Still Glowing After All These Years:
Storage Life of a Complete Bovine Sexing PCR Mix

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Abstract

We developed a complete PCR reaction mix (YCD) for use in field sexing. Our study compared 14-year-old bovine YCD that had been stored in a standard freezer to a freshly prepared PCR reaction mix. The 14-year-old stored YCD and freshly prepared reaction mix both produced strong signals. We further investigated effects of stability of two reagents: 8-year-old *AmpliTaq* and 8-year-old deoxyribonucleotides. Nucleotides and Taq p have a labeled shelf life of two years. Sexing bands were observed in gels from the reaction mix with 8-year-old deoxyribonucleotides, but no signals were observed with the use of 8-year-old *AmpliTaq*.

Introduction

Generally, PCR reaction mixes are made fresh on the day of use. This is impractical for use in field sexing. Mistakes often occur due to the required measuring precision and to contamination from environmental DNA. Therefore, we developed a complete reaction mix (YCD). Storage and transport of YCD required liquid N_2. No study to date has determined storage life of PCR reaction mixes in standard freezers. Our study compared 14-year-old bovine YCD (AB Technology, Pullman, WA, USA) that had been stored in a standard freezer, to freshly prepared bovine YCD. We further investigated effects of stability of two reagents: 8-year-old *AmpliTaq* and 8-year-old deoxyribonucleotides (Boehringer Mannheim, Basel, Switzerland). Nucleotides and *Taq* p have a labeled shelf life of two years and are costly to replace. By determining if their shelf life is actually longer than stated by the manufacturer, lab resources can be saved.

Materials & Methods

The working concentration of bovine YCD was: buffer (50 mM Tris, 1% dextran T-500, 50 mM KCl, 2.5 mM $MgCl_2$, and 0.035% 2-mercaptoethanol), deoxyribonucleotides (5 µM) (Boehringer Mannheim, Basel, Switzerland), two sets of primers (sexing primers, 5′-GAACTTTCAAGCAGCTGAGGC-3′ and 5′-GATTGTTGATCCCACAGAAGG-3′ (2.5 µM), and control primers, 5′-TTGAGGCATGGAACTCCGCT-3′ and 5′-GGTGGTTCCACATTCCGTAGG-3′ (0.25 µM) (custom synthesis, IDT Inc, Coralville IA, USA)), and *Taq* polymerase (*Taq* p) (2 IU) (*AmpliTaq* DNA Polymerase, Stoeffel Fragment, Perkin Elmer, Branchburg, NJ, USA). The concentrations in the complete reaction mix were twice the working concentration. The freshly prepared mix was the same as YCD except: deoxyribonucleotides (C01581, GenScript Corp., Piscataway, NJ, USA) and *Taq* p (M0273L, New England BioLabs, Ipswich, MA, USA). Male and female bovine lymphocytes (100 cells/2 µL) were used as the DNA source. The DNA replication occurred in a Corbett Rapid Thermocycler (Model FTS-IS, Corbett Research, Montlake, Australia) in 20-µL volumes. All assays were run with positive and negative DNA controls. The PCR products were separated using polyacrylamide gel electrophoresis (PAGE). A 6% gel with Tris as the buffer was formed in an agarose gel chamber (M12 Electrophoresis Unit, Edvotek, Bethesda, MD, USA) under argon gas. The gel was run at 200 volts (PS500ST, Hoefer Scientific Instruments, San Francisco, CA, USA) for 30 min, and then stained with 5 µL ethidium bromide in 100 mL of Tris buffer for 30 min. The gel was destained for 30 min in H_2O. The gel was viewed using a transilluminator (3-300, Fotodyne, Hartland, WI, USA) and photographed.

Results

The 14-year-old stored YCD and freshly prepared reaction mix both produced strong signals. Sexing bands were observed in gels from the reaction mix with 8-year-old deoxyribonucleotides, but no signals were observed with the use of 8-year-old *AmpliTaq*.

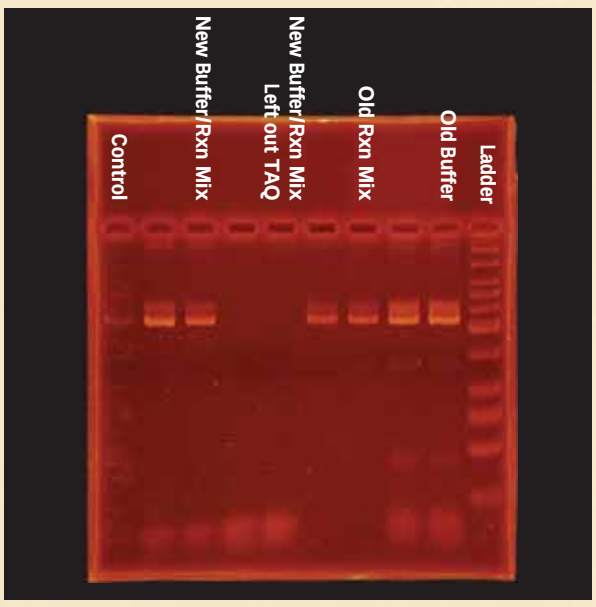

Figure 1: Reaction mix from 14 years ago still produces results.

Discussion

These results suggest that *Taq* p is the most likely candidate to cause failure in stored PCR reaction mixes. As supplied, *Taq* p is liquid even when stored in the freezer. Our hypothesis is that because the *Taq* p was frozen solid in YCD instead of being kept in a liquid form, the denaturing of *Taq* p was prevented. We conclude that storage and transport of PCR reaction mix could become more convenient: ship on dry ice and transport to the field in a mobile freezer.

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