

# Isolation of Active *Caenorhabditis elegans* Nuclear Extract and Reconstitution for *In Vitro* Transcription

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jove.com/video/62723

## Materials

Name	Company	Catalog Number	Comments
<b>Consumables and reagents</b>			
0.2 mL 8-Strip Tubes & Flat Strip Caps, Clear	Genesee Scientific	24-706	
0.2 mL Individual PCR tubes	Genesee Scientific	24-153G	
1.7 mL sterile microtubes	Genesee Scientific	24-282S	
100% absolute molecular grade ethanol	Fisher Scientific	BP2818	
100% ethanol, Kopectec	Decon Labs	V1001	
10 mL serological pipet	VWR international	89130-898	
150 mm petri plates	Tritech Research	T3325	
15 mL conical centrifuge tubes	Genesee Scientific	28-103	
20 mL plastic syringes	Fisher Scientific	14955460	
2 mL Norm-Ject syringes	Henke-Sass Wolf GmbH	4020	
500 mL vacuum filter cup 0.22 µm PES, Stericup Millipore Express Plus	Millipore Sigma	SCGPU10RE	
50 mL conical centrifuge tubes	ThermoFisher Scientific	339652	
50 mL serological pipet	VWR international	89130-902	
5 mL serological pipet	VWR international	89130-896	
Agar, Criterion	VWR International	C7432	
Agarose	Denville Scientific	CA3510-6	
Alcohol proof marker	VWR International	52877-310	
Bacto peptone	VWR International	90000-264	
<i>Caenorhabditis elegans</i>	CGC	N2	
Calcium dichloride	Millipore Sigma	C4901	
Cholesterol	Millipore Sigma	C8667	
Control DNA temple cloning primers, Forward 5'- ctc atg ttt gac agc tta tcg atc cgg gc -3'			

Control DNA temple cloning primers, Forward 5'- aca gga cgg gtg tgg tcg cca tga t -3'			
Deionized water			
Dithiothreitol	Invitrogen	15508-013	
DNA gel stain, SYBR safe	Invitrogen	S33102	
DNA ladder mix, O'gene ruler	Fisher Scientific	SM1173	
DNA Loading Dye, 6x TriTrack	Fisher Scientific	FERR1161	
DNase, Baseline-ZERO	Lucigen	DB0715K	
Dry ice			
<i>Escherichia coli</i> OP50 strain	CGC	OP50	
Glacial acetic acid	Fisher Scientific	A38	
Glycerol	Millipore Sigma	G6279	
HeLa nuclear extract in vitro transcription system, HeLaScribe	Promega	E3110	
Hepes Solution, 1 M Gibco	Millipore Sigma	15630080	
Hydrochloric acid 37%	Millipore Sigma	P0662	
Hypochlorite bleach	Clorox		
LB Broth	Millipore Sigma	L3022	
Magnesium dichloride	Millipore Sigma	M8266	
Magnesium Sulfate	Millipore Sigma	M7506	
Medium weigh dishes	Fisher Scientific	02-202-101	
microscope slides, Vista vision	VWR International	16004-368	
molecular grade water, Hypure	Hyclone Laboratories	SH30538	
Nystatin	Millipore Sigma	N1638	
PCR system, FailSafe with premix A	Lucigen	FS99100	
Potassium chloride	Millipore Sigma	P39111	
Potassium phosphate dibasic	Millipore Sigma	P3786	
Potassium phosphate monobasic	Millipore Sigma	P0662	
Protease inhibitor, Halt single use cocktail 100x	ThermoFisher Scientific	78430	
protein assay kit, Qubit	ThermoFisher Scientific	Q33211	
reverse transcription kit, Sensiscript	Qiagen	205211	
RNA extraction kit RNeasy micro kit	Qiagen	74004	
RNase Inhibitor	Applied Biosystems	N8080119	
Sodium Chloride	VWR International	BDH9286-12KG	
Sodium hydroxide	Millipore Sigma	1-09137	
Sterile syringe filter with 0.2 µm Polyethersulfone membrane	VWR international	28145-501	
Sucrose	VWR International	200-334-9	
transcription primers, Forward 5'- gcc ggg cct ctt gcg gga tat -3'			
transcription primers, Reverse 5'- cgg cca aag cgg tcg gac agt-3'			
Tris-Base	Fisher Scientific	BP152	
Tween20	Millipore Sigma	P2287	
<b>Equipment</b>			
-20 °C incubator	ThermoFisher Scientific		
20 °C incubator	ThermoFisher Scientific		
37 °C incubator	Forma Scientific		

4 °C refrigerator	ThermoFisher Scientific		
-80 °C freezer	Eppendorf		
Autoclave	Sanyo		
Balch homogenizer, isobiotec cell homogenizer	Isobiotec		
Benchtop Vortexer	Fisher Scientific	2215365	
Centrifuge, Eppendorf 5418 R	Eppendorf	5401000013	
Centrifuge, VWR Clinical 50	VWR International	82013-800	
Dissection microscope, Leica M80	Leica Microsystems		
Fluorometer, Qubit 2.0	Invitrogen	Q32866	
Gel imaging system, iBright FL1500	ThermoFisher Scientific	A44241	
Gel system	ThermoFisher Scientific		
Heat block	VWR International	12621-048	
Microcentrifuges, Eppendorf 5424	Eppendorf	22620401	
PIPETBOY acu 2	Integra	155017	
Pipette L-1000 XLS+, Pipet-Lite LTS	Rainin	17014382	
Pipette L-10 XLS+, Pipet-Lite LTS	Rainin	17014388	
Pipette L-200 XLS+, Pipet-Lite LTS	Rainin	17014391	
Pipette L-20 XLS+, Pipet-Lite LTS	Rainin	17014392	
Rocking platform	VWR International		
Thermocycler, Eppendorf Mastercycler Pro	Eppendorf	950030010	