

## マイクロビーズへの濃縮効果を利用した HCR による核酸検出の高感度化

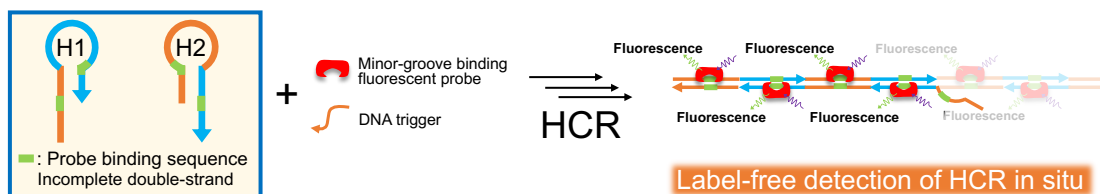
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Increased sensitivity of HCR-based nucleic acid detection using the concentration effect of microbeads (<sup>1</sup>*Faculty of Systems Engineering, Wakayama University*, <sup>2</sup>*Graduate School of Systems Engineering, Wakayama University*) Yu Yasuhara,<sup>1</sup> ○Takashi Sakamoto,<sup>1,2</sup>

In hybridization chain reaction (HCR)<sup>1</sup>, the signal can be amplified non-enzymatically under isothermal conditions to detect the target nucleic acid, so that simple and sensitive detection of nucleic acids is possible. In addition, by applying the label-free HCR (LF-HCR, Figure 1)<sup>2</sup> that developed recently and that does not require fluorescent labeling of oligo DNA, we can perform inexpensive on-site clinical testing (POCT; point of care testing). However, the detection sensitivity of LF-HCR is far from that of the PCR-based method, and a further improvement in detection sensitivity has been required. Therefore, in this study, we tried to improve the detection sensitivity by adsorbing HCR products on microbeads and increasing the local concentration. As the results, we report that we succeeded in significantly improving the detection sensitivity (about a 10<sup>6</sup>-fold increase in detection limit) by adding microbeads having an amino group to the reaction solution of LF-HCR and quantifying the fluorescence of the microbeads under a fluorescence microscope.

**Keywords:** *Nucleic acids detection; Hybridization chain reaction; Microbeads*

ハイブリダイゼーション連鎖反応 (HCR)<sup>1</sup>では等温条件下、非酵素的に信号を増幅し標的核酸を検出できることから、核酸の簡便な高感度検出が可能である。また、我々が独自に開発したオリゴ DNA の蛍光ラベルを必要としないラベルフリーHCR (LF-HCR, Figure 1)<sup>2</sup>を適用することで、核酸配列に基づく安価な臨床現場即時検査 (POCT; point of care testing) を実現できると期待できる。しかし、LF-HCR の検出感度は PCR 法には到底及ばず、さらなる検出感度の改善が求められていた。そこで本研究では HCR 産物をマイクロビーズに吸着し、局所濃度を増大させることによる検出感度の改善を試みた。結果、LF-HCR の反応溶液に、アミノ基を持ったマイクロビーズを添加し、顕微鏡下でマイクロビーズの蛍光を定量することで、顕著な検出感度の改善 (10<sup>6</sup> 倍程度の検出限界の向上) に成功したので報告する。



**Figure 1.** Schematic drawing of label-free HCR<sup>2</sup>

- 1) R. M. Dirks, N. A. Pierce, *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, 101, 15275–15278.
- 2) T. Sakamoto, R. Yamada, *ChemBioChem*, **2019**, 20, 1242–1245.