

Materials List for

Ortho- and Ectopic Zebrafish Xeno-Engraftment of Ocular Melanoma to Recapitulate Primary Tumor and Experimental Metastasis Development

Arwin Groenewoud¹, Jie Yin¹, B. Ewa Snaar-Jagalska¹¹Institute of Biology, Leiden University

Corresponding Author

B. Ewa Snaar-Jagalska

b.e.snaar-jagalska@biology.leidenuniv.nl

Citation

Groenewoud, A., Yin, J., Snaar-Jagalska, B.E. Ortho- and Ectopic Zebrafish Xeno-Engraftment of Ocular Melanoma to Recapitulate Primary Tumor and Experimental Metastasis Development. *J. Vis. Exp.* (175), e62356, doi:10.3791/62356 (2021).

Date Published

September 4, 2021

DOI

10.3791/62356

URL

jove.com/video/62356

Materials

Name	Company	Catalog Number	Comments
2.5mm box filament	Science products	FB255B	for pulling micro injection needles using a Sutter P97 or P1000
3mL transfer pipettes	Merck	Z350796	for transfer and selection of zebrafish embryos
Agarose	Milipore	2120	1.5% (w/v) in eggwater, 1.5 g in 100 mL DPBS, microwave to dissolve, for injecting and stereofluorescence imaging of zebrafish larvae
Capillaries: borosilicate glass outer	World precision instruments	BF100-78-10	Borosilicate glass capillaries used for needle preparation
DMSO	Sigma	D8418	Often used as solvent in drug treatments, should be stored at 2-8°C in the dark.
DPBS	Thermo Fischer Scientific	14190144	Dulbecco's phosphate buffered saline, without Mg ²⁺ and Ca ²⁺ for washing the cells, lack of Ca ²⁺ impairs cell-cell adhesion through cadherins and prevents cell aggregation during injection
Egg water	Instant ocean	SS15-10	0.6 mg/L final concentration sea salt in demineralized water
GFP encoding lentiviral transfer plasmid	Addgene	Plasmid #106172	Generated in Snaar lab, available at Addgene
Hek293T	ATCC	CRL-3216	Stable cell line for generating lentiviral particles, contains SV40-T antigen required for the generation of lentiviral particles
Leica sp8 confocal	Leica	Leica TCS SP8	automated stage confocal microscope with 405/488/514/635nm lasers
LipodD293	Signagen	SL100668	Highly efficient HEK293t optimized transfection reagent

Low-melting agarose	Milipore	2070	1% (w/v) in eggwater 1.5 g in 100 mL DPBS, microwave to dissolve, for embedding zebrafish larvae for confocal imaging
Micro loader tips	Fischer scientific	10289651	flexible microloader tips
Micro manipulator	World precision instruments	M3301R	x/y/z manual micro manipulator for microinjection
Needle puller: P-97 or P-1000	Sutter	P-97	needle puller used for generating standardized micro engraftment needles
Nr.5 watchmakers forceps	VWR	HAMMHSC818-11	fine watchmakers forceps used for breaking back needles
Picopump	World precision instruments	SYS-PV820	pulse controller supplying pressure for microinjection
pMD2.G	Addgene	plasmid #12259	Gifted by Didier Trono, 2nd generation lentiviral virulence plasmid
psPAX2	Addgene	plasmid #12260	Gifted by Didier Trono, 2nd generation lentiviral packaging plasmid
PVP40	Sigma-Aldrich	PVP40	Polyvinylpyrrolidone average mol wt 40,000) PVP40 2% (w/v) in DPBS, 1 g PVP40 in 50 mL DPBS. Vortex and incubate at 37°C to facilitate dissolving. Store at room temperature.
tdTomato encoding lentiviral transfer plasmid	Addgene	Plasmid #106173	Generated in Snaar lab, available at Addgene
transmitted light stereo microscope	Leica	leica M50 with (MDG33 base)	leica transmitted light microscope with mirror adjustable illumination.
Tricaine	Sigma-Aldrich	E10521	Ethyl 3-aminobenzoate methanesulfonate or MS-222
TrypLE	Thermo Fischer Scientific	12604-01	Synthetic trypsin replacement, less damaging to the cells and allows for the gentle dispersion of strongly adherent cells. (Thermo-
willco dish	WillCo wells	GWST-5040	50mm glass bottom dishes, allow for the embedding of up to 20 zebrafish larvae, enabling the imaging of multiple conditions in one dish due to its large optical glass surface