Biopsy method

There are a few methods to obtain a biopsy sample from bovine fertilized embryo for sexing. Here we will demonstrate an example of biopsy method.

1. Biopsy method
The sample is taken from blastocyst embryo using a micromanipulator.

<Instruments and reagents>
-Petri dish (6 cm diameter)
-Micromanipulator (with inverted microscope)
-Micro-blade (K-715; FEATHER)
-Stereo microscope
-PBS；Phosphate Buffered Saline
-Fluid paraffin

<Procedure>
1) Select the blastocyst embryo showing a clear inner cell mass and trophoectoderm.
2) A few drops of PBS (50uL) are spotted along the circumference of petri dish and are overlaid by the fluid paraffin (Fig. 1.).
3) Transfer the embryo to PBS drop by glass capillary or pipette. Wash the embryo several times with PBS drops to remove such as culture medium. Biopsy operation becomes easier when the embryo is attached to the bottom of the dish.
4) Inverted microscope with micromanipulator is used to conduct biopsy by micro-blade (K-715, FEATHER). When blastocele can be observed, cut the portion of trophoectoderm so as not to hurt the inner cell mass (Fig. 2). It is recommended adjusting the cutting position to take about 10~20 cells for sexing.
5) Under stereo microscope, use glass capillary or pipette to collect the biopsy cells with operation solution (6uL) and put into the micro-tube as the sample for sexing. To eliminate the inhibiting materials to reactions and avoid contamination, it is recommended to wash the embryo after conducting biopsy.

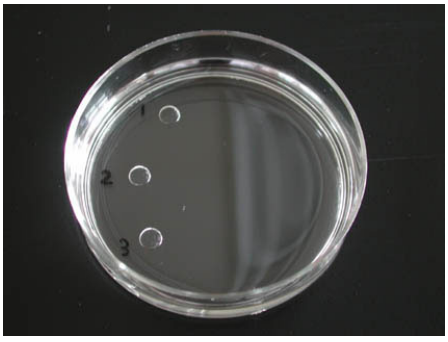

Fig. 1. Preparing the drops of PBS

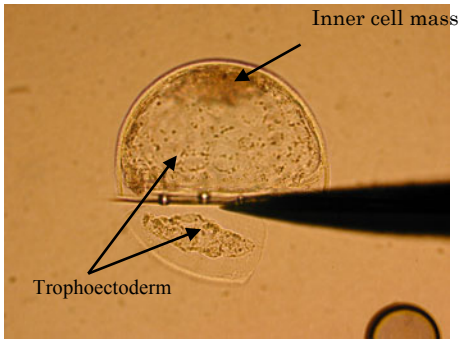

Fig. 2. Biopsy of blastocyst embryo