particles; Hybridization; UV irradiation; PCR

Novel coronavirus disease (COVID-19) is a newly discovered human infectious disease that causes severe respiratory distress. Since there is no specific drug for COVID-19, early diagnosis, control of infected patients, and establishment of highly accurate virus testing techniques are necessary to stop the pandemic. Currently, COVID-19 testing is performed by PCR due to its specificity and sensitivity. However, false negatives occur due to the yield of target RNA in the extraction process, inhibition of reverse transcription reaction by impurities, and degradation by RNase during sample processing.

Spin column is popular extraction method. However, the target RNA purity is low in the purified product because the product contains all nucleic acids derived from the specimen.

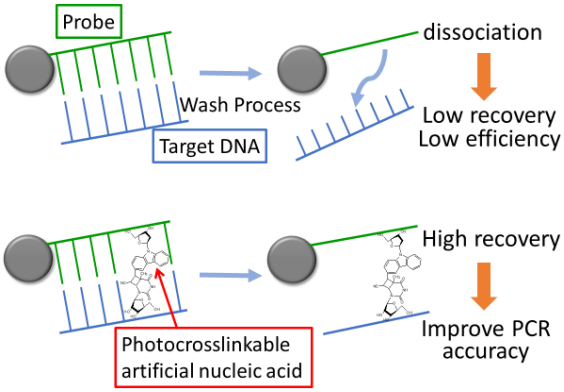

Fig.1 Recovery of target nucleic acids using photo-cross-linkable probes

In this study, we designed the magnetic particle that can reliably capture target RNA. The magnetic particles were modified with probes containing photo-cross-linkable artificial nucleic acid (CNV-K). The hybridized probe and target RNA are covalently bound by UV irradiation. Capture by covalent bonds offers improved RNA recovery efficiency and reliable removal of impurities.

The synthetic DNA with specific sequence (120 bp) of the SARS-Cov2 was used as simulated target. One pmol/μL of target DNA was added to the probe (5'-GTT CTG GAC CAC GTC TGC CGA XAG-3', X: CNV-K) modified magnetic particle solution and incubated at 55°C for 90 minutes to hybridize the target DNA and probe. After hybridization, UV 366 nm was irradiated for 5 min to the magnetic particle solution. The probe was covalently bound to the target DNA by UV irradiation. The magnetic particles were collected by magnetic separation, washed. The target DNA bound to the recovered magnetic particles was amplified by PCR and confirmed by agarose gel electrophoresis.

As a result of electrophoresis, the band of target DNA was identified around 100 bp. In contrast, no bands were observed when particles without UV irradiation were used. The uncross-linked target DNA was removed by washing.

The photo-cross-linkable probe modified magnetic particles developed in this study were able to specifically and strongly recover the target DNA. In the future, we plan to try to apply this method to viral RNA recovery.