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# FLAM-seq (with Kapa mRNA enrichment)\_CMS\_edit-2024-09-25

Forked from a private protocol

DOI

### dx.doi.org/10.17504/protocols.io.j8nlk8q46l5r/v1

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Protocol status: In development We are still developing and optimizing this protocol

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#### **Abstract**

fork\_\_CMS\_edit-2024-09-25



#### **Materials**

## Kapa mRNA HyperPre Kit Illumina (cat. KK8440, Roche; Replaces TruSeq mRNA)

- mRNA Capture Beads
- mRNA Bead Binding Buffer (BBB)
- mRNA Bead Wash Buffer(BWB)

### Poly(A) Tail Length Assay Kit (cat. 764551KT, Thermo Fisher)

- 5X tail buffer mix
- 10X tail enzyme mix
- Universal Reverse (Univ. RV Primer; used in Sect 4: cDNA Amplification)

## SMARTer PCR cDNA cDNA Synthesis Kit (cat. 634926, Takara)

- 5X First strand buffer
- DTT 20mM
- dNTP mix 10 mM
- RNase Inhibitor
- SMARTScribe RT
- 5' PCR Primer II A (used in Sect 4: cDNA Amplification)

## Advantage 2 DNA polymerase mix (cat 639207, Takara)

- 10X Advantage 2SA PCR buffer
- dNTP Mix (10 mM each)

# RNAClean XP Beads (cat. A63987, Beckman Coulter)

XP DNA beads (cat. A63881, Beckman Coulter)

#### **Custom Oligonucleotides**

isoTSO - Template switch oligo (where "i" indicates stereoisomers of dC and dG as in the associated publication):

*iCiGiCAAGCAGTGGTATCAACGCAGAGTACATrGrGrG* 

#### RT primer 1:

GGTAATACGACTCACTATAGCGAGANNNNNNNNNNNCCCCCCCCCTTT

PCR primer 1 (to use in combination with RT primer 1; not used; replaced by 5' PCR Primer II A):

GGTAATACGACTCACTATAGCGAG

### RT primer 2 (to use in alternative to 1):

TGAGTCGGCAGAGAACTGGCGAANNNNNNNNNNNCCCCCCCCCTTT,

PCR primer 2 (to use in combination with RT primer 2; not used; replaced by 5' PCR Primer II A):

TGAGTCGGCAGAGAACTGGCGAA



## Poly(A)+ RNA preparation (using Kapa mRNA Beads) 10m 10s Prepare mRNA Beads 1.1 52.5 μL mRNA Beads can be scaled for multiple samples **45** 500 rpm, 00:01:00 1.2 Room temperature (\*) 00:05:00 magnet 5m Discard supernatant 1.3 Remove from magnet **₹**500 rpm, 00:01:00 1.4 Room temperature (\*) 00:05:00 magnet Discard supernatant 1.5 Remove from magnet Δ 52.5 μL BBB **45** 500 rpm, 00:01:00 2 & ds DNA in 0.2 mL PCR tube Δ 50 μL QS; NAF 3 Δ 50 μL re-suspended mRNA Beads (from 1.5) **(**5 1000 rpm, 20°C, 00:01:00 4 **₽** 65 °C 00:02:00 7m 10s 00:05:00 ₽ 20 °C undetermined, 00:00:10, pulse 5 Room temperature (\*) 00:05:00 magnet 5m Discard supernatant







17 Δ 16 μL 🔊 ds DNA to new 0.2 mL PCR tube

## GI Tailing (using USB poly(A) tail length assay, Thermo Fisher).

- 18 Prepare on ice (20 uL total):
  - Δ 14 μL RNA poly(A)+
  - 4 µL 5X tail buffer mix
  - Δ 2 μL 10X tail enzyme mix
- 19 **3**7 °C 01:00:00

20 Δ 1.5 μL Stop Solution

- \$ 4 °C (2) 00:02:00
- 21 Δ 38.7 μL XP RNA Beads (1.8X)
- Room temperature 00:05:00
- 22 Room temperature 00:03:00 magnet

Discard supernatant

23 Δ 50 μL 80% EtOH 🕚 00:00:30

Discard supernatant

- 24 <u>so to step #23</u> x1

Discard supernatant

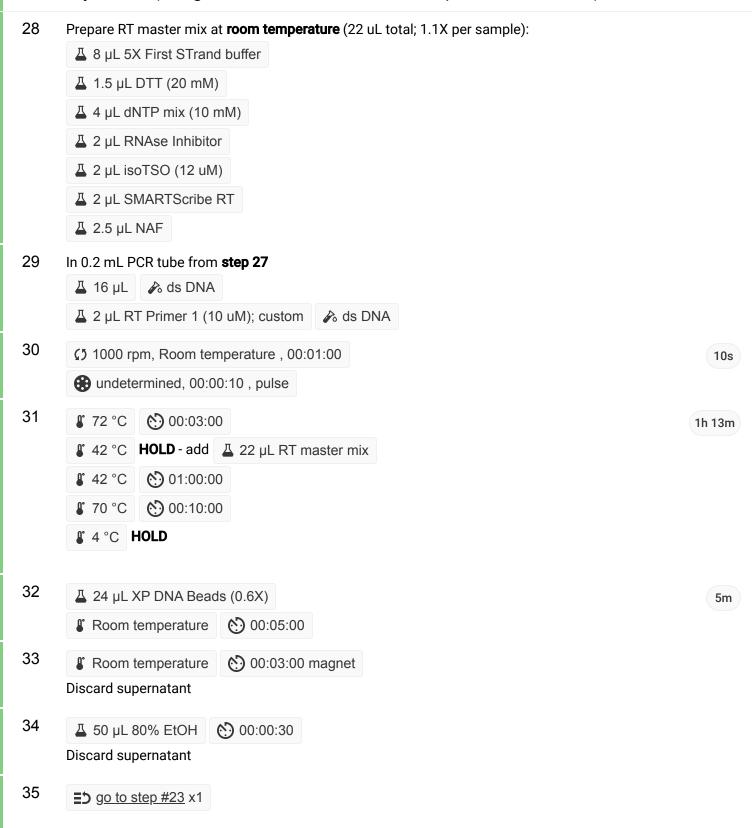
- 26 Remove from magnet
  - 4 17 µL NAF

    A 17 µL NAF
  - **(**5 1000 rpm, 20°C, 00:01:00
- 27 Δ 16 μL 🔊 ds DNA to new 0.2 mL PCR tube

1h



## cDNA synthesis (using SMARTscribe reverse transcriptase kit, Clontech).





36 undetermined, 00:00:10 , pulse Discard supernatant 37 Remove from magnet

42 μL NAF **(**5 1000 rpm, 20°C, 00:01:00

38 & ds DNA to new 0.2 mL PCR tube 40 µL

# cDNA library amplification (using Advantage 2 PCR enzyme system, Clontech).

11m 25s

39 Prepare the cDNA library amplification rxn:

Δ 10 μL 10X Advantage 2SA PCR buffer

Δ 2 μL dNTP (10 mM)

Δ 2 μL 5' PCR Primer II A (SMARTer PCR kit)

Δ 2 μL Univ. RV Primer (Poly(A) Tail Kit)

42 μL NAF

Δ 2 μL Advantage 2 Polymerase Mix

40 Perform PCR

11m 25s

1. **§** 98 °C **HOLD** - add cDNA rxn

2. **\$** 98 °C **(**) 00:01:00

3. **\$** 98 °C **6** 00:00:10

4. **\$** 63 °C **6** 00:00:15

5. \$\cdot 68 \cdot \cdot \cdot \cdot 00:03:00 \text{ repeat 40.3 -40.5 24X}

6. **\$** 68 °C **(S)** 00:07:00

7. **4** °C **HOLD** 

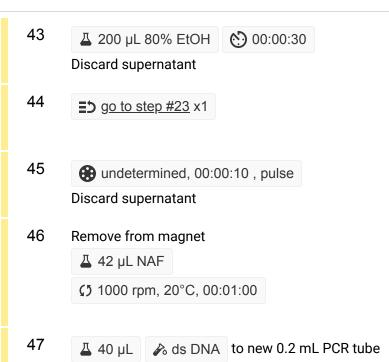
41 Δ 60 μL XP DNA Beads (0.6X)

> Room temperature 00:05:00

42 Room temperature (\*) 00:03:00 magnet

Discard supernatant





# Sequencing library preparation (using the SMRTbell™ Template Prep Kit)

Performed in PacBio sequencing core 48