Optimizing copper-catalyzed azide-alkyne cycloaddition for DNA chemical ligation

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DNA amplification has been widely used in many areas, including point-of-care diagnostic tests for detection of diseases. The traditional use of PCR for this purpose is slowly being replaced by other methods due to its expensive need for thermal cycling. Lesion-induced isothermal DNA amplification (LIDA) is efficient, cost-effective and easy to perform at one optimized temperature. However, the presence of background reaction poses limitations on the application of this class of amplification reactions. The amplification of desired target and the absence of background are essential for a sensitive and accurate diagnostic test. Moreover, the use of enzymes is impractical in third world countries because they are costly and require very low temperatures for the storage. Thus, we have employed new method in which this can be carried out, by using copper-catalyzed azide-alkyne cycloaddition (CuAAC) that has been shown to be region-specific, highly efficient, and tolerable in mild conditions. In order to optimize this class of chemical reaction into DNA ligation, different parameters were varied, including temperature, concentration of the target DNA sequence and the probes, and concentration of the reducing agent sodium ascorbate. The effects of inert atmosphere were also examined by flushing argon gas through the reaction mixture. Aminoguanidine, an agent known to prevent crosslinking of the proteins by oxidation by byproducts in CuAAC reaction, was also added to study whether it is helpful for reducing noise in the DNA chemical ligation. Finally, fluorescein was found to be affected by the byproducts formed during the reaction.