TITLE Quick DNA/RNA Zymo Protocol – Emma Protocol, modified by Kristina

Project No. B10

Book No. 1

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* Based on Kristina Terpis training, who uses a modified Emma Strand protocol (github lab notebook May 31, 2019) based on K. Wong & E. Chille protocols

First time using kit: Add 96 mL to Wash Buffer, reconstitute Pro K & DNAse as directed & store aliquots in freezer (-20 °C)

Preparation: This protocol is for clippings in Zymo DNA/RNA shield (vol ~1 mL). These were stored @ -80 °C. Thaw samples to RT on benchtop.

Clean bench w/ 10% Bleach → DI water → 70% EtOH → RNAse Zap
Clean pipettes, tip boxes, & centrifuge/heat block controls w/ 70% EtOH → RNAse Zap (w/ kimwipe, don't spray)

Notes: This protocol will start with taking 300 µL of the DNA/RNA shield from the sample and performing a Prok digestion.

* If the liquid looks very dark (ex: P. asperoides), you can take 150 µL of the shield and add 150 µL of clean DNA/RNA shield. Proceed as is.

* If you want to add tissue from the clipping into the extraction, example protocol from kristina: (but see github for other bead beating exs)
  - → 100 µL of clean DNA/RNA shield
  - → 300 µL of sample DNA/RNA shield
  - → some tissue + beads
  - ↳ bead beat for 1–2 minutes
  - ↳ transfer 300 µL to a new tube and proceed.

① On a clean work surface, stand up the tubes in numerical order & photograph. (for github post).

② Into clean 1.5mL tubes, transfer 300 µL of the DNA/RNA shield from the sample tube after inverting the tube to mix.
Add: 30 µL Prok digestion buffer
15 µL of Prok (freezer)
Invert 3x + incubate 15 minutes

③ Put samples back in -80 °C freezer (-70°C)

④ Turn on heating block to 70°C and put a 1.5mL tube w/ Tris-EDTA for DNA elution into the heating block to be warmed.

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TITLE Zymo quick DNA/RNA extraction protocol

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(5) Invert / flick tubes & centrifuge @ 9000 rcf for 3 minutes to pellet
(6) Transfer all supernatant to a new 1.5mL tube and add 345 (1:1) µL of DNA/RNA lysis buffer
↳ pipette up and down and add whole volume (700µL max) to labelled yellow DNA Spin-away column in collection tube.
(7) Spin 30s @ 15,000 rcf and SAVE FLOW THROUGH (transfer to a new 1.5mL tube for RNA extraction)
(8) Place column in new collection tube. Add 400µL of prep buffer & spin. Discard FT.
DNA extraction
(9) Add 700µL of ~~DNA~~ wash buffer & spin. (30s, 15000 rcf). Discard flow through.
↳ Repeat this step.
(10) Spin columns (dry) for 2 min @ 15,000 rcf to dry column. LABEL
(11) Discard collection tube & transfer column to final DNA tube [sample #, gDNA, initials, project info, date]
- Add 50µL of warmed Tris directly to column. & incubate for 5 min @ RT.
- Spin @ 15000 rcf for 30 seconds.
- Repeat elution step into same tube.

(12) Aliquot 10µL of DNA into labelled strip tubes & place DNA tubes in -20°C.
↳ keep strip tubes on cold block until QC (same day)

RNA extraction
(13) Add 700µL (1:1 ratio) of 100% Ethanol to the RNA tube (flow through step 7)
(14) Pipette up & down to mix & add 700µL of mixture to labelled green RNA column in collection tube
↳ spin for 30 s @ 15000 rcf & discard flow through
↳ repeat step w/ rest of mixture.
(15) Add 400µL of wash buffer & spin. discard flow through
(16) DNAse digestion:
Make soln: - per sample, mix 5µL DNAse I (-20°C) with 75µL of DNA digestion buffer in a 1.5mL (or larger) tube for all samples. Mix gently (NO VORTEX)
- add 80µL of this mixture directly onto each column.
- incubate @ RT for 15 min

(17) Add 400µL of prep buffer. Spin & discard FT.
(18) Add 700 µL of wash buffer Spin & discard FT.
↳ Repeat this step.
(19) Dry membrane by spinning @ 15000 rcf for 2 min.
(20) Same as for DNA, elute twice w/ 50 µL of DNAse/RNAse free H_2O (not warmed Tris)
- before spinning, incubate ~2 minutes instead of the whole 5 for DNA.

(21) Aliquot 10 µL for QC & put final tubes into -80°C.

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TITLE ENCORE DNA/RNA Extractions

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Qubit Results 10/3/22

	DNA 1	DNA 2	RNA 1	RNA 2
DLAB D1	4.20	4.08	12.4	12.2
DLAB F6	2.20	2.14	nd	nd
MCAV B1	3.70	3.70	14.8	14.8
MCAV C4	6.86	6.78	12.2	12.2
MDEC A7	15.0	14.9	18.4	18.2
MDEC C6	6.24	6.08	16.8	16.6
PAST D7	2.34	2.24	nd	nd
PAST F4	3.26	3.14	14.4	14.4

DNA		RNA	
S1	201.76	S1	415.70
S2	23899.02	S2	9890.95

10/3/22		
DLab D1 ①	DD	
DLab F6 ②	DF	
MCav B1 ③	CB	
MCav C4 ④	CC	
MDec A7 ⑤	MA	
MDec C6 ⑥	MC	
Past D7 ⑦	PD	
Past F4 ⑧	PF	

Day 2		10/5/22
PLAB A4	DA	1
DLab C7	DC	2
MCav E6	CE	3
MCav F7	CF	4
MDec D4	MD	5
MDec E1	ME	6
PAst A1	PA	7
Past B6	PB	8

Extraction Notes: PAST-F4 looks bad @ lysis buffer step. Mucus-y & not wanting to mix w/ the lysis buffer. Filter pigmented throughout RNA extraction & RNA @ end pale brown.

Notes on Qubit & Gels (Kristine training):

- Qubit: for each DNA & RNA, make enough reactions for # samples + 2 stds + 1 = n
  - standards are in basket in fridge, reagent & buffer in drawer HD11 (RT)
  - standard tubes get 190 µL of master mix & sample tubes get 199 µL
    Master mix: (199 x n) µL buffer + (1 x n) µL reagent (make 1 DNA mm & 1 RNA mm)
  - add 10 µL of standards & 1 µL of samples. Vortex, spin, & s.t 2 min.
  - Qubit: read standards once then read all samples 2x.

- Gel: 1.5% thin gel (50 mL TAE (NEW) + 0.75 g agarose). Microwave 45s & cool
  - add 1 µL of gel green. Swirl & pour. pop bubbles & add 2 combs.
  - s.t for 30 mins.

Purple Tri-track (RT, where gel area)

Loading: • add 1 µL of loading dye to remaining DNA/RNA (in 9 µL) in strip tube.
• spin & load whole volume into gel. Load 3 µL of Gene Ruler 1kb ladder (-20°C)

Run: 400 amps, 60 V, 60 minutes. Image using phone & orange filter.

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