

Materials List for:

Split-BioID — Proteomic Analysis of Context-specific Protein Complexes in Their Native Cellular Environment

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Materials

Name	Company	Catalog Number	Comments
Acetic acid (glacial)	VWR	20104.298	To make TAE buffer
Agarose	Sigma	A9539	Take TAE-agarose gels for DNA analysis and extraction
Ammonia solution 25% NH ₃	Bernd Kraft	6012	To dissolve biotin
Ampicillin	Sigma	A9518	To select transformed bacteria
Bioruptor plus sonification device	Diagenode	B01020001	Other sonification devices are also ok
Biotin	Sigma	B4639	To be added to the growth medium to stimulate efficient biotinylation
Bovine serum albumin fraction V	Carl Roth	8076	Used in Western blot buffers and a protein standard in Bradford assays
Bradford Ultra reagent	Expedeon	BFU05L	Any other method/kit for protein determination is fine, this particular reagent is more tolerant to detergent than other Bradford reagents
Cell scrappers	TPP	99002	Any other model is also fine
ClaI	New England Biolabs	R0197	Restriction enzyme for cloning into the split-BioID plasmids (NBirA* fusion)
DMEM medium	Sigma	D6046	If using another cell line, use the corresponding optimal growth medium
DNA miniprep kit	Sigma	PLN350	Any other kit is also fine
Doxycycline	Applichem	A2951	Dox is light sensitive
DTT	Applichem	A2948	Make 1M stock solution, store at -20 °C and always use fresh
DyLight 680-conjugated streptavidin	Thermo scientific	21848	to use with a LiCor Western blot scanning device
Dynabeads MyOne Streptavidin C1	Invitrogen	65002	The C1 beads are not BSA coated which is preferable for downstream MS applications (no leakthrough of BSA in the final elution)
EDTA	Applichem	A5097	Make a 500 mM stock, adjust pH to 8 while dissolving the EDTA powder
Ethanol	Sigma	32205	Make a 70% stock solution in which Doxycycline can be dissolved at 10 mg.mL ⁻¹

Fastgene Gel/PCR DNA Extraction Kit	Nippon Genetics	FG-91302	Any other kit is also fine
HCl 37%	Merck	1.00317.1000	To adjust pH of biotin stock solution
HEPES	Carl Roth	6763	Make a 500 mM stock solution, adjust the pH to 7.4
Immobilon-FL PVDF membrane, 0.45 µm	Millipore	IPFL00010	This membrane shows minimal autofluorescence when used with a LiCor Western blot scanning device
LiCl	Grüssing GmbH	12083	Make a 5M stock solution
Odyssey CLx imaging system	LI-COR	N/A	To scan Western blot membrane decorated with fluorophore-labeled antibody
Linear polyethylenimine (PEI)	Polysciences	23966-2	Any other transfection reagent is also fine
Milk powder	Carl Roth	T145	To block Western blot membranes
MluI-HF	New England Biolabs	R3198	Restriction enzyme for cloning into the split-BioID plasmids (NBirA* fusion)
Na-deoxycholate	Sigma	30970	Make a 10% (w/v) stock solution
NaCl	Sigma	31434	Make a 5M stock solution
NP-40 (Nonidet P40 substitute)	Sigma	74385	Make a 20% (v/v) stock solution
Pacl	New England Biolabs	R0547	Restriction enzyme for cloning into the split-BioID plasmids (CBirA* fusion)
Phosphate buffer saline (PBS)	Sigma	806552	To wash cells before scrapping
PmeI	New England Biolabs	R0560	Restriction enzyme for cloning into the split-BioID plasmids (CBirA* fusion)
Protease inhibitor cocktail	Roche	4693132001	Added to the lysis buffer to prevent protein degradation
pSF3-Flag-CBir-FRB_Myc-NBir-FKBP	Addgene	90003	Split-BioID plasmid, mediates the co-expression of NBirA*-FKBP and CBirA*-FRB, FKBP and FRB can be replaced by two other ORFs
pSF3-Flag-CBir-FRB_FKBP-Myc-Nbir	Addgene	90004	Split-BioID plasmid, mediates the co-expression of FKBP-NBirA* and CBirA*-FRB, FKBP and FRB can be replaced by two other ORFs
pSF3-Flag-FRB-Cbir_Myc-NBir-FKBP	Addgene	90008	Split-BioID plasmid, mediates the co-expression of NBirA*-FKBP and FRB-CBirA*, FKBP and FRB can be replaced by two other ORFs
pSF3-Flag-FRB-Cbir_FKBP-Myc-Nbir	Addgene	90009	Split-BioID plasmid, mediates the co-expression of FKBP-NBirA* and FRB-CBirA*, FKBP and FRB can be replaced by two other ORFs
Q5 High-Fidelity PCR kit	New England Biolabs	E0555S	To amplify the ORF coding for the proteins to be tested. Any other thermostable DNA polymerase is fine.
Quick ligation kit	New England Biolabs	M2200S	To ligate DNA fragments into the split-BioID plasmids, any other DNA ligation system is fine.
RunBlue 4-20% SDS precast gels	Expdeon	BCG42012	To use when running samples for MS analysis
RunBlue LDS Sample Buffer	Expdeon	NXB31010	Running buffer for the RunBlue precast gels

SDS	Sigma	5030	Comes as a 20% stock solution
tet-free serum	Biowest	S181T	we use tet-free serum to minimize basal expression of the fusion proteins
Trans-Blot Turbo Transfer system	Bio-Rad	1704150	High speed Western blotting transfer system, any other transfer system is also fine
Tris	Carl Roth	4855	Make 1M stock solutions with adequate pH (7.4 and 8)
Triton X-100	Applichem	A4975	Make a 20% (v/v) stock solution
Tween-20	Carl Roth	9127	Used in Western blot buffers, Tween 20 leads to high background fluorescence and should be omitted in the blocking and last wash step