Sexing bovine preimplantation embryos by PCR

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Introduction

This protocol for using PCR to sex bovine embryos are based on Y-specific and autosomal targets described by Park et al, Theriogenology 2001; 55: 1843-1853 but with updates according to current bovine genomic database in NCBI as of 2019. Note that assembly of the bovine Y chromosome is still in progress.

Primers

Y specific primers

The Y-specific region targeted for PCR is BOV97M located at CM001061.2 (GenBank ID):

gatcactatacatacaccactctcatcctacctaatagattccagctgataatttgcaactatactccaaagttttgtggaaatgtgaaagaaaatc attcagctcatctgtttcagagacagaatttgtgtagcatagccttttttatcacaagatc

Forward: 5'-gatcactatacatacaccact-3'

Reverse: 5'-aaggctatgctacacaaattctg-3'

size of amplicon = 143bp

Targeted autosome: Chromosome 19

Autosomal primers (5' → 3'), size of amplicon 217bp*:

Forward: 5'tggaagcaaagaaccccgct-3'

Reverse: 5'tcgtgagaaaccgcaccctg-3'

Size of amplicon = 217bp (other bands at 1623 and 3029bp).

Embryo Collection

1. Wash blastocysts with DEPC-treated Dulbecco's phosphate buffered saline containing 0.2% (w/v) polyvinylpyrrolidone (DPBS-PVP) three times.
2. Remove zona with Acid Tyrode's (Sigma T1788).
3. Repeat step 1.
4. Move a blastocyst with minimum amount solution (<0.5 µl) into a PCR tube with 1 µl of resuspension buffer from the CellsDirect kit 11753 (ThermoFisher Scientific).

5. Snap freeze and store at -80°C.

Note: If you work with hatched blastocysts (blastocysts older than 7 days-old), we strongly recommend exposing embryos to drops of 50 µL Dissociation Reagent (TrypLE ™ Select 10x, Gibco, REF: A12177-01) to ensure full cell digestion. Exposure to Acid Tyrode's is not required when using the Dissociation Reagent.

Embryo lysis and RNase treatment

1. Add 0.5 µl of lysis enhancer from the CellsDirect kit 11753 (ThermoFisher Scientific) into each tube containing an embryo under a stereoscope.
2. Digest at 70°C for 20 min in a thermocycler. At 10 min, tap the tubes to mix and then continue digestion.
3. Make sure embryos are fully dissolved under a stereoscope. If not, digest for 10 min more at 70°C.
4. Add 1 µl of 100 µg/mL RNase A (19101, Qiagen; diluted with nuclease free water). Incubate at 37°C for 0.5 h.

PCR

1. Prepare 10mM dNTP:
   Add 10 µl of dATP, dGTP, dCTP, dTTP (10297-018, Invitrogen) into 60 µl nuclease free water.
2. Prepare mastermix as follow:

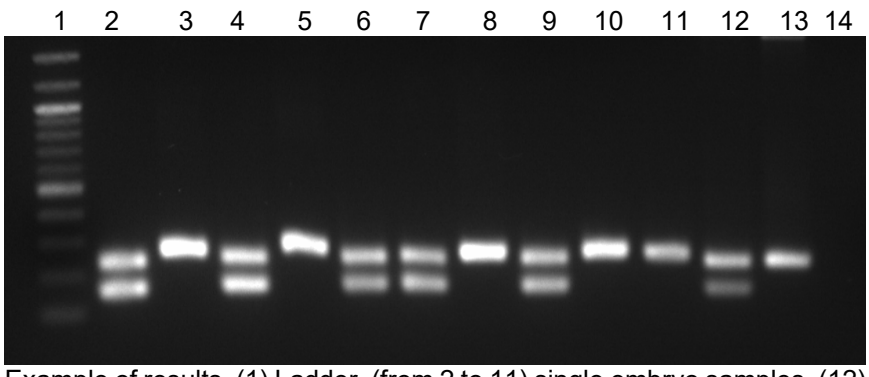
Items	Volume (µl)
5X Green GoTap Flexi Buffer (M891A, Promega)	4
25 mM $MgCl_2$ (A351H, Promega)	4
10 mM dNTP	1
10 µM sex primers (forward + reverse)	0.4
GoTaq hot start polymerase (M500B, Promega)	0.2
Nuclease-free water	6.6
TOTAL	**16.2**

3. Add 16.2 µl of mastermix into each tube containing a single embryo. The final volume will be ~ 19 µl.

4. As a positive control, add 16.2 µl of mastermix to a tube containing 2.8 µl of 10 ng/µl genomic DNA isolated from blood of male and female.
5. As a negative control, add 16.2 µl of mastermix to 2.8 µl of nuclease free water.
6. 1st round PCR for sex primers: 95ºC, 5 min; (95ºC, 15s; 58ºC, 15s; 72ºC, 15s) X 20; 72ºC, 10 min.
7. Add 1 µl of 4 µM of autosomal primer into each tube, and perform a 2nd round of PCR: 95ºC, 5 min; (95ºC, 15s; 58ºC, 15s; 72ºC, 15s) X 17; 72ºC, 10 min.

Gel electrophoresis

1. Prepare a 2% (w/v) agarose gel: add 2 g of low EEO agarose (BP160, ThermoFisher) into 100 mL of TAE buffer 1X (diluted from 10X, T9650, Sigma). Heat in a microwave until it is clear.
2. Pour the gel with a comb, allow to solidify, and then remove the comb.
3. Submerge the gel beneath the TAE buffer.
4. Place 2 µl drops of Diamond Nucleic Acid Dye (H1181, Promega) onto a paraffin strip and mix with 8 µl of PCR products or PCR controls. For ladder: Place 2 µl drops of Diamond Nucleic Acid Dye onto a paraffin strip and mix with 10 µl DNA ladder.
5. Load the samples into the wells of the gel.
6. Run at 100 V, 160 mA for 40-60 min.
7. Take images using a photoimager.



Example of results. (1) Ladder, (from 2 to 11) single embryo samples, (12) positive control for male (217+143 bp), (13) positive control for female (217 bp), (14) no template control.

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