Materials List for:

Measuring Gene Expression in Bombarded Barley Aleurone Layers with Increased Throughput

Grace Uwase[1], Taylor P. Enrico[1], David S. Chelimo[1], Benjamin R. Keyser[1], Russell R. Johnson[1]

[1]Department of Biology, Colby College

Correspondence to: Russell R. Johnson at russ.johnson@colby.edu

URL: https://www.jove.com/video/56728
DOI: doi:10.3791/56728

Materials

Name	Company	Catalog Number	Comments
GeneElute HP plasmid Maxiprep kit	Sigma	NA0310-1KT	
UV-vis spectrophotometer	Nanodrop	ND-1000	
Himalaya barley grains	/	/	A variety of hulless barley (store in the dark at 4° C)
sodium succinate	Sigma	S2378	Reagent for Imbibing Solution
calcium chloride (dihydrate)	Fisher	C79-500	Reagent for Imbibing Solution
Imbibing Solution	home made	/	20 mM sodium succinate, 20 mM calcium chloride, pH 5.0. Sterilize by autoclaving before use.
chloramphenicol	Sigma	C0378	Prepare a 10 mg/mL stock solution in 70% ethanol.
vermiculite	Fisher	NC0430369	Used for vermiculite plates.
filter paper circles (90 mm)	Whatman	1001 090	Used for vermiculite and for pre-bombardment grain preparation
Vermiculite Plates	home made	/	Add 50 mL of vermiculite to a glass petri dish. Place a 90 mm paper circle on top of the vermiculite. Autoclave.
forceps (fine pointed)	Fisher	13-812-42	Used for removing seed coat from barley grains.
forceps (ultra fine point)	Fisher	12-000-122	Used for removing seed coat from barley grains.
gold microcarriers (1.6 µm)	BioRad	1652264	
macrocarriers	BioRad	1652335	
calcium chloride (dihydrate)	Fisher	C79-500	Prepare a 2.5 M stock solution and store 1 mL aliquots at -20° C.
spermidine	Sigma	S0266	Prepare a 100 mM stock solution and store 500 µL aliquots at -20° C (use within 2 months).
rupture discs (1550 psi)	BioRad	1652331	
stopping screens	BioRad	1652336	
macrocarrier holders	BioRad	1652322	
Biolistic particle delivery system	BioRad	PDS-1000/He	
sodium phosphate monobasic monohydrate	Sigma	S9638	Reagent for 1M sodium phosphate pH 7.2
sodium phosphate dibasic	Sigma	S9763	Reagent for 1M sodium phosphate pH 7.2
1M sodium phosphate pH 7.2	home made	/	Combine 6.9 g of sodium phosphate monobasic

			monohydrate with 7.1 g of sodium phosphate dibasic. Add water to 100 mL. Add NaOH to get pH 7.2.
dithiothrietol (DTT)	Sigma	43819	Dissolve in water to 1 M. Store at -20° in 1 mL aliquots.
leupeptin	Sigma	L2884	Dissolve in water to 10 mg/mL. Store at -20° C.
glycerol	Sigma	G5516	Prepare a 50% solution in water.
Grinding Buffer	home made	/	Combine 10 mL of 1 M sodium phosphate pH 7.2, 500 µL of 1 M DTT, 100 µL of 10 mg/mL leupeptin, and 40 mL of 50% glycerol. Add water to 100 mL.
stainlesss steel beads (5 mm)	Qiagen	69989	
2.0 mL tubes	Eppendorf	22363352	This specific model of tube is recommended for use with the homogenizer.
bead homogenizer (TissueLyser)	Qiagen	85210	
12mm x 75 mm glass test tubes	Fisher		
luciferin	Goldbio	LUCK-100	Prepare a 25 mM stock solution and store 1 mL aliquots at -20° C.
ATP	Sigma	A7699	Prepare a 100 mM stock solution and store 250 µL aliquots at -20° C.
Tris base	Sigma	T1503	Reagent for 1M Tris sulfate pH 7.7.
sulfuric acid	Sigma	258105	Reagent for 1M Tris sulfate pH 7.7.
1M Tris sulfate pH 7.7	home made	/	Dissolve 12.1 g Tris base in 100 mL of water. Adjust pH to 7.7 with sulfuric acid.
magnesium chloride	Sigma	M9397	Dissolve in water to 2 M.
Luciferase Assay Buffer (LAB)	home made	/	Combine 3 mL of 1 M Tris sulfate pH 7.7, 500 µL of 2 M magnesium chloride, 1 mL of 1 M DTT, and 200 µL of 0.5 M EDTA. Add water to 50 mL.
Luciferase Assay Mixture	home made	/	Combine 15 mL of LAB, 800 µL of 25 mM luciferin, 200 µL of 100 mM ATP, and 4 mL of water. This makes enough assay mixture (20 mL) for 100 luciferase assays.
luminometer (Sirius)	Berthold	/	
4-methylumbelliferyl-β-D-glucuronide (MUG)	Goldbio	MUG1	Dissolve in DMSO to 100 mM.
sodium azide	Sigma	S8032	Prepare a 2% stock solution in water and store 1 mL aliquots at -20° C.
96 well plates (standard)	Fisher	12565501	
GUS assay buffer	home made	/	Combine 2.5 mL of MUG, 5 mL of 1 M sodium phosphate pH 7.2, 400 µL of 0.5 M EDTA, 1 mL of 1 M DTT, 100 µL of 10 mg/ml leupeptin, 20 mL of methanol, and 1 mL of 2% sodium azide. Add water to 100 mL.
TempPlate sealing film	USA Scientific	2921-1000	
96 well plates (black)	Costar	3916	

sodium carbonate	Sigma	S7795	Prepare a 200 mM solution in water.
4-methylumbelliferone	Sigma	M1381	Prepare a 100 µM solution in water. Freeze 1 mL aliquots at -20° C.
microplate fluouresence reader	Bio-Tek	FLX-800	