

Materials List for:

# Laser-assisted Cytoplasmic Microinjection in Livestock Zygotes

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URL: <https://www.jove.com/video/54465>

DOI: [doi:10.3791/54465](https://doi.org/10.3791/54465)

## Materials

| Name                     | Company              | Catalog Number | Comments  |
|--------------------------|----------------------|----------------|---|
| Micropipette puller      | Sutter Instrument    | P-97           |   |
| Glass capillary          | Sutter instruments   | B100-75-10     | These capillaries are used for making the holding and injecting pipettes. Any thick/standard wall borosilicate tubing without filament can be used.   |
| Microforge               | Narishige            | MF-9           | Equipped with 10X magnification lense.  |
| Micromanipulator         | Nikon/ Narishige     | NT88-V3        |   |
| Inverted microscope      | Nikon                | TE2000-U       | Equipped with 4X, 20X lenses and with a laser system.   |
| Laser                    | Research Instruments | 7-47-500       | Saturn 5 Active laser.  |
| Microdispenser           | Drummond             | 3-000-105      | The microdispenser is used to move the embryos. A p10 pipette can also be used but loading as minimal volume as possible.   |
| 100 x 15 mm culture dish | Falcon               | 351029         | Use the lid of the dish to make the injection plate since they have lower walls and will make positioning and moving of the micromanipulator easier. The lid of a 60 mm culture dish can also be used.  |
| 35 mm culture dish       | Corning              | 430165         | These dishes are used for culturing the embryos in 50 µl drops covered with mineral oil. Alternatively, a 4 well dish can also be used. Regardless of the dish chosen to culture the embryos, they always have to be equilibrated in the incubator for at least 4 hr prior to transferring the embryos to them. |
| Incubator                | Sanyo                | MCO-19AIC      | Any incubator that can be set to 38.5 °C, 5% CO <sub>2</sub> conditions can be used.  |
| Stereomicroscope         | Nikon                | SMZ800         | Used for visualizing the embryos in the culture drops and during washes. Any stereomicroscope with a 10X magnification can be used.   |
| Control Unit HT          | Minitube             | 12055/0400     | Heating system attached to the stereomicroscope.  |
| Heated Microscope Stage  | Minitube             | 12055/0003     | Heating system attached to the stereomicroscope.  |
| Dextran-Red              | Thermo Scientific    | D1828          | A sterile 10 mg/ml solution is used to inject.  |

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| Mineral Oil                          | sigma      | M8410    | Keep the mineral oil at room temperature and protected from light using foil paper.  |
| KSOMaa Evolve Bovine                 | Zenit      | ZEBV-100 | Supplemented with 4 mg/ml BSA. KSOM plates for embryo culture should be equilibrated in an incubator for at least 4 hr before use. |
| FBS                                  | Gemini-Bio | 100-525  | Use a stem-cell qualified FBS.   |
| Zygotes                              |            |          | Zygotes are injected 17 - 20 hpf and can be <i>in-vitro</i> - or <i>in-vivo</i> -derived.  |
| NaCl                                 | Sigma      | S5886    | Final concentration: 107.7 mM. Component of SOF-HEPES medium.  |
| KCl                                  | Sigma      | P5405    | Final concentration: 7.16 mM. Component of SOF-HEPES medium.   |
| KH <sub>2</sub> PO <sub>4</sub>      | Sigma      | P5655    | Final concentration: 1.19 mM. Component of SOF-HEPES medium.   |
| MgCl <sub>2</sub> 6H <sub>2</sub> O  | Sigma      | M2393    | Final concentration: 0.49 mM. Component of SOF-HEPES medium.   |
| Sodium DL-lactate                    | Sigma      | L4263    | Final concentration: 5.3 mM. Component of SOF-HEPES medium.  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | Sigma      | C7902    | Final concentration: 1.71 mM. Component of SOF-HEPES medium.   |
| D-(-)-Fructose                       | Sigma      | F3510    | Final concentration: 0.5 mM. Component of SOF-HEPES medium.  |
| HEPES                                | Sigma      | H4034    | Final concentration: 21 mM. Component of SOF-HEPES medium.   |
| MEM-NEAA                             | Sigma      | M7145    | Final concentration: 1x. Component of SOF-HEPES medium.  |
| BME-EAA                              | Sigma      | B6766    | Final concentration: 1x. Component of SOF-HEPES medium.  |
| NaHCO <sub>3</sub>                   | Sigma      | S5761    | Final concentration: 4 mM. Component of SOF-HEPES medium.  |
| Sodium pyruvate                      | Sigma      | P4562    | Final concentration: 0.33 mM. Component of SOF-HEPES medium.   |
| Glutamax                             | Gibco      | 35050    | Final concentration: 1 mM. Component of SOF-HEPES medium.  |
| BSA                                  | Sigma      | A-3311   | Final concentration: 1 mg/ml. Component of SOF-HEPES medium.   |
| Gentamicin                           | Sigma      | G-1397   | Final concentration: 5 µg/ml. Component of SOF-HEPES medium.   |
| Water for embryo transfer            | Sigma      | W1503    | Component of SOF-HEPES medium.   |

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| SOF-HEPES medium | Made in the lab |  | pH 7.3 - 7.4, 280 ± 10 mOs. Filter sterilized through a 22 µm filter can be stored in the fridge at 4 °C for 1 month. Warm in 37 °C water bath before use. |
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