Review of: "An improved nucleic acid sequence-based amplification method mediated by T4 gene 32 protein"

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The merit of isothermal amplifications over PCR is that a complex device such as thermal cycler is not required. Various nucleic acid amplification technologies have been devised including NASBA. However, the most widely used nucleic acid amplification technique is still PCR. This may be attributed to that primer annealing is much easier in PCR than in isothermal amplification methods that require T7 promoter sequence as long as 25 nucleotides in one primer. In this paper, T4 phage-derived single-stranded binding protein, gp32, increases the reaction efficiency of NASBA and eliminates the need of incubation of RNA at 65ºC before addition of enzyme cocktail. The optimum concentration of gp32 was 100 ng/mL. The authors speculate that gp32 improves primer annealing by stabilizing the single-stranded cDNA. This is an important discovery because it enables one-step NASBA.

As the authors stated, gp32 is used in recombinase polymerase amplification (RPA) that amplifies the target DNA sequence at constant temperature around 41ºC, along with T4 recombinase uvsX and T4 recombinase mediator protein uvsY. We reported that in the RPA reaction with 400 ng/mL uvsX and 40 ng/mL uvsY, the optimum gp32 concentration was 400 ng/mL (1). In RPA, the balance of the binding and dissociation between recombinase and DNA primer is important. uvsX binds to DNA primer to form the nucleoprotein with the aid of uvsY. Upon hydrolysis of ATP, uvsX dissociates from DNA primer and is replaced by gp32. If the binding of gp32 to primer is too high, the nucleoprotein cannot be formed. If the binding of gp32 to primer is not high enough, uvsX remains occupied even after the elongation starts, preventing another nucleoprotein from binding to the target sequence and starting the elongation. I think that the importance of this balance is applicable to NASBA. Last year, we developed a novel isothermal and one-step amplification technique, RICCA, by combining NASBA and RPA (2). It was thought that high~~er~~ sensitivity of RICCA over NASBA results from a co-assisted and coupled approach of RNA amplification cycle in NASBA and DNA amplification cycle in RPA. But, as this paper suggests, stabilization of cDNA by gp32 might also be important. I hope that addition of gp32 improves the sensitivity, specificity, rapidness, and accuracy of nucleic acid amplification generally, and that the one-step isothermal amplification meets the demand of point-of-care diagnosis.

1. Juma, K.M. et al. *Biochem. Biophys. Res. Commun.* **567**, 195-200 (2021)
2. Biyani, R. et al. *Sci. Rep.* **11**(1), 15997 (2021)