Dec 13, 2024

JAX-Sen: Mouse Heart and Kidney dissociation for bulk RNA sequencing

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl8d24rg2w/v1

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Cellular Senescence Net...

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Protocol Citation: Patrick Fleming, Jessica Garofalo, Ramalakshmi Ramasamy, Juliana Alcoforado Diniz, Paul Robson 2024. JAX-Sen: Mouse Heart and Kidney dissociation for bulk RNA sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8d24rg2w/v1

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

Created: December 13, 2024

Last Modified: December 13, 2024

Protocol Integer ID: 115152

Keywords: JAX-Sen, kidney dissociation for bulk rna, bulk rna, rna, sequencing, senescence, dissociation of the heart, kidney dissociation, mouse heart, sen project in the sennet consortium, sennet consortium

Funders Acknowledgements:
National Institute on Aging (NIA) JAX-Sen Senescence Tissue Mapping Center Grant
Grant ID: U54 AG079753

Abstract

These samples are part of the JAX-Sen project in the SENNET Consortium. We aim to study and characterize senescence in the C57Bl/6 mouse heart and kidney. This protocol describes the dissociation of the heart and kidney before library preparation and sequencing for Bulk RNA-seq.

Reagents and Materials

1
- Beadbug™ 6 Homogenizer (recommended)
- BeadBug™ prefilled tubes, 2.0 mL capacity (Sigma Aldrich, Z763772-50EA) (2 tubes per sample)
- RNeasy Plus Mini Kit (Qiagen, 74134) (50 reactions)
- Qiashredder tubes (Qiagen, 79654) (50 tubes total)
- 2-mercaptoethanol (Gibco, 21985023)
- 2 mL tubes (1 tube per sample)
- 6cm dishes
- Scalpel/tweezers

Procedure

2 Thaw fresh frozen sample on ice for 30 minutes.

2.1 Prepare two Beadbug tubes per sample by adding 600 uL RLT buffer (supplied) to each tube. Ensure that 2-mercaptoethanol has been added to RLT buffer before use as per RNeasy Plus Mini Kit instructions.

2.2 On a dish, mince individual organs to ~2 mm pieces using a scalpel

2.3 Add half of the minced organ to each prepared beadbug tube

2.4 Using the Beadbug tissue homogenizer, run the sample through two cycles of 90 seconds set at 3000 speed

2.5 Remove the lysate from the beadbug tube and collect in a 2 mL tube. Avoid pieces of tissue. Lysate will likely contain many bubbles, collect as much sample as possible. Briefly spinning the beadbug tube using a minifuge may aid in lysate collection

2.6 Add 600 uL RLT buffer with 2-mercaptoethanol to the beadbug tube containing the residual of each sample.

2.7 Using the Beadbug tissue homogenizer, run the sample through two cycles of 90 seconds set at 3000 speed.

2.8 Remove the residual lysate from the beadbug tube and combine the lysate in the same 2 mL tube as initial tissue lysate. Avoid pieces of tissue. Lysate will likely contain many bubbles, collect as much sample as possible. Briefly spinning the beadbug tube using a minifuge may aid in lysate collection.

2.9 At this step in the protocol, whole samples (~1200 uL) have been pooled together in a single 2 mL tube.

2.10 For kidney, freese a half volume (~600 uL) of tissue lysate at -80°C. For heart, freese three quarters a volume (~900 uL) of tissue lysate at -80°C.

2.11 Begin to follow the RNeasy Plus Mini Kit Protocol as directed by manufacturer. For kidney, proceed with ~600 uL (about half) of sample lysate. For heart, proceed with ~300 uL (about a quarter) of sample lysate.

2.12 Add lysate to a Qiashredder tube.

2.13 Centrifuge 30 seconds x 8000g.

2.14 Transfer flowthrough to a gDNA binding column.

2.15 Centrifuge 30 seconds x 8000g.

2.16 Discard gDNA column, save flowthrough.

2.17 Add 600 uL of 70% ethanol to the sample and mix by pipetting.

2.18 Transfer no more than 700 uL of sample to a RNeasy spin column (supplied).

2.19 Centrifuge 30 seconds x 8000g.

2.20 Repeat steps 19-20 until all sample volume has passed through the RNeasy spin column.

2.21 Add 600 uL buffer RW1 (supplied) to RNeasy spin column. Ensure to add pure ethanol to buffer RW1 as directed in manufacturer instructions.

2.22 Centrifuge 30 seconds x 8000g, discard flowthrough.

2.23 Add 500 uL buffer RPE (supplied) to RNeasy spin column.

2.24 Centrifuge 30 seconds x 8000g, discard flowthrough.

2.25 Add 500 uL buffer RPE to RNeasy spin column.

2.26 Centrifuge 2 minutes x 8000g, discard flowthrough.

2.27 Transfer RNeasy spin column to a new 2 mL tube (supplied).

2.28 Centrifuge 1 minute at full speed.

2.29 Transfer spin column to a final 1.5 mL collection tube (supplied).

2.30 Add 30 uL of RNase-free water (elution buffer, supplied).

2.31 Centrifuge 1 minute at 8000g.

2.32 Measure RNA concentration and record.