

Materials List for

Evaluating the Impact of Hydraulic Fracturing on Streams using Microbial Molecular Signatures

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Citation

Chen See, J.R., Wright, O., Unverdorben, L.V., Heibeck, N., Techtmann, S.M., Hazen, T.C., Lamendella, R. Evaluating the Impact of Hydraulic Fracturing on Streams using Microbial Molecular Signatures. *J. Vis. Exp.* (), e61904, doi:10.3791/61904 (2021).

Date Published

April 4, 2021

DOI

10.3791/61904

URL

jove.com/video/61904

Materials

Name	Company	Catalog Number	Comments
200 Proof Ethanol	Thermo Fisher Scientific	A4094	400 mL need to be added to Buffer PE (see Qiagen QIAQuick Gel Extraction kit protocol) and 96 mL needs to be added to the DNA/RNA Wash Buffer (see ZymoBIOMICS DNA/RNA Miniprep kit protocol). Additional ethanol is needed for the ZymoBIOMICS DNA/RNA Miniprep and NEBNext® Ultra™ II RNA Library Prep with Sample Purification Beads kits.
Agarose	Thermo Fisher Scientific	BP1356-100	100 g per bottle. 0.6 g of agarose would be needed to make one 2% 30 mL gel.
Disinfecting Bleach	Walmart (Clorox)	No catalog number	Use a 10% bleach solution for cleaning the work area before and after lab procedures
DNA gel loading dye	Thermo Fisher Scientific	R0611	Each user-made (i.e. non-e-gel) should include loading dye with all of the samples in the ratio of 1 µL dye to 5 µL sample
DNA ladder	MilliporeSigma	D3937-1VL	A ladder should be run on every gel/e-gel
DNA/RNA Shield (2x)	Zymo Research	R1200-125	3 mL per sediment sample (50 mL conical) and 2 mL per water sample (filter)
Ethidium bromide	Thermo Fisher Scientific	BP1302-10	Used for staining user-made e-gels
Forward Primer	Integrated DNA Technologies (IDT)	51-01-19-06	0.5 µL per PCR reaction
Isopropanol	MilliporeSigma	563935-1L	Generally less than 2 mL per library. Volume needed varies by mass of excised gel fragment (see Qiagen QIAQuick Gel Extraction kit protocol).

PCR-grade water	MilliporeSigma	3315932001	13 μ L per PCR reaction (assuming 1 μ L of sample DNA template is used)
Platinum Hot Start PCR Master Mix (2x)	Thermo Fisher Scientific	13000012	10 μ L per PCR reaction
Reverse Primer	Integrated DNA Technologies (IDT)	51-01-19-07	0.5 μ L per PCR reaction
TBE Buffer (Tris-borate-EDTA)	Thermo Fisher Scientific	B52	1 L of 10x TBE buffer (30 mL of 1x TBE buffer would be needed to make one 30 mL gel)
1 L bottle	Thermo Fisher Scientific	02-893-4E	One needed per stream (the same bottle can be used for multiple streams if it is sterilized between uses)
1.5 mL Microcentrifuge tubes	MilliporeSigma	BR780400-450EA	5 microcentrifuge tubes are needed per DNA extraction and an additional 3 are needed to purify RNA (see ZymoBIOMICS DNA/RNA Miniprep kit protocol)
2% Agarose e-gel	Thermo Fisher Scientific	G401002	Each gel can run 10 samples (so 9 with a PCR negative and 8 if the extraction negative is run on the same gel)
50 mL Conicals	CellTreat	229421	1 50 mL conical needed per sediment samples
500 mL Beaker	MilliporeSigma	Z740580	Only 1 needed (for flame sterilization)
Aluminum foil	Walmart (Reynolds KITCHEN)	No number	Aluminum foil can be folded and autoclaved. The part not exposed to the environment can then be used as a sterile, DNA and RNA free surface for processing filters (one folded piece per filter to avoid cross-contamination)
Autoclave	Gettinge	LSS 130	Only one needed
Centrifuge	MilliporeSigma	EP5404000138-1EA	Only 1 needed
Cooler	ULINE	S-22567	Just about any cooler can be used. This one is listed due to being made of foam, making it lighter and thus easier to take along for field sampling.
Disruptor Genie	Bio-Rad	3591456	Only one needed
Electrophoresis chamber	Bio-Rad	1664000EDU	Only 1 needed
Electrophoresis power supply	Bio-Rad	1645050	Only 1 needed
Freezer (-20 C)	K2 SCIENTIFIC	K204SDF	One needed to store DNA extracts
Freezer (-80 C)	K2 SCIENTIFIC	K205ULT	One needed to store RNA extracts
Gloves	Thermo Fisher Scientific	19-020-352	The catalog number is for Medium gloves.
Heat block	MilliporeSigma	Z741333-1EA	Only one needed
Lab burner	Sterlitech	177200-00	Only one needed
Laminar Flow Hood	AirClean Systems	AC624LFUV	Only 1 needed
Library purification kit	Qiagen	28704	One kit has enough for 50 reactions
Magnet Plate	Alpaqua	A001219	Only one needed
Microcentrifuge	Thermo Fisher Scientific	75004061	Only one needed
Micropipette (1000 μ L volume)	Pipette.com	L-1000	Only 1 needed
Micropipette (2 μ L volume)	Pipette.com	L-2	Only 1 needed
Micropipette (20 μ L volume)	Pipette.com	L-20	Only 1 needed
Micropipette (200 μ L volume)	Pipette.com	L-200R	Only 1 needed
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	New England BioLabs Inc.	E7775S	One kit has enough reagents for 24 samples.

Parafilm	MilliporeSigma	P7793-1EA	2 1" x 1" squares are needed per filter
PCR Tubes	Thermo Fisher Scientific	AM12230	One tube needed per reaction
Pipette tips (for 1000 μ L volume)	Pipette.com	LF-1000	Pack of 576 tips
Pipette tips (for 20 μ L volume)	Pipette.com	LF-20	Pack of 960 tips
Pipette tips (for 200 μ L volume)	Pipette.com	LF-250	Pack of 960 tips
PowerWulf ZXR1+ computer cluster	PSSC Labs	No number	This is just an example of a supercomputer powerful enough to perform metatranscriptomics analysis in a timely manner. Only one needed.
Qubit fluorometer starter kit	Thermo Fisher Scientific	Q33239	Comes with a Qubit 4 fluorometer, enough reagent for 100 DNA assays, and 500 Qubit tubes
Scoopula	Thermo Fisher Scientific	14-357Q	Only one needed
Sterile blades	AD Surgical	A600-P10-0	One needed per filter
Sterivex-GP Pressure Filter Unit	MilliporeSigma	SVGP01050	1 filter needed per water sample
Thermocycler	Bio-Rad	1861096	Only one needed
Vise-grip	Irwin	2078500	Only one needed (for cracking open the filters)
Vortex-Genie 2	MilliporeSigma	Z258415-1EA	Only 1 needed
WHIRL-PAK bags	ULINE	S-22729	1 needed per filter
ZymoBIOMICS DNA/RNA Miniprep kit	Zymo Research	R2002	One kit has enough reagents for 50 samples.