

Materials List for:

Culture and Transfection of Zebrafish Primary Cells

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Materials

Name	Company	Catalog Number	Comments
<u>Fish lines</u>			
AB (wild-type)			established by Streisinger and colleagues, available from the Zebrafish International Resource Center (ZIRC)
Tg(<i>ptf1a</i> :eGFP)jh1			stable transgenic line in which the enhancer of the zebrafish gene <i>ptf1a</i> drives expression of the fluorescent protein EGFP (Parsons <i>et al.</i> , 2007)
Tg(XITubb:DsRed)zf148			stable transgenic line in which the <i>Xenopus</i> neural-specific beta tubulin promoter drives expression of the fluorescent protein DsRed (Peri and Nüsslein-Volhard, 2008)
Name	Company	Catalog Number	Comments
<u>Equipment</u>			
centrifuge	Eppendorf		model 5804 R
ChemiDoc MP imaging system	BioRad		model XRS+, used to acquire black-and-white images of Petri dishes containing 1 da embryos
confocal laser scanning microscope	Leica microsystems		model SP8, equipped with 28 °C temperature box and a 63X objective
epifluorescent microscope	Leica microsystems		model DM5500B, equipped with 28 °C temperature box and a 40X objective
Gene Pulser Xcell with capacitance extender	BioRad	1652661	electroporation device
Horizontal shaker	GFL		model 3011
incubator for cell culture (28 °C)	Memmert		model incubator I
incubator for embryos (28 °C)	Heraeus		type B6120
light microscope	Zeiss		model TELAVAL 31
micro pipettes	Gilson		
sterile work bench	Bio Base		with laminar flow and UV light
tweezers	Dumont		Style 5, Inox
vertical tube rotator	Labinco B.V.		model LD-79
Name	Company	Catalog Number	Comments
<u>Software</u>			
Image Lab Software	BioRad		for the ChemiDoc MP imaging system from BioRad

ImageJ	National Institutes of Health		used for counting 1 dpf embryos by applying the Count particles-tool to the respective black-and-white images; Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/ . (1997-2016).
LAS X	Leica Microsystems		for both confocal and epifluorescent microscopes from Leica Microsystems
Name	Company	Catalog Number	Comments
Plasmids			
pCS-DCX-tdTomato	Köster Lab	# 1599	based on the backbone pCS2+ (Rupp <i>et al.</i> , 1994)
pCS-eGFP	Köster Lab	# 7	based on the backbone pCS2+ (Rupp <i>et al.</i> , 1994)
pCS-H2B-mseCFP	Köster Lab	# 2379	based on the backbone pCS2+ (Rupp <i>et al.</i> , 1994)
pCS-mClover	Köster Lab	# 3865	based on the backbone pCS2+ (Rupp <i>et al.</i> , 1994)
pCS-MitoTag-YFP	Köster Lab	# 2199	based on the backbone pCS2+ (Rupp <i>et al.</i> , 1994)
pCS-ss-RFP-KDEL	Köster Lab	# 4330	based on the backbone pCS2+ (Rupp <i>et al.</i> , 1994)
pCS-VAMP1-mCitrine	Köster Lab	# 2291	based on the backbone pCS2+ (Rupp <i>et al.</i> , 1994)
pSK-UAS:mCherry	Köster Lab	# 1062	based on the pBluescript-backbone of Stratagene
Plasmid numbers refer to the database entries of the Köster lab. Plasmids are available upon request.			
Name	Company	Catalog Number	Comments
Plastic and glass ware			
BD Falcon Cell Strainer (40 µm)	FALCON	REF 352340	distributed by BD Bioscience, used as "landing net" to dip deyolked embryos into ethanol and to transfer them quickly to fresh cell culture medium
1.5 mL reaction tubes	Sarstedt	72690550	
24-well plate	Sarstedt	83.3922	
50 mL falconic tube	Sarstedt	62.547.004	
96-well plate	Sarstedt	83.3924.005	
EasyStrainer (40 µm)	Greiner Bio-One	542 040	with venting slots; used to filter cells after collagenase-mediated dissociation
electroporation cuvette (0.4 cm)	Kisker	4905022	
glass coverslips	Heinz Herenz Medizinalbedarf GmbH	1051201	
Microscope slides	Thermo Fisher Scientific (Menzel Gläser)	631-0845	
Neubauer chamber	Henneberg-Sander GmbH	9020-01	
Pasteur pipettes (plastic; 3 mL)	A. Hartenstein	PP05	
Petri dishes (plastic; diameter 10 cm)	Sarstedt	821473	for zebrafish embryos

pipette tips	Sarstedt	Blue (1000 µl): 70762; Yellow (200 µl): 70760002; White (10 µL): 701116	
sterile cell culture dishes (plastic; diameter 3 cm)	TPP Techno Plastic Products AG	93040	
sterile cell culture dishes (plastic; diameter 6 cm)	Sarstedt	72690550	
sterile Petri dishes (plastic; diameter 10 cm)	Sarstedt	83.3902	for brain dissection
Name	Company	Catalog Number	Comments
<u>Chemicals and Reagents</u>			
sodium chloride	Roth	0601.1	
4 % paraformaldehyde in 1x PBS	Sigma-Aldrich	16005	
4',6-diamidino-2-phenylindole (DAPI)	Thermo Fisher Scientific	D1306	
calcium nitrate tetrahydrate	Sigma-Aldrich	C1396	
ethanol p.a. 100%	Sigma-Aldrich	46139	
goat α-mouse IgG (Fc specific) FITC conjugated	Thermo Fisher Scientific	31547	
HEPES	Roth	9105.4	
high vacuum grease	DOW CORNING	3826-50	silicon grease used for self-made glass bottom dishes
magnesium sulfate heptahydrate	Merck	105886	
methylene blue	Serva	29198.01	
Monoclonal Anti-Tubulin, Acetylated antibody	Sigma-Aldrich	T6793	
Aqua-Poly/Mount (mounting medium)	Polyscience	18606	
poly-L-lysine	Biochrom	L 7240	
potassium chloride	Merck	104938	
Skim milk	Roth	68514-61-4	
Texas Red-X Phalloidin	Thermo Fisher Scientific	T7471	
Tricaine	Sigma-Aldrich	E10521	Synonym: Ethyl 3-aminobenzoate methanesulfonate
Triton X-100	BioRad	1610407	
Trypan Blue	Gibco by Life Technologies	15250061	
Name	Company	Catalog Number	Comments
<u>Enzymes</u>			
collagenase (Type 2)	Thermo Fisher Scientific	17101015	dissolve powder in cell culture medium (8 mg/mL) and sterile-filter the solution, store aliquots at -20 °C
pronase (from Streptomyces griseus)	Roche	11459643001	distributed by Sigma-Aldrich, dissolve in 30% Danieau (10 mg/mL) and store aliquots at -20 °C
Name	Company	Catalog Number	Comments
<u>Medium and solutions for cell culture</u>			
1x PBS (Dulbecco's Phosphate Buffered Saline)	Gibco by Life Technologies	14190-169	distributed by Thermo Fisher Scientific
CO ₂ -independent medium	Gibco by Life Technologies	18045054	distributed by Thermo Fisher Scientific

filtrated bovine serum (FBS)	PAN-Biotech		individual batch
glutamine 100x	Gibco by Life Technologies	25030081	distributed by Thermo Fisher Scientific
Leibovitz's L-15 medium	Gibco by Life Technologies	11415049	distributed by Thermo Fisher Scientific
PenStrep (10,000 U/mL)	Gibco by Life Technologies	15140148	distributed by Thermo Fisher Scientific