AFT™ Linked-Reads Single-Cell Whole-Genome Library Preparation Kit - 24 rxn

Protocol

PN: 6729002

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.
Information in this document is subject to change without notice.

By FortiusSeq
https://www.fortiusseq.com
info@fortiusseq.com

Introduction

AFT™ Linked-Reads Single-Cell Whole-Genome Library preparation technology utilizes multiple strand-displacement amplification by the strand-displacing enzymes to seamlessly integrate the Amplification, Fragmentation, and Tagging of the single cell DNA into one reaction, achieves ultimate efficiency in single cell DNA sequencing Library preparation.

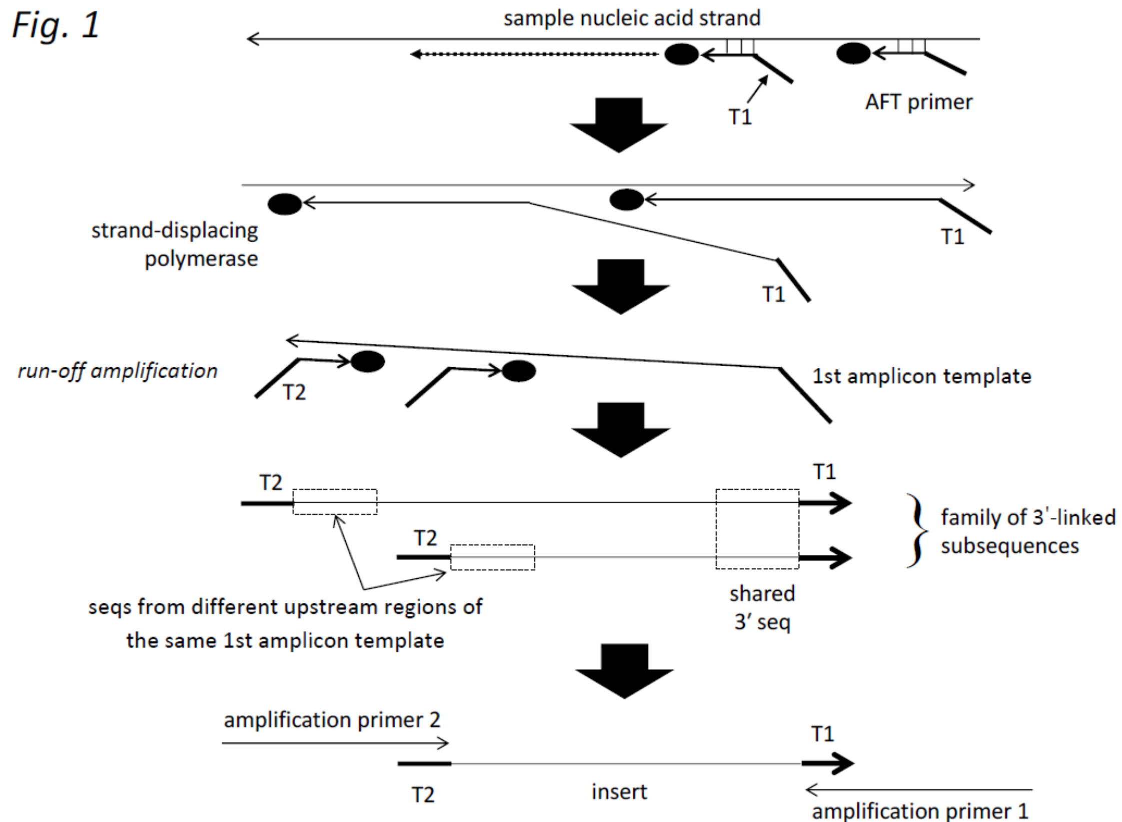
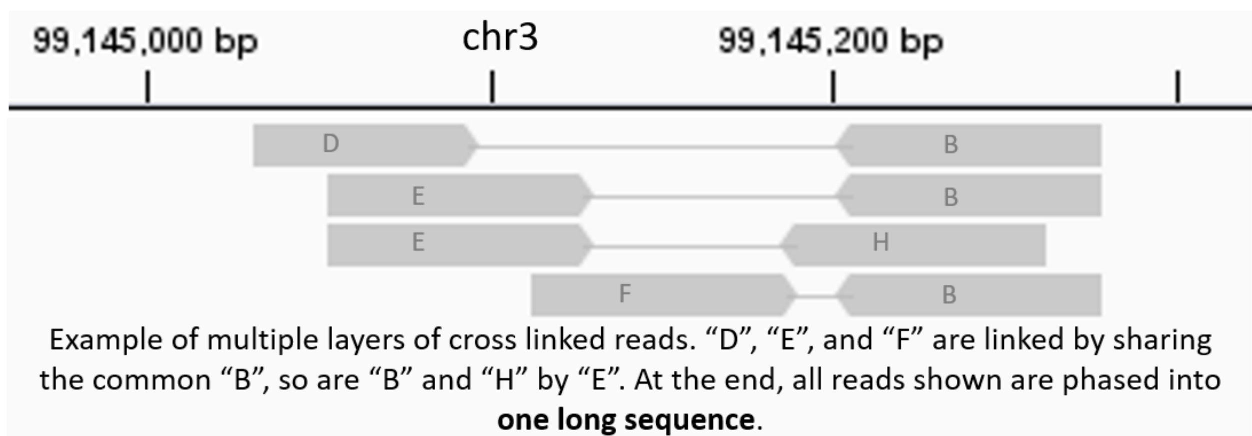


FIG. AFT™ primers are degenerate primers with 5' tag of a fixed sequence. AFT™ primers randomly bind to multiple sites of the target DNA. Strand-displacing DNA polymerase initiates the amplification from AFT™ primers; the front amplicons are displaced by the running-up amplicons and in turn become template for next iteration of AFT™ amplification. The new amplicons all run off at the 5' terminus of the template amplicon and share the same sequence at 3'. In the pair-end sequencing, the common read serves as a native barcode to link all these that amplify from the same template amplicon.

Introduction (Continued)

As a unique feature, groups of AFT™ reads are linked by one common pair-end read that arise from the running off at the 5' terminus of the same template. The linked reads facilitate the long scaffolding, de novo assembly, and chromosome phasing.



Example of multiple layers of cross linked reads. "D", "E", and "F" are linked by sharing the common "B", so are "B" and "H" by "E". At the end, all reads shown are phased into **one long sequence**.

Currently, no bioinformatics software package is provided to utilize the linked-reads feature.

Kit contents and storage

The kit is shipped on gel ice for within US and dry ice for international delivery.

The kit should be stored at -20 °C on receipt.

Component	Volume (µL)
Lysis Solution	84
Stop Solution	12
DNA Denaturant	12
2x AFT™ buffer	120
AFT™ enzymes	12
2x PCR Master Mix	600
i501 primer, 10 µM	60
i701 primer, 10 µM	60

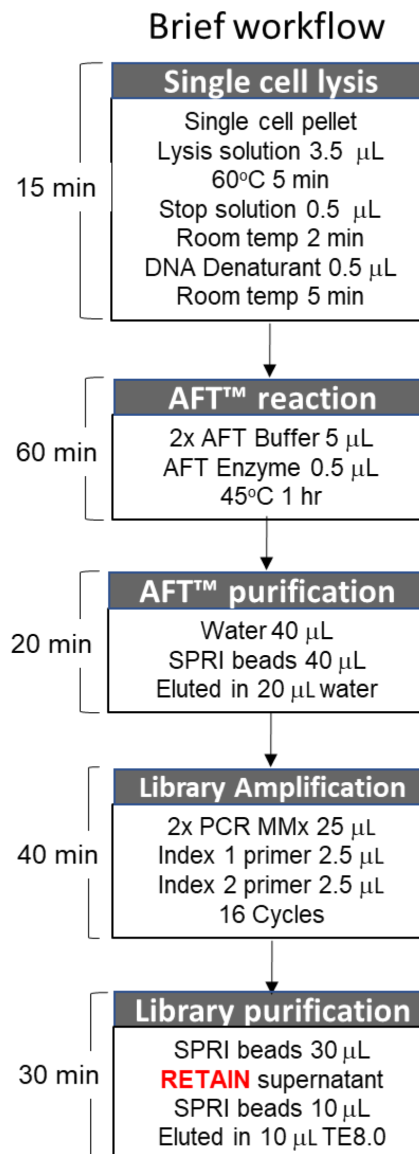
Materials and equipment required but not supplied

Item
SPRI beads
Ethanol
Multiplexing index primers, if multiplexing
Magnetic stand
1x TE Buffer, pH 8.0
Nuclease-free water
Thermal cycler
Microcentrifuge
Microcentrifuge tubes
Pipettors
Pipette tips, nuclease-free

Workflow_Overview

The AFT™ Linked-Reads Single-Cell Whole-Genome Library preparation kit workflow comprises of 3 functional steps: Single cell lysis, AFT™ reaction, and Library PCR Amplification, there is also a SPRI beads-based purification step before and after Library PCR.

The total time is less than 3 hours, with limited hands-on time.



Workflow_AFT™ reaction

A. Amplification, Fragmentation, and Tagging of Genomic DNA

I. Preparation

- Thaw Lysis Solution
- Thaw Stop Solution
- Thaw DNA Denaturant
- Bring the 2X AFT™ Buffer to room temperature and vortex briefly.

II. Procedure

1. Add 3.5 µL of Lysis Solution to the tube with the cell pellet.
2. Briefly vortex and centrifugate to mix.
3. Use PCR machine with heated lid to heat the tube at 60 ° C for 5 min.
4. Add 0.5 µL of Stop Solution.
5. Briefly vortex and centrifugate to mix, sit at room temp for 2 min.
6. Add 0.5 µL of DNA Denaturant.
7. Briefly vortex and centrifugate to mix, sit at room temp for 5 min.
8. Add 5 µL of 2x AFT™ buffer.
    - 2x AFT™ buffer is viscous, allow at least 5 seconds for each aspiration and dispense.
    - Add 2x AFT™ buffer individually, do not make it into master mix
9. Add 0.5 µL of AFT™ enzyme.
10. Briefly vortex and centrifugate to mix.
    - Do not pipette, pipetting produces bubbles.
11. Incubate at 45 ° C for 1 hour.

By FortiusSeq
https://www.fortiusseq.com
info@fortiusseq.com

Workflow_AFT™ Product Purification

B. AFT™ Product Purification

I. Preparation

- Bring SPRI beads to room temp.
- Vortex to resuspend SPRI beads.
- Prepare fresh 80% ethanol.

II. Procedure

1. Add 40 µL of nuclease-free water.
2. Add 40 µL of SPRI beads.
3. Briefly vortex and centrifugate to mix.
4. Sit for 5 min.
5. Pellet on a magnet stand for 5 min.
6. Discard the supernatant.
7. Keep the tube on the magnet stand.
8. Wash 2 times with 180 µL 80% ethanol, completely remove ethanol.
9. Keep the tube on the magnet stand, dry the beads for 5 min.
10. Take the tube away from the magnet stand.
11. Resuspend beads in 23 µL of nuclease-free water with pipetting.
12. Sit for 3 minutes for elution.
13. Place the tube on a magnet stand for 3 minutes.
14. Recover 20 µL of supernatant for Library PCR.
    - We recommend to use ring magnetic stand, for the better and clear recovery of supernatant.

Workflow_Library PCR

C. Library PCR

I. Preparation

- Thaw 2x PCR Master Mix and i501 and i701 primers.
- (Optional) If multiplexing, pick and record the index primers from index primer kits (third-party vendor, NEB E7600S, etc.). Be careful in selecting third-party index primers; some index primers are based on Nextera transposase recognition sites, not the standard Illumina Read1 and Read2 primer sequences. The correct index primer structures should be like below:
  - P5(AATGATACGGCGACCACCGAGATCTACAC)-i5-Read1(ACACTCTTTCCCTACACGACGCTCTTCCGATCT)
  - P7(CAAGCAGAAGACGGCATACGAGAT)-i7-Read2(GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT)
- Set up thermal cycler as following

  98 ° C 30s
  [98° C 10s, 55° C 30s, 72° C 30s]x 16
  72 ° C 3 min
  4 ° C on hld

II. Procedure

1. Add 20 µL of purified AFT™ product.
2. Add 25 µL of 2x PCR Master Mix.
3. Add 2.5 µL of 10 µM i5 index primer.
4. Add 2.5 µL of 10 µM i7 index primer.
5. Briefly vortex and centrifugate to mix.
6. Place on thermal cycler and run the program.

Workflow Library Purification

D. Library Purification

I. Preparation

- Vortex to resuspend SPRI beads.

II. Procedure

1. Add 30 µL of SPRI beads.
2. Vortex briefly to mix.
3. Sit for 5 min.
4. Pellet on a magnet stand for 5 min.
5. RETAIN and transfer 70 µL of the supernatant to a new tube.
   - It's more than rare that the supernatant from this step is inadvertently discarded, we found it helps to dispense the 10 µL of SPRI beads that's going to be used in the next step in a tube ahead of time, and transfer the retained supernatant into it.
6. Add 10 µL of SPRI beads.
7. Briefly vortex and centrifugate to mix.
8. Sit for 5 min.
9. Pellet on a magnet stand for 5 min.
10. Keep the tube on the magnet stand.
11. Wash 2 times with 180 µL 80% ethanol, completely remove ethanol.
12. Keep the tube on the magnet stand, dry the beads for 5 min.
13. Take the tube away from the magnet stand.
14. Resuspend beads in 13 µL of TE pH8.0 with pipetting.
15. Sit for 3 minutes for elution.
16. Place the tube on a magnet stand for 3 minutes.
17. Recover 10 µL of supernatant for quantification and sequencing.

Workflow_Library Quantification

E. **Library Quantification**

Quantify using Agilent BioAnalyzer or TapeStation, set region at 250 – 700 bp.

Troubleshooting

No library

1. If the single cell is aliquoted from bulk cell suspension, by Poisson distribution, there is more than half of the chance that no cell will be aliquoted at all with the calculated volume. To guarantee the harvest of a single cell, make at least three aliquotes and make libraries for them at the same time.
2. If the single cell is collected with cell sorting, the sorted cell might not be captured in the tube. To guarantee to get a library, make at least three attempts of collecting the cell and make libraries from each of the collections.
3. The cell DNA might be degraded and reduced to less than 1 kb in length; AFT ™ technology prefers to work on DNA longer than 1 kb. Try conduct the cell isolation procedure at low temperature and proceed immediately after the collection.
4. Excessive pipetting and vortexing during the workflow might reduce the target DNA to shorter than 1 kb, avoid excessive pipetting or vortexing.
5. Stop solution does not get into the cell lysates, make sure dip the pipette tip into the lysate to dispense the 0.5 μL of the stop solution.
6. When recovering the elution from SPRI beads, leave at least 3 μL behind, don't try to recover all.
7. The third-party index primers are not compatible; some index primers are based on Nextera transposase recognition sites, not the standard Illumina Read1 and Read2 sequences. The correct index primer structures should be like below:
    - P5(AATGATACGGCGACCACCGAGATCTACAC)-i5-Read1(ACACTCTTTCCCTACACGACGCTCTTCCGATCT)
    - P7(CAAGCAGAAGACGGCATACGAGAT)-i7-Read2(GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT)

By FortiusSeq
https://www.fortiusseq.com
info@fortiusseq.com