

Materials List for:

The *Drosophila* Imaginal Disc Tumor Model: Visualization and Quantification of Gene Expression and Tumor Invasiveness Using Genetic Mosaics

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Materials

Name	Company	Catalog Number	Comments
Agar	Gewürzmühle Brecht, Eggenstein, Germany	00262-0500	Fly food recipe: Prepare 20 L fly food with 160 g agar, 360 g yeast, 200 g soy flour, 1.6 kg yellow cornmeal, 1.2 L malt extract, 300 ml light corn syrup, 130 ml propionic acid and 200 ml 15% nipagin. Fly food should be cooked for 1 hour at 85 °C.
Corn syrup	Grafschafter Krautfabrik Josef Schmitz KG, Meckenheim, Germany	01939	
Propionic acid	Carl Roth GmbH, Karlsruhe, Germany	6026.1	
Cornmeal	ReformKontor GmbH, Zarrentin, Germany	4010155063948	
Malt extract	CSM Deutschland GmbH, Bremen, Germany	728985	
Soy flour	Stockmeier Food GmbH, Herford, Germany	1000246441010	
Yeast	Werner Ramspeck GmbH, Schwabach, Germany	210099K	
Methyl-4-benzoate/ Nipagin	Sigma-Aldrich, Deisenhofen, Germany	H5501	Prepare a 15% stock solution with 70% EtOH
Drosophila fly food vials	Kisker Biotech, Steinfurt, Germany	789008	
Vial plugs	K-TK e.K., Retzstadt, Germany	1002	
Drosophila fly food bottles	Greiner Bio-One, Frickenhausen, Germany	960177	
Bottle plugs	K-TK e.K., Retzstadt, Germany	1002 S	
Poly(vinyl alcohol) 4-88/[CH ₂ CHOH-] _n	Sigma-Aldrich, Deisenhofen, Germany	81381	Mounting medium recipe: Dissolve 6 g Poly(vinyl alcohol) 4-88 in 39 ml Millipore H ₂ O, 6 ml 1 M Tris (pH 8.5) and 12.5 ml glycerol. Stir overnight at 50 °C and centrifuge 20 min at 5,000 rpm. Add DABCO to the supernatant to get a final concentration of 2.5%. Store aliquots at -20 °C.
1,4-Diazabicyclo[2.2.2]octane/ DABCO	Sigma-Aldrich, Deisenhofen, Germany	D2522	
Triton X-100	Sigma-Aldrich, Deisenhofen, Germany	9002-93-1	

Phosphate-buffered saline/ PBS			137 mM NaCl, 2.7 mM KCl, 10 mM Na ₂ HPO ₄ and 1.8 mM KH ₂ PO ₄ , pH 7.4 in Millipore H ₂ O
PBST			0.1% Triton X-100 in PBS
Paraformaldehyde/ PFA	Sigma-Aldrich, Deisenhofen, Germany	158127	4% PFA fixative recipe: Dissolve 4 g PFA in 80 ml Millipore H ₂ O on a magnetic stirrer plate heated to 55 °C. Add 1 M NaOH dropwise until all PFA particles are dissolved. Add 10 ml 10x PBS and adjust the pH to 7.4 with 1 M HCl. Mix in 100 µl Triton X-100 and fill up with Millipore H ₂ O to 100 ml. Store aliquots at -20 °C. Avoid repeated thawing. (CAUTION: PFA is highly toxic. Prepare the PFA fixative in a fume hood. Wear a self-contained breathing apparatus, gloves and clothing. Avoid contact with skin, eyes or mucous membranes.)
Bovine Serum Albumin/ BSA	Sigma-Aldrich, Deisenhofen, Germany	A3059	Blocking solution recipe: Dissolve 0.3% BSA in PBST
Fluorescently labeled phalloidin	Invitrogen, Karlsruhe, Germany, Molecular Probes	A12379, A22283, A22284	Phalloidin stock solution: Dissolve powder (300 U) in 1.5 ml Methanol. Store at -20 °C. For staining, use in dilution 1:250 (A12379, A22283) and 1:100 (A22284) in PBST.
4',6-Diamidino-2-phenylindol Dihydrochlorid/ DAPI	Carl Roth GmbH, Karlsruhe, Germany	6335	DAPI staining solution: Prepare a stock solution of 5 mg/ml in Millipore H ₂ O and store aliquots at 4 °C. Used in dilution 1:1,000 in PBST.
Mouse anti-H2 antibody	Kurucz et al., 2003		Used in dilution 1:500 in blocking solution
Cy5 AffiniPure Donkey Anti-Mouse IgG (H+L)	Jackson ImmunoResearch, Suffolk, UK	715-175-151	Used in dilution 1:500 in blocking solution
Dumont #5 forceps	Fine Science Tools, Heidelberg, Germany	11295-10	
Glass embryo dish (30 mm)	Thermo Scientific, Schwerte, Germany	E90	
Tungsten needles	Fine Science Tools, Heidelberg, Germany	10130-20	
Nickel Plated Pin Holder	Fine Science Tools, Heidelberg, Germany	26018-17	
Microscope slides	VWR, Darmstadt, Germany	631-1553	
Coverslips 22 x 22 mm (#1 Menzel-Gläser)	VWR, Darmstadt, Germany	631-1336	
Coverslips 24 x 50 mm (0.13-0.16 mm)	Thermo Scientific, Schwerte, Germany	1076371	
Kimtech Science Precision Wipes	Thermo Scientific, Schwerte, Germany	06-677-70	
Dissecting stereomicroscope	Olympus, Hamburg, Germany	SZX7 (DF PLAPO 1X-4 Japan)	
Fluorescent stereomicroscope	Olympus, Hamburg, Germany	SZX16 (SDF PLAPO 0.8X Japan) with DP72 CCD camera	Equipped with the narrow blue bandpass filter set for excitation of GFP other blue excitable fluorochromes (excitation filter BP460-480 nm, barrier filter BA495-540 nm) and narrow green excitation and longpass barrier

			filter for RFP and other green excitable fluorochromes (excitation filter BP530-550, barrier filter BA575IF). Software: Olympus cellSens Standard 1.11.
Confocal microscope	Olympus, Hamburg, Germany	FV1000	Equipped with inverted IX81 microscope. Objectives: 20× UPlan S-Apo (NA 0.85), 40× UPlanFL (NA 1.30) and 60× UPlanApo (NA 1.35). Lasers: UV laser Diode LD405 (50 mW), Argon laser, multi-line 457/(476)/ 488/515 (40 mW), Yellow/Green laser diode LD560 (15 mW) and Red Laser diode 635 (20 mW). Software: Fluoview 2.1c Software
Drosophila cooled incubator	Ewald Innovationstechnik GmbH, Bad Nenndorf, Germany	Sanyo MIR553	
Squirt bottle	VWR, Darmstadt, Germany	215-8105	
BD Clay Adams Nutator Mixer	VWR, Darmstadt, Germany	15172-203	
ELMI Digital Rocking Shaker	VWR, Darmstadt, Germany	DRS-12	
5 PRIME Isol-RNA Lysis Reagent	VWR, Darmstadt, Germany	2302700	The protocol is compatible with other TRIzol-based reagents, e.g., TRIreagent from Sigma (Cat. Nr.T9424), TRIzol reagent from ThermoFisher (Cat. Nr. 15596).
Diethyl pyrocarbonate/ DEPC	Sigma-Aldrich, Deisenhofen, Germany	D5758	Dilute DEPC 1:1,000 in Millipore H ₂ O, stir overnight and autoclave.
UltraPure Phenol:Chloroform:Isoamyl Alcohol (25:24:1)	ThermoFischer Scientific, Invitrogen, Karlsruhe, Germany	15593-031	
Chloroform	Merck, Darmstadt, Germany	102445	
TURBO DNase (2 U/μl)	Thermo Scientific, Schwerte, Germany	AM2238	
Invitrogen UltraPure Glycogen	ThermoFischer Scientific, Invitrogen, Karlsruhe, Germany	10-814-010	
Sodium acetate trihydrate	VWR, Darmstadt, Germany	27652.232	Prepare a 3 M Sodium acetate solution with DEPC-H ₂ O and adjust the pH to 5.2
2-Propanol	Merck, Darmstadt, Germany	109634	
Ethanol	Merck, Darmstadt, Germany	100983	Prepare a 75% EtOH dilution with DEPC-H ₂ O.
UV/Vis-Spectrophotometre NanoDrop ND-8000	Thermo Scientific, Schwerte, Germany	ND-8000	
Eppendorf Microcentrifuge (Refrigerated)	Thermo Scientific, Schwerte, Germany	5417R	
Experion RNA StdSens Analysis Kit	Bio-Rad Laboratories GmbH, München, Germany	7007103	
Experion Automated Electrophoresis Station	Bio-Rad Laboratories GmbH, München, Germany	7007010	
SuperScript III Reverse Transcriptase	Thermo Scientific, Schwerte, Germany	18080044	
Oligo d(T) Primer	Integrated DNA Technologies, Leuven, Belgium		Prepare 100 μM stock solutions in DEPC-H ₂ O and store at -20 °C
dNTP Mixture	Takara Bio Europe/Clonetech, Saint-Germain-en-Laye, France	4030	Store aliquots of 25 μl at -20°C

CFX96 Touch Real-Time PCR Detection System	Bio-Rad Laboratories GmbH, München, Germany	1855195	
iQ SYBR Green Supermix	Bio-Rad Laboratories GmbH, München, Germany	170-8882	
Hard-Shell PCR Plates 96-well, thin wall	Bio-Rad Laboratories GmbH, München, Germany	HSP9601	
Microseal 'B' Film	Bio-Rad Laboratories GmbH, München, Germany	MSB1001	
<i>rp49</i> Forward primer	Integrated DNA Technologies, Leuven, Belgium		5' TCC TAC CAG CTT CAA GAT GAC 3'
<i>rp49</i> Reverse primer	Integrated DNA Technologies, Leuven, Belgium		5' CAC GTT GTG CAC CAG GAA CT 3'
<i>mmp1</i> Forward primer	Integrated DNA Technologies, Leuven, Belgium		5' AGG GCG ACA AGT ACT ACA AGC TGA 3'
<i>mmp1</i> Reverse primer	Integrated DNA Technologies, Leuven, Belgium		5' ACG TCT TGC CGT TCT TGT AGG TGA 3'