

Enhancement of the PCR with the Trace Element

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Abstract

For the forensic DNA testing, we must often perform DNA analysis from a very small amount of samples with poor quality. As a result, it becomes often non-testable without succeeding in PCR. To overcome this problem, we looking for a trace element with catalytic effect for PCR.

13 kinds of the following element were examined, Si, Pb, V, Zn, Cu, Cd, Ca, Bi, Tl, Sn, Hg, Se, As. The concentration of trace element to final PCR solution was in the range of 1 ppb~10 ppm, respectively, and compared it with the additive-free control PCR. We showed an acceleration effect of the PCR to vanadium and copper. The addition of vanadium and copper showed the highest acceleration effect by the concentration of 10 ppm from 1 ppm in final mixture. The combination of vanadium 10 ppm and copper 1 ppm was highest rate of detection using PPY23 for personal identification, and showed the amplification was accelerate at least 4 cycles (equivalent to 32 times increase in quantity) rather than control PCR.

Keywords: Forensic DNA testing; DNA analysis; Metallic elements

Introduction

We already reported that VCL4 has a PCR acceleration effect, and successfully applied to challenging cases [1-3]. So, if one drop of vanadium chloride is added to the standard reaction mixture, the enzymatic amplification of DNA can be enhanced in various kinds of PCR.

To improving PCR sensitivity, there is a method to reinforce the improvement of the PCR condition and mini-STR, a testing system specially designed primers for relatively small amplicon, thereby increasing the chances of successful amplification from the degraded DNA [4]. On the other hand, this method is high in amplification sensitivity, there is a fault that irregular band is easy to increase [5].

However, specificity fell to when we raised sensitivity, we did not avoid the nature that background noise was increased by these methods.

Therefore, we tried to review a PCR acceleration effect of the trace element widely without raising background.

Materials and Method

We examined an acceleration effect of a lot of microelement by the PCR of the Y-STR region using control DNA that concentrations were exactly measured by 5 pg template DNA (extremely trace amounts), 96°C for 2 min then, 94°C for 10 s, 61°C for 1 min 72°C for 30 s for 30 cycles, then, 60°C for 20 min, 4°C soak. The estimated total cycle time is 1 h and 40 min.

We widely examined 13 kinds of the following element, Si, Pb, V, Zn, Cu, Cd, Ca, Bi, Tl, Sn, Hg, Se, As. The concentration of microelement to final PCR solution was in the range of 1 ppb~10 ppm, respectively, and compared it with the additive free control PCR.

PCR products were confirmed by agarose gel electrophoresis.

The confirmation of the acceleration effect was performed by 7500 Real time PCR System (Applied Biosystems) using PCR of DYS390 (Y-STR) locus [6].

Results

We found no effects by 11 kinds of listed elements. However, Copper had an augmentation effect in addition to vanadium. We showed an acceleration effect of the PCR to vanadium and copper. The addition

of vanadium and copper showed the highest acceleration effect by the concentration of 10 ppm from 1 ppm in final mixture.

What we want to emphasize in particular, combination of vanadium and copper showed the acceleration effect that was at least relatively 32 times of the control PCR. We could confirm amplified products by enhanced multiplex PCR (PPY23) in the electrophoresis of the agarose gel, whereas we could not see the bands by the normal PCR (Figures 1 and 2).

Discussion

Vanadium and copper are the metallic elements which are located in a periodic table for the third period. In addition, elements associated with the life activity are arranged in the third period.

It has been already reported that copper provides much physiology [7].

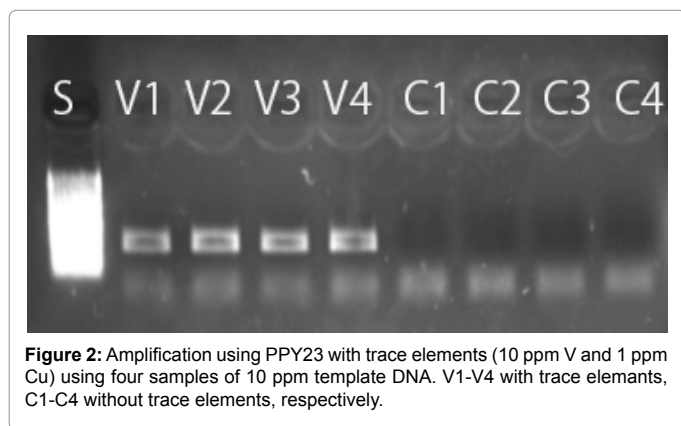
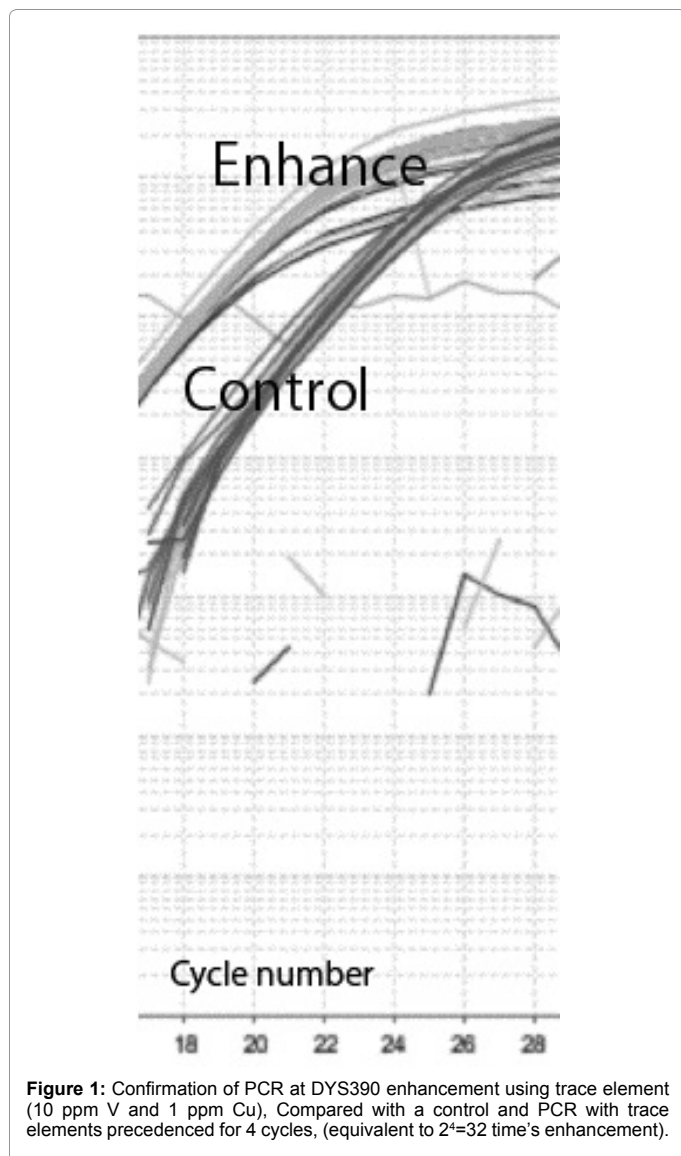
In particular, many biochemical effects of vanadium have been investigated in the past research [8]. It was demonstrated that vanadium ions stimulate DNA synthesis, because vanadium ions produced additive increases in [3H] thymidine incorporation in mouse cells. The mitogenic effect of the vanadium compounds was similar to that of colchicine [9]. JB Smith also discovered that vanadyl sulfate and sodium orthovanadate in the concentration range between 5 and 50 μM is shown to be mitogenic for quiescent cultures of 3T3 and 3T6 mouse cell line. Another experiment suggests that vanadium can interact with enzymes involved in nucleic acid metabolism [10]. It was already studied that promoting effects of BSA (Bovine serum albumin) were seen in the first cycles of the PCR, regardless of the size of the DNA to amplify [11].

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Conclusion

According to our experiment, we suggest that the effect of the vanadium raises annealing efficiency in early stage of PCR.

We concluded that one drop of vanadium and cooper addition would be another choice for a challenging DNA testing.

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