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DNA/RNA extraction from fresh-frozen tissue, AllPrep DNA/RNA/miRNA Universal Kit V.3

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for combined RNA and DNA extraction from fresh-frozen tissue using the AllPrep DNA/RNA/miRNA Universal Kit.

Guidelines

DNase I stocks can be used 4 weeks after being thawed but should not be frozen again.
Never thaw tissue when processing.
Always use a fresh scalpel and plate when cutting tissues to prevent cross contamination.

Materials

AllPrep DNA/RNA/miRNA Universal Kit (50) **Qiagen Catalog #**80224
Genomic DNA ScreenTape **Agilent Technologies Catalog #**5067-5365
Qubit™ dsDNA BR Assay Kit **Thermo Fisher Scientific Catalog #**Q32853
Qubit RNA BR Assay Kit **Thermo Fisher Scientific Catalog #**Q10211
RNA ScreenTape and Reagents **Agilent Technologies**
EB buffer **Qiagen Catalog #**19086
ß-ME
EtOH
Isoprop
1.5 and 2 ml LoBind tubes
TissueLyser Beads 5 mm

Protocol materials

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- EB buffer **Qiagen Catalog #**19086
- RNase-Free DNase Set **Qiagen Catalog #**79254

Safety warnings

All steps with ß-ME should be done under the hood.
Reduce use as sharps when working with primary tissue and notify Bettina Ergün in case of an accident.

Ethics statement

Primary patient tissue can only be used after appropriate ethics approval has been obtained. Only use tissue for which an EVE has been signed and which has been approved to be used for the study you are working on.

Before start

Preparations:
FRN buffer: Add 42 ml Isoprop to new bottle
RPE buffer: Add 44 ml EtOH to new bottle
AW1 buffer: Add 25 ml EtOH to new bottle
AW2 buffer: Add 30 ml EtOH
DNAse I stocks: 550 µl RNase-free water to lyophilised DNAse I, aliquot and store at -20°C for 9 months)

Prepare Working Solutions

1 From RNase-Free DNase Set **Qiagen Catalog #**79254

DNAse I working solution: 70 µL RDD + 10 µL DNase I working solution per sample

2 Included within
AllPrep DNA/RNA/miRNA Universal Kit (50) **Qiagen Catalog #**80224

Proteinase K working solution for DNA isolation: 60 µL AW1 + 20 µL Proteinase K per sample

3 Included within
AllPrep DNA/RNA/miRNA Universal Kit (50) **Qiagen Catalog #**80224
Add 10 µL ß-Mercaptoethanol (not included in kit) per 1 mL of RLT plus buffer

Tissue preparation

4 This protocol is for Sample *

4.1 Optional: Weigh tissue before start to decide for volume

4.2 Enter a complete list of samples used for each experiment below:

	SampleID	Type (TURB, T1, T2)	Optional: Weight (mg)	Comment	DNA (ng/ul)

List of tissue sample used in experiment

5 Keep tubes with tissue on dry ice and make sure that they do not thaw during processing.
Transfer the tissue piece into a 6-well plate or small petry dish placed on dry ice and cut an appropriate piece of tissue (ca. 10-30 mg) with a safety scalpel.

6 Transfer tissue in 350 µL for up to 10 mg of tissue
600 µL for 10 to 30 mg or stabilised tissue RLT + ß-ME in 2 ml DNA LoBind tube.

7 Add a 5mm bead to each tube and lyse tissue in TissueLyser for 00:02:00 @ 20 Hz 2m

Equipment

TissueLyser II	NAME
Bead Mill	TYPE
QIAGEN	BRAND
85300	SKU
https://www.qiagen.com/us/products/human-id-and-forensics/automation/tissuelyser-ii/#orderinginformation	LINK

8 Spin down for 00:01:00 @ 9000 x g 1m

9 Turn tube rack and lyse tissue for 00:02:00 @ 20 Hz 2m

10 Spin down for 00:01:00 @ 9000 x g 1m

11 Add lysed product to DNA Mini Spin Column

12 Spin 00:00:30 @ max x g , repeat if any liquid remains on column 30s

13 Transfer column to a new collection tube and store tube at 4 °C until DNA extraction

14 Transfer flow-through to new 2 ml LoBind tube

RNA extraction

15 Add 50 µL for up to 10 mg tissue 80 µL for 10 to 30 mg or stabilised tissue
Proteinase K (not diluted), mix by pipetting

16 Add 200 µL for up to 10 mg tissue 350 µL for 10 to 30 mg or stabilised tissue
EtOH abs., mix by inverting, spin down liquid

17 Incubate 00:10:00 @ Room temperature 10m

18 Add 400 µL for up to 10 mg tissue 750 µL for 10 to 30 mg or stabilised tissue
EtOH abs. , mix by pipetting

19 Add 700 µL to RNeasy spin column and spin for 00:00:30 @ max x g 30s
and discard flow-through

20 Repeat until all liquid passed through the column

21 Add 500 µL RPE

22 Spin 00:00:30 @ max x g and discard flow-through 30s

23 Add 80 µL DNase I working solution directly onto the membrane and incubate 00:15:00 at Room temperature 15m

24 Add 500 µL FRN

25 Spin 00:00:30 @ max x g and ***don't*** discard flow-through and place column in new collection tube 30s

26 Add flow-through again to column

27 Spin 00:00:30 @ max x g and discard flow-through 30s

28 Add 500 µL RPE

29 Spin 00:00:30 @ max x g and discard flow-through 30s

30 Add 500 µL EtOH abs

31 Spin 00:02:00 @ max x g and place column in new collection tube 2m

32 Spin 00:02:00 @ max x g and place column in new 1.5 ml collection tube 2m

33 Add 30 µL of RNase-free H_2O

34 Incubate 00:01:00 @ Room temperature 1m

35 Spin down 00:01:00 @ 8000 x g 1m

36 QC: Qubit Qubit RNA BR Assay Kit **Thermo Fisher Scientific Catalog #Q10211** and tape station RNA ScreenTape and Reagents **Agilent Technologies**

36.1

Note

Upload results from Tapestation here

DNA extraction 11m

37 350 µL AW1 auf DNA Mini Spin Column geben

38 Spin 00:00:30 @ max x g and discard flow-through 30s

39 80 µL Proteinase K working solution directly onto membrane

40 Incubate 00:05:00 @ Room temperature 5m

41 Add 350 µL AW1

42 Spin 00:00:30 @ max x g and discard flow-through 30s

43 Add 350 µL AW2

44 Spin 00:02:00 @ max x g and place column in new 2 ml collection tube 2m

45 Spin 00:01:00 @ max x g and place column in new 1.5 ml collection tube 1m

46 Add 50 µL EB buffer **Qiagen Catalog #**19086 directly onto membrane

47 Incubate 00:01:00 @ Room temperature 1m

48 Spin down 00:01:00 @ 8000 x g 1m

49 QC: Qubit DNA
Qubit™ dsDNA BR Assay Kit **Thermo Fisher Scientific Catalog #**Q32853 and tape station Genomic DNA ScreenTape **Agilent Technologies Catalog #**5067-5365

49.1

Note

Upload Tapestation results as pdf here.