

Ampli-Y™

Rapid non-electrophoretic

sex determination

of bovine embryos

Working
on
Science



FINNZYMES

PRODUCTS FOR PCR AND MOLECULAR BIOLOGY

AMPLI - Y™

Bovine Embryo Sexing Kit

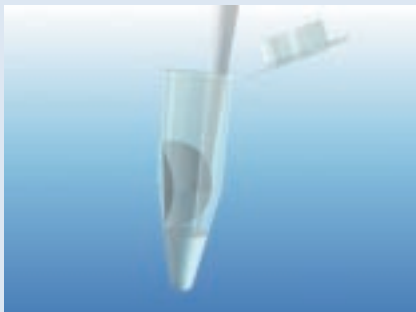
Non-electrophoretic sex determination of bovine pre-implantation embryos

DESCRIPTION

The Ampli-Y™ bovine embryo sexing kit is a non-electrophoretic method based on amplification of male (Y-chromosomal) DNA. The protocol is suitable for biopsies (1 to 300 cells) of *Bos taurus* or *Bos indicus* pre-implantation embryos. The kit consists of a lysis solution (A-solution), PCR solution (B-solution) and reaction tubes including control tubes. After PCR the samples are diagnosed using UV illumination of the PCR tubes. Pink fluorescence indicates the presence of a male sample.

1. Transfer biopsy into tube

Each biopsy is transferred in 2µl biopsy medium into a reaction tube prefilled with 10µl lysis mixture. Note the piece of wax on the left inside wall.



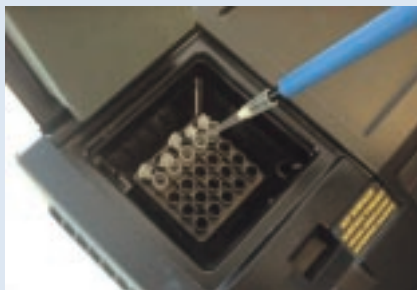
2. Lyse cells in PCR cycler

The tubes are placed in the PCR cycler (5 min at 55°C, 5 min at 95°C). DNA will be released and the wax will melt and form a layer preventing evaporation.



3. Add B-mix and run PCR

The PCR program is started and 15 µl of amplification mixture is added into each tube. The program takes about 70 minutes.



4. Inspect tubes to determine sex

The tubes are taken out of the PCR cycler and inspected in UV light (preferably 302-312 nm, but 254 nm can also be used). Tubes fluorescing pink contain Y-chromosomal DNA. Female samples do not show fluorescence. (from left to right: F, F, M, blank, M, M, M)



MORE INFORMATION AT

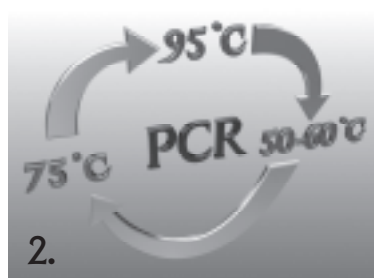
METHOD COMPARISON

Electrophoretic method



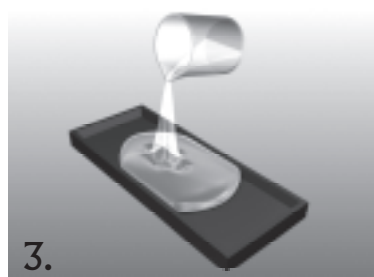
1.

*DNA release
from biopsy*



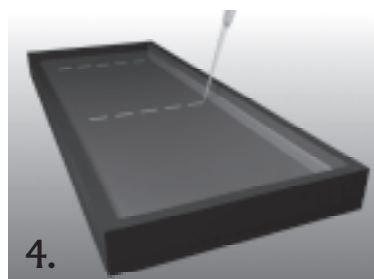
2.

PCR cycling



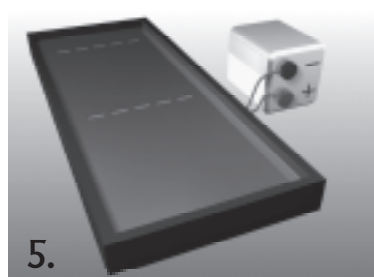
3.

Preparation of gel



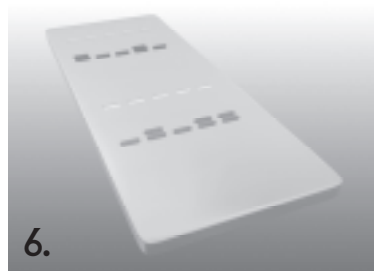
4.

*Gel loading of
PCR products*



5.

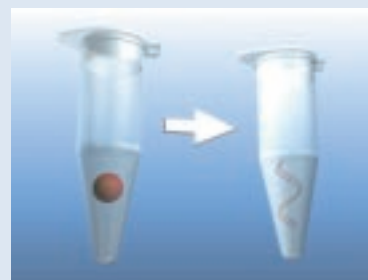
*Running the
electrophoresis*



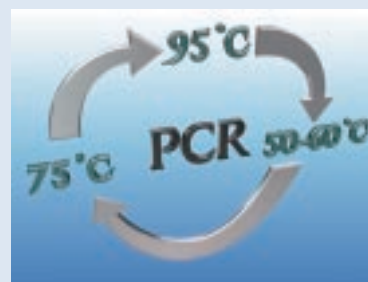
6.

UV detection

Ampli-Y™ method from Finnzymes



*DNA release
from biopsy*



PCR cycling



UV detection

Steps 3-6 are replaced with
ONLY one step

ADVANTAGES

The Ampli-Y method is a major simplification of bovine embryo sexing. After PCR the detection of Y-chromosomal DNA can be made directly from the tubes, making electrophoresis unnecessary. This reduces significantly the risk of PCR contamination.

- Simplest and fastest PCR-based method on the market.
- Convenient for sexing on the farm
- Low cost method
- No need for electrophoresis
- Minimal risk of contamination
- High accuracy (95%)

KIT COMPONENTS

Solution A (release of DNA)

Solution B (amplification of Y-DNA)

Reaction tubes, female and male control tubes

A detailed Ampli-Y™ Instruction Manual is included in the kit

STORAGE CONDITIONS

The A-solution can be stored for several weeks at +4°C. The B-solution is usable for about 1 week at +4°C. If you store longer, store at -20°C. Make aliquots as necessary.

LITERATURE

Bredbacka, P., Kankaanpää, A. and Peippo, J. (1995) PCR-sexing of bovine embryos: A simplified protocol. *Theriogenology* 44: 167-176.

Bredbacka, P. (1998) Recent developments in embryo sexing and its field application. *Reprod. Nutr. Dev.* 38: 605-613.

PCR LICENCE STATEMENT

The PCR process is covered by patents owned by Hoffmann-La Roche Inc. and F- Hoffmann-La Roche Ltd. This product is offered under a license for the manufacture and sale of veterinary PCR products from Roche.

The purchase of this product allows the purchaser to use it for amplification of nucleic acid sequences for veterinary *in vitro* diagnostics. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

ORDERING INFORMATION

F-800S Ampli-Y™ kit for 25 reactions

F-800L Ampli-Y™ kit for 100 reactions

USER-SUPPLIED MATERIALS AND EQUIPMENT

Micropipettes, microblade, micromanipulator (optional), thermal cycler, UV illuminator.

RELATED PRODUCTS

F-801 AmpliTester

Training kit for bovine embryo biopsy detection.

F-802 Ampli-BLAD

Simultaneous diagnosis of sex and bovine leucocyte adhesion deficiency (BLAD). Available during year 2000.

F-803 Ampli-Red

Simultaneous determination of sex and red/black coat color (MSHr locus). Available during year 2000.

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