Materials List for:

RNA Interference in Aquatic Beetles as a Powerful Tool for Manipulating Gene Expression at Specific Developmental Time Points

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Materials

Name	Company	Catalog Number	Comments
1. RNA isolation and de novo transcriptome assembly			
liquid nitrogen	University Stockroom		Typically locally available at research institutions
pipette tips	Fisher Scientific	size dependent	Products from other vendors would work equally well
RNeasy lipid tissue mini kit (RNA isolation kit)	Qiagen	74804	Detailed specific protocol is provided with the kit
spectrophotometer (NnanoDrop)	Thermo Fisher	9836674	Other models would work equally well
1.2. De novo transcriptome assembly.			
BUSCO.v3	https://busco.ezlab.org/		Any freely available software for assessing trasncriptome completeness can be used.
CLC Genomics workbench	Qiagen	832021	Other equivalent software packages are also available
Galaxy workbench	https://usegalaxy.org/		An open source online transcriptome assembly & annotation pipeline
NCBI BLASTx	https://blast.ncbi.nlm.nih.gov/Blast.cgi?LINK_LOC=blasthome&PAGE_TYPE=BlastSearch&PROGRAM=blastx		An open source online alignment and annotation pipeline
2. cDNA synthesis, Cloning & Miniprep isolation			
ampicillin sodium salt	Gibco	11593-027	Products from other vendors would work equally well
glycerol	Fisher Scientific	G33-500	Products from other vendors would work equally well
LB broth	Fisher Scientific	BP1426-500	Products from other vendors would work equally well
Omniscript RT (reverse trasncription) kit	Qiagen	205111	Detailed specific protocol is provided with the kit
One Shot™ TOP10 Chemically Competent E. coli	Invitrogen	C404010	Detailed specific protocol is provided with the kit
Petri dishes	Fisher Scientific	FB0875713	Products from other vendors would work equally well

PureLink Quick Plasmid Miniprep Kit	ThermoFischer	771471	Detailed specific protocol is provided with the kit
QIAquick PCR purification kit	Qiagen	28104	Detailed specific protocol is provided with the kit
shaker-incubator	Labnet	211DS	Other models would work equally well
spectrophotometer (NnanoDrop)	Thermo Fisher	9836674	Other models would work equally well
thermal cycler for PCR	BioRad	T100	Other models would work equally well
TOPO TA Cloning kit	Invitrogen	1845069	Detailed specific protocol is provided with the kit
X-Gal Solution	Thermo Scientific	R0941	Products from other vendors would work equally well
2.2 PCR amplification & in vitro dsRNA synthesis			
Centrifuge	Fisher Scientific	accuSpin Micro 17R	Other models would work equally well
ethanol	Fisher Scientific	A4094	Products from other vendors would work equally well
FastTaq DNA Polymerase, dNTPack	Roche	13873432	This kit contains all the reagents necessary for a PCR
MEGAclear Transcriiption clean up kit	ThermoFischer	AM1908	Detailed specific protocol is provided with the kit
MEGAscript T7 Transcription kit	ThermoFischer	AM1334	Detailed specific protocol is provided with the kit
nuclease-free water	Fisher Scientific	AM9932	Products from other vendors would work equally well
QIAquick PCR purification kit	Qiagen	28104	Detailed specific protocol is provided with the kit
sodium acetate	Fisher Scientific	BP333-500	Make 3M working solution
Spectrophotometer (NnanoDrop)	Thermo Fisher	9836674	Other models would work equally well
3. Collecting and preparing early stage T. marmoratus embryos for dsRNA injections			
agarose	Fisher Scientific	9012-36-6	Products from other vendors would work equally well
distilled water	Fisher Scientific	9180	Products from other vendors would work equally well
forceps (Dumon #4 Biology)	Fine Science Tools	11242-40	Products from other vendors would work equally well
glass cavity dish (3 well-dish)	Fisher Scientific	50-243-43	Products from other vendors would work equally well
microwave	Welbilt	turn-table	Other models would work equally well
natural hair paintbrush	Amazon		Any fine brush will do
P1000 micro-pipetter	Gilson	F123602	Other models would work equally well
Petri dishes	Fisher Scientific	FB0875713	Products from other vendors would work equally well
stereomicroscope	Microsocope Central	10446293	Any good stereomicroscope will work

transfer pipettes	Fisher Scientific	21-200-109	Products from other vendors would work equally well
4. dsRNA micro-injections in early stage T. marmoratus embryos			
digital camera	Edmund optics (Qimaging)	Retiga 2000R	Other models would work equally well
ethanol	Fisher Scientific	A4094	Products from other vendors would work equally well
food dye	Kroger		Any available food dye should work fine
humidity chamber			take any plastic box with a lid, sterilize it with 70% ethanol and let it dry
incubator	Labline	203	Other models would work equally well
injection buffer			Prepared for 1 mL following reference #22 : Mix 10 µL of 0.1 M sodium phosphate buffer, 100 µL of 0.5 M potassium chloride solution, 100 µL of food dye and 790 µL of double-distilled water. Store in 4 °C.
intracellular Microinjection Systems (Picospritzer)	Parker	052-0500-900	Currently the model III is available, but older models work also
micro needle holder	A-M Systems	672441	Other products would work equally well
microinjection needles (1.2 mm x 0.68 mm, 4 inches)	A-M Systems	603000	Other models would work equally well
micromanipulator	Drummond Scientific Company	3-000-024-R	Any quality micromanipulator will work
monosodium phosphate	Fischer scientific	7558-80-7	To make 10 mL of 1 M working solution: Add 1.2 g of monosodium phosphate powder to 10 mL of Double distilled water and mix until clear solution is obtained
P-1000 Micropipette Puller	Sutter Instrument	P-1000	Puller settings:Heat 575, Pull 60, Velocity 75 Delay 110, Pressure 700.
P10 micro-pipetter	Gilson	F144802	Other models would work equally well
P1000 micro-pipetter	Gilson	F123602	Other models would work equally well
potassium chloride	Fischer scientific	7447-40-7	To make 100 mL of 0.5 M potassium chloride solution: Add 3.73 g of potassium chloride crystals to 100 mL of double distilled water and mix until clear solution is obtained.
sodium phosphate buffer (0.1M)			To make 10 mL of 0.1 M of this buffer: Mix 8.5 mL of 1 M sodium phosphate dibasic solution with 1.5 mL of 1 M monosodium phosphate solution. Check the pH with a pH meter and adjust accordingly to pH 7.6 at room temprature.
sodium phosphate dibasic	Fischer scientific	7558-79-4	To make 10 mL of 1 M working solution: Add 1.42 g of sodium phosphate dibasic powder to 10

			mL of Double distilled water and mix until clear solution is obtained
stereomicroscope	Microsocope Central	10446293	Any good stereomicroscope will work
5. Preparing T. marmoratus larvae for dsRNA injections			
agarose	Fisher Scientific	9012-36-6	Products from other vendors would work equally well
forceps (Dumon #4 Biology)	Fine Science Tools	11242-40	Products from other vendors would work equally well
Petri dishes	Fisher Scientific	FB0875713	Products from other vendors would work equally well
6. dsRNA micro-injections in T. marmoratus larvae			
injection buffer (10x)			See section 4
microinjection needles (1.2 mm x 0.68 mm, 4 inches)	A-M Systems	603000	Other models would work equally well
microinjection syringe	A-M Systems	603000	Other models would work equally well
micro needle holder	A-M Systems	672441	Other products would work equally well
P-1000 Micropipette Puller	Sutter Instrument	P-1000	Puller settings:Heat 575, Pull 60, Velocity 75 Delay 110, Pressure 700.
stereomicroscope	Microsocope Central	10446293	Any good stereomicroscope will work