Room Temperature Stable PCR Reagents Preparation Using Capillary-Mediated Vitrification Process

Sankar Renu, Yolanda Peris-Taverner, Jenny Sharpe, Shruti Amle, Laura Bronsart, Pravansu Mohanty, Mary Shank-Retzlaff
Upkara, Inc, 1600 Huron Parkway Bldg 520, Rm 2390, Ann Arbor, MI 48109, USA

ABSTRACT

Polymerase chain reaction (PCR) is a sensitive, and most used molecular technique for disease diagnosis. Reagents used for PCR are available as a one-step and ready-to-use PCR master mix, which contains Hot Start DNA polymerase, dNTPs, $MgCl_2$, and PCR reaction buffer, and quantitative PCR (qPCR) along with has SYBR Green I, enhancers, stabilizers, and a blend of passive reference dyes. To preserve bioactivity, PCR reagents must be stored and distributed as frozen. Thus, desiccation of PCR reagents can avoid cold storage and improper handling of wet reagents and is associated errors. We discovered capillary-mediated vitrification (CMV) processes that can stabilize different biomolecules and allow them to be stored at ambient temperature. The CMV process can be performed at the bench in under an hour, does not require reagent specific optimization, and eliminates the need for freezing and cold storage. In this report, we demonstrate that the CMV process can be used to preserve both conventional and qPCR reagents.

CMV process was performed by mixing PCR master mix and 16S rRNA-F&R primers with Upkara's BioFix™ Buffer and adding the mixture to a porous scaffold. The loaded scaffolds were placed into our vitrification chamber and dried for 30 min. After drying, the CMV-stabilized sample was tested or packed in mylar pouches and stored at 37°C for up to 84 days. On the day of testing, the stabilized samples were eluted with nuclease-free water and E. coli genomic DNA was added, and the 50 or 20 uL reaction was tested by conventional PCR. The PCR product was visualized under agarose gel electrophoresis.

The CMV processed PCR master mix and primer samples stored at 37°C for 7, 28, 56, and 84 days outperformed liquid samples (negative control), which were stored at 37°C. Notably compared to frozen reagent (positive control), CMV-master mix samples stored up to 84 days at 37°C had similar DNA amplification profile. The negative control sample had reduced amplification at 7 days and no detectable signal from 28 days storage at 37°C (Fig.1). The post CMV-processed qPCR master mix reagent observed comparable amplification signal of frozen stored reagent under agarose gel electrophoresis (Fig.2).

Study results indicate that the CMV process stabilized and improved thermal stability of PCR master mix reagents, which will reduce PCR processing time and improve the testing throughput. The study suggests that the CMV technology has the potential to enable ambient storage and shipping of PCR reagents for molecular diagnostic and other related applications.

BACKGROUND

- ✓ Capillary-mediated vitrification (CMV) is a novel method that leverages the naturally-occurring process of capillary evaporation to rapidly remove moisture from an aqueous matrix without freezing or boiling, transitioning biological reagents into a stable, glassy state.
- ✓ The pores within the scaffold act as capillaries, increasing the surface area and surface tension. The increase in surface tension prevents boiling, allowing the material to be dried under vacuum without a freezing step.
- ✓ The quantity of material processed can be selected to match the assay requirements and the final, dried product typically exhibits enhanced thermal stability and performance, allowing the material to be stored under ambient conditions (1-4).

Current storage and distribution issues reduced by the CMV process

Waste
- ✗ Concentrated formats
- ✗ Limited shelf life

Efficiency
- ✗ Time intensive
- ✗ Risk of error
- ✗ Reagent defrosting
- ✗ Documentation

Storage
- ✗ Expensive
- ✗ Unsustainable
- ✗ Deviation risk
- ✗ Material loss risk

RESULTS

Thermal Stability of CMV-Stabilized PCR Master Mix

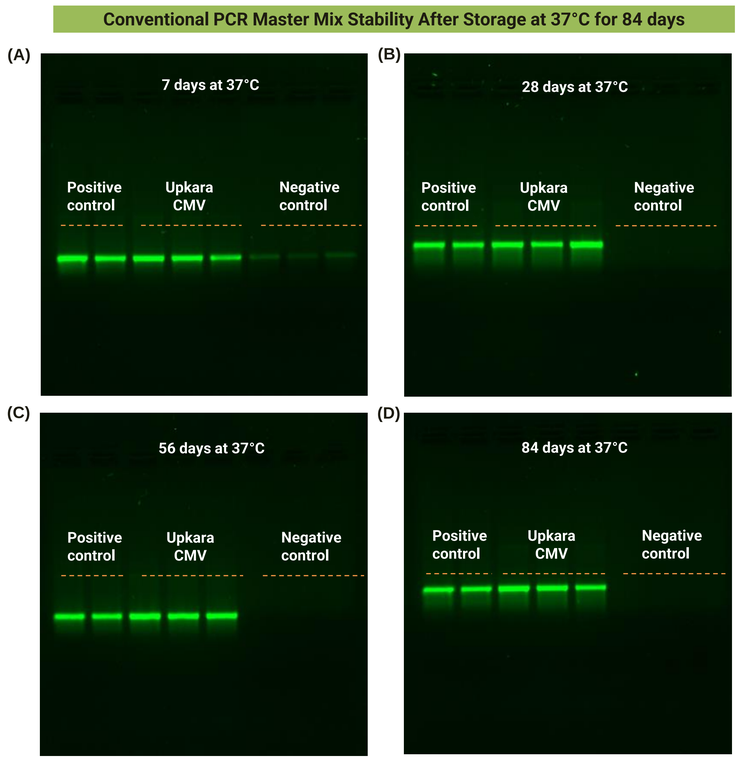

Figure 1. CMV Preserved Conventional PCR Master Mix Stability. PCR master mix reagents and primers vitrified and stored at 37°C for different timepoints. On the day of testing samples were eluted and mixed with DNA and run on PCR. The PCR product amplification profile under agarose gel electrophoresis of CMV samples stored at 37°C for (A) 7 days; (B) 28 days; (C) 56 days; and (D) 84 days.

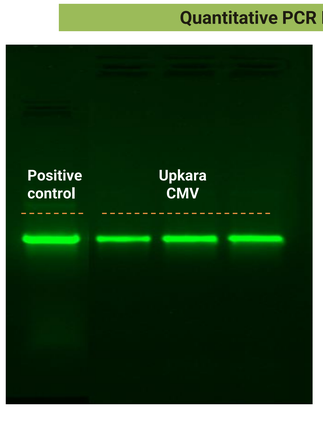

Figure 2. CMV Preserved Quantitative PCR Master Mix Stability. The qPCR master mix reagents and primers vitrified, and the eluted reagent were mixed with DNA and run on PCR. The PCR product amplification profile of frozen and post-CMV processed samples were examined under agarose gel electrophoresis.

Preparation and use of CMV samples

CONCLUSIONS

- ✓ Capillary-mediated vitrification (CMV) is an innovative and simple process for preserving different types of biomolecules.
- ✓ Using the CMV process, we have efficiently preserved and improved the stability of PCR master mix.
- ✓ The CMV process is expected to be easily scalable, fit into current workflows, adaptable to a wide range of biomolecules and may enable storage, distribution, and deployment of reagents, clinical samples, and therapeutics which currently require cold chain logistics.

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*Figures created with BioRender.com

We are actively recruiting collaborators with an interest in exploring our technology.

If interested, please contact: Mary Shank-Retzlaff: mretzlaff@upkara.com or Laura Bronsart: lbronsart@upkara.com