

Materials List for:

# Structure-function Studies in Mouse Embryonic Stem Cells Using Recombinase-mediated Cassette Exchange

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## Materials

Name	Company	Catalog Number	Comments
ROSALUC Mice	made in house		frozen sperm available upon request
R26-iPSC mice	made in house		frozen sperm available upon request
Vessel probe	Fine Science Tools	10160-13	to check for copulation plugs
M2 medium	Sigma-Aldrich	M7167	make aliquots and store at -20 °C
Fine forceps (Dumont #5 Standard tip Student forceps)	Fine Science Tools	11251-10	spray with 70% EtOH before use (do not autoclave)
23 G needles	Fine-ject	8697	
1-mL syringes	Soft-ject	6680	
60-mm bacterial grade plates (for flushing)	Gosselin	BB60-01	
Mouth pipette	made in house		see discussion
Mouse embryonic fibroblasts (MEFs, TgN (DR4)1 Jae strain)	ATTC	SCRC-1045	
TgN (DR4)1 Jae mice	The Jackson Laboratory	3208	
Mitomycin C	Sigma-Aldrich	M0503	
Phosphate buffered saline (PBS) without calcium or magnesium	Gibco	14190-094	
Tg(DR4)1Jae/J mice	JAX	3208	mice that contain four drug-selectable genes and DR4 MEFS confers resistance to neomycin, puromycin, hygromycin and 6-thioguanine
0.1% Gelatin	Sigma-Aldrich	G1393	Dissolve 0.5 g in 500 mL distilled water, autoclave and store at 4 °C.
Trypsin (0.25%)	Gibco	25200-056	
2 µM pluripotin	Cayman Chemical	10009557	
pRMCE-DV1	BCCM/LMBP collection	LMBP 08870	public available from the BCCM/LMBP collection ( <a href="http://bccm.belspo.be">http://bccm.belspo.be</a> )

cre-excised pRMCE-DV1	BCCM/LMBP collection	LMBP 08195	public available from the BCCM/LMBP collection ( <a href="http://bccm.belspo.be">http://bccm.belspo.be</a> )
pCAG-FlpE-IRES-Puro-pA	Addgene	20733	
heat-shock competent DH5 $\alpha$ bacteria	made in house		
Gateway pDONR221 vector	Thermo Fisher	12536-017	contains kanamycin-resistance gene
BP clonase II mix	Thermo Fisher	11789-020	
LR clonase II mix	Thermo Fisher	11791-020	
Luria Broth (LB)			
Ampicillin			
Applied Biosystems 3730XL DNA Analyzer	Thermo Fisher	3730XL	
G418	Thermo Fisher	11811-023	
Lipofectamine 2000 transfection reagent	Thermo Fisher	11668027	
Lipofectamine LTX transfection reagent	Thermo Fisher	15338100	
Effectene transfection reagent	Qiagen	301425	
GATEWAY pENTR 1A vector	Thermo Fisher	A10462	recombination-compatible vector
mouse monoclonal anti-p120ctn antibody	BD Transduction Laboratories	610134	
mouse monoclonal anti-Ecadherin antibody	BD Transduction Laboratories	610181	
<b>General equipment</b>			
Sterile dissection tools			fine scissors and forceps for dissecting the uterus
Sterile pipettes: 5 mL, 10 mL and 25 mL			
15-mL and 50-mL conical centrifuge tubes			
96-well culture plates V-shaped bottom and flat bottom)			
Culture dishes: 24 wells, 12 wells and 6 wells			
Multichannel pipettes (to pipette 30, 50, 100 and 200 $\mu$ L)			
Sterile multichannel reservoirs			
Access to a laminar air flow			
Access to an incubator at 37 °C with 5% CO <sub>2</sub>			
Access to an inverted microscope			
Access to a bench-top centrifuge			
Access to a stereo microscope with transmitted-light			
<b>Culture media</b>			
<b>MEF Medium</b>			stored at 4 °C; warm 30 min at 37 °C before use
Dulbecco's modified Eagle's medium (DMEM)	Gibco	41965-062	
10% fetal bovine serum (FBS)	Sigma-Aldrich	F-7524	

L-glutamine (2 mM)	Gibco	25030-024	
Sodium pyruvate (0.4 mM)	Gibco	11360-039	
penicillin (100 U/mL)	Gibco	15140-122	
streptomycin (100 µg/mL)	Gibco	15140-122	
<b>SR-based mESC medium</b>			stored at 4 °C; warm 30 min at 37 °C before use
DMEM/F12	Gibco	31330-038	mixed in a 1:1 ratio
15% knock-out serum replacement (SR)	Gibco	10828-028	
L-glutamine (2 mM)	Gibco	25030-024	
0.1 mM non-essential amino acids	Gibco	11140-050	
penicillin (100 U/mL)	Gibco	15140-122	
streptomycin (100 µg/mL)	Gibco	15140-122	
β-mercaptoethanol (0.1 mM)	Sigma-Aldrich	M 3148	
2,000 U/mL recombinant mouse LIF	(IRC/VIB Protein Service facility)		
<b>FBS-based mESC medium (similar to SR-based mESC medium)</b>			stored at 4°C; warm 30 min at 37°C before use
Knockout DMEM	Gibco	10829-018	
15% FBS	Hyclone	SH30070.03E	
<b>Differentiation Medium</b>			stored at 4 °C; warm 30 min at 37 °C before use
Iscove's Modified Dulbecco's Medium (IMDM)	Gibco	21980-032	
15% FBS	Hyclone	SH30070.03E	
5% CD Hybridoma Medium(1x) liquid	Gibco	11279-023	
2 mM L-glutamine	Gibco	25030-024	
0.4 mM 1-thioglycerol	Sigma-Aldrich	M-6145	
50 µg/mL ascorbic acid	Sigma-Aldrich	A-4544	
penicillin (100 U/mL)	Gibco	15140-122	
streptomycin (100 µg/mL)	Gibco	15140-122	
<b>2i</b>			
1 µM Erk inhibitor PD0325901	Axon Medchem	Axon 1408	
3 µM Gsk3 inhibitor CHIR99021	Axon Medchem	Axon 1386	