Materials List for:

Laser-assisted Cytoplasmic Microinjection in Livestock Zygotes

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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 transfering the embryos to them.
Incubator	Sanyo	MCO-19AIC	Any incubator that can be set to 38.5 °C, 5% CO_2 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.

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 *in-vitro*- or *in-vivo*-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_2PO_4	Sigma	P5655	Final concentration: 1.19 mM. Component of SOF-HEPES medium.
$MgCl_2$ $6H_2O$	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.
$CaCl_2$-$2H_2O$	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.
$NaHCO_3$	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.

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.