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Bovine Embryo Sexing by Loop-Mediated Isothermal Amplification (LAMP

S. Kojiya and K. Watanabe

Eiken Chemical Co., Ltd., Tokyo, Japan

In the bovine embryo transfer industry, sexing preimplantation embryos is an important management tool. Several methods for bovine embryo sexing utilizing polymerase chain reaction (PCR) have been developed. However, they were not popularized because the methods requiretechnical skills and expensive instruments, and are time consuming. PCR also has the risk of false positives due to DNA contamination during the electrophoresis. Therefore, simple, rapid and precise method for sexing is eagerly anticipated.

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method that amplifies DNA (or RNA) with high specificity, efficiency and rapidity under isothermal conditions using a set of four specially designed primers and a DNA polymerase with strand displacement activity. One of the characteristics of the LAMP is its ability to synthesize extremely large amount of DNA. Accordingly, a large amount of by-product, pyrophosphate ion, is produced, yielding white precipitate of magnesium pyrophosphate in the reaction mixture. Due to its high specificity, by using four primers recognizing six distinctregions on the target nucleic acid, judgment of the presence or the absence of this white precipitate allows easy distinction of whether nucleic acid was amplified by LAMP.

We have developed Loopamp Bovine Embryo Sexing Kit for the LAMP-based sexing of bovine embryo using a portion of fertilized egg. Extraction of DNA from biopsied trophectodem cells can be easily conducted by simply mixing it with extraction solution and incubate it at room temperature for 5 minutes. The kit employs two sets of primers for male specific and male-female common reactions, which increases reliability of the test. The LAMP reaction is conducted at a constant temperature of 63C for 35 minutes by simply mixing master mix containing primers, dNTPs and *Bst* DNA polymerase and sample solution. This LAMP reaction and heat inactivation at 80℃ for 2 minutes after LAMP reaction as well as turbidity reading of reaction mixture can be conducted by Loopamp End Point Turbidimeter.

Recent study by Hokkaido Animal Research Center, etc.(Theriogenology in press) demonstrated high sensitivity and accuracy of LAMP-based bovine embryo sexing. When more than 3-cell-samples were used, the sex of embryos was correctly determined. Sixty-one fresh sexed embryos (23 male and 38 female) were transferred to recipients. Thirty-five (57.4%) of the recipients were diagnosed as pregnant, but two animals aborted. The remaining 33 recipients gave birth to 12 male and 21 female calves, all with the predicted sex. This indicates that sexing by LAMP is a reliable method suitable for use in the field.

One of the advantages of LAMP is that specific nucleic acid amplification can be conducted under isothermal conditions, i.e. no thermal cycler is needed. The end-point turbidity measurement is a convenienttechnique for the detection of nucleic acid amplification. Furthermore, this helps to prevent contamination since amplified products are not removed from the reaction tube, i.e. no electrophoresis is needed. While PCR requires expensive thermal cycler and ultraviolet transilluminator, LAMP needs only inexpensive simple incubator or incubator with absorbance reader.

In conclusion, LAMP-based bovine embryo sexing is a rapid method with high sensitivity and accuracy. Sexing including DNA extraction, amplification and detection can be completed within about an hour. This method makes it possible to easily determine the sex without thermal cycler and electrophoresis. We believe that LAMP-based bovine embryo sexing is suitable for field applications and will contribute to the improvement of sexing technology and breeding and management of herds.