

Materials List for:

Genetic and Biochemical Approaches for *In Vivo* and *In Vitro* Assessment of Protein Oligomerization: The Ryanodine Receptor Case Study

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URL: <https://www.jove.com/video/54271>

DOI: [doi:10.3791/54271](https://doi.org/10.3791/54271)

Materials

Name	Company	Catalog Number	Comments
PART 1 yeast two-hybrid			
Plate incubator	Hereaus	B6120	Used at 30 °C
Orbital shaker incubator	New Brunswick Scientific	INNOVA 4300	Used at 30 °C with shaking at 230 - 250 rpm
Spectrophotometer	Perkin Elmer	Lambda Bio+	To measure OD ₆₀₀ of yeast culture; to measure Absorbance at 420 nm in liquid b-Gal assay
Matchmaker Two-Hybrid System 2 Kit	Takara Clontech	K1604-1	Contains bait vector pGBKT7, prey vector pACT2 and yeast strain Y190
Yeast Nitrogen Base	Sigma-Aldrich	Y0626	To prepare minimal SD growth medium
Dropout supplement lacking leucine and tryptophan	Sigma-Aldrich	Y0750	To prepare minimal SD growth medium
Dropout supplement lacking leucine, tryptophan, histidine	Sigma-Aldrich	Y2001	To prepare minimal SD growth medium
Herring testes carrier DNA	Takara Clontech	630440	For yeast transformation
Whatman filter paper Grade 5	Sigma-Aldrich	WHA1005070	for use in colony-lift filter paper b-Gal assay
X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)	Sigma-Aldrich	B4252	for use in colony-lift filter paper b-Gal assay
ONPG (o-nitrophenyl-β-D-galactopyranoside)	Sigma-Aldrich	N1127	for use in liquid b-Gal assay
PART 2. Protein expression in a mammalian cell line			
HEK293 cell line	ATCC	ATCC® CRL-1573™	
Humidified incubator (5% CO ₂ , 37 °C)	SANYO	MCO-18AIC	To culture mammalian cells
DMEM (Dulbecco's Modified Eagle's medium)	Invitrogen (ThermoFisher)	41966	To prepare growth medium for mammalian cells
Fetal Bovine Serum	Invitrogen (ThermoFisher)	10500	To prepare growth medium for mammalian cells
Glass beads	Sigma-Aldrich	G8772	To homogenise cells
Needle 23 G (0.6 x 30 mm)	BD Microlance	300700	To homogenise cells
Protease inhibitor cocktail (Complete)	Roche	11873508001	To prevent proteolysis
PART 3. In vitro biochemical methods			
Mini-PROTEAN Tetra Cell (SDS-PAGE system)	Bio-Rad	1658000EDU	For polyacrylamide gel electrophoresis

Trans-Blot SD (Semi-dry transfer system)	Bio-Rad	1703940	For electrophoretic transfer
Compact X-ray film processor	Xograph	X4	For use in immunoblotting
Glutaraldehyde	Sigma-Aldrich	G5882	For use in chemical cross-linking
Protein-A sepharose beads	GE Healthcare Life Sciences	17-5280-01	For use in co-IP assay
Anti-HA (Y-11 rabbit polyclonal IgG)	Santa Cruz Biotechnology	sc-805	For use in co-IP assay
Non-immune rabbit IgG	Santa Cruz Biotechnology	sc-2027	For use in co-IP assay
Anti-cMyc (9E10 mouse monoclonal IgG)	Santa Cruz Biotechnology	sc-40	For use in immunoblotting
Anti-HA (16B12 mouse monoclonal IgG)	Covance	MMS-101P	For use in immunoblotting
Anti-mouse IgG-HRP	Santa Cruz Biotechnology	sc-2005	For use in immunoblotting
Hyperfilm ECL	GE Healthcare Life Sciences	28906836	For use in immunoblotting
ECL Western Blotting Substrate	Pierce (ThermoFisher)	32106	For use in immunoblotting