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CycloneSEQ long-read RNA-seq Library Preparation and Sequencing Protocol

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

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Abstract

This protocol is designed for full-length transcriptome library preparation and sequencing of extracted animal RNA on the CycloneSEQ platform.

Genomic DNA Removal

1 Treat 100–500 ng total RNA with 5× gDNA Digester Mix in a nuclease-free tube.
- Incubate at 42°C for 2 min.
- Centrifuge briefly to collect liquid.

First-Strand cDNA Synthesis

2 Prepare 20 µL reaction mix containing:
- 4× Hifair SuperMix
- Random Primers (50 µM)
- Treated RNA template

Incubate at 72°C for 5 min.
- Heat-inactivate at 85°C for 5 sec.

Note: For high-GC RNA templates, increase incubation temperature to 60°C.

CycloneSEQ Library Preparation

3 Process cDNA into sequencing libraries using the CycloneSEQ Universal Library Prep Set:
- DNA Repair (end-repair/dA-tailing)
- Adapter Ligation (CycloneSEQ-specific adapters)

Follow the standard whole-genome sequencing workflow steps provided in the kit.

Library Quality Control

4 Quantify libraries using a Qubit fluorometer.

Assess fragment size distribution (e.g., using Agilent TapeStation).

Sequencing

5 Load libraries onto the CycloneSEQ WuTong02 platform.

Run sequencing using manufacturer-recommended parameters (e.g., 48-hr run, >Q20 accuracy).