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Arraystar G4 CUT&Tag Library Prep Kit

Cat#: AS-TN-006

Instruction Manual version 1.3

For research use only. Not for diagnostic procedures.

Background

G-quadruplexes (G4s) are four-stranded intramolecular structures that arise from the self-stacking of two or more guanine quartets (G-quartets). G4s are distributed in specific genomic regions and implicated in several essential cellular processes. This kit uses G4 CUT&Tag (Cleavage Under Targets and Tagmentation) to profile native DNA G4s at high resolution, specificity, and sensitivity.

Components

Component	Volume	Storage	
Wash Buffer	1.5 mL	4°C	Box 1
Antibody Buffer	1.5 mL		
Dig-150 Buffer (10x)	2.6 mL		
Dig-300 Buffer (10x)	1.5 mL		
Tagmentation Buffer	20 uL		
Stop Buffer	80 uL		
Neutralize Buffer	20 uL		
60 mM Spermidine	400 uL		
1% NP40	500 uL		
DNA Beads	2 mL		
Antibody 1	75 uL	-80°C	Box 2
Assembled pA-Tn5 Transposome	25 uL	-20°C	
Antibody 2	14 uL		
Antibody 3	14 uL		
P5/P7 PCR Primers (20 uM)	7 x 12 uL		
PCR Mix (2x)	280 uL		
1% Digitonin	2 mL		

Note: *Store Antibody 1 separately at -80°C upon receipt. Avoid freeze-thaw cycles for 1% Digitonin.*

Additional Materials

- Nuclease-free ddH_2O
- 80% ethanol
- BSA dry power
- Vortexer
- Magnetic plate rack
- Centrifuge
- Orbital shaker
- Water bath or incubator
- Thermal cycler
- Analytical balance

Important Notes

• This kit is suitable for cell samples. The recommended cell viability is > 85%.

• To avoid cell loss during wash processes, leave 3~5 uL liquid when removing the supernatant. A horizontal centrifuge is easier for cell collection.

• For all steps involving cell resuspension, it is recommended to gently pipette up and down to mix the cells slowly, avoiding vigorous agitation.

G4 CUT&Tag Protocol

The entire protocol takes three days to complete.

DAY 1

Prepare fresh buffers according to the tables below. Keep the buffers on ice.

10% BSA	
Component	**Amount per sample**
BSA dry power	50 mg
Nuclease-free ddH_2O	500 uL

Note: *Store the freshly prepared BSA at 4°C. It will be used for buffer prep on Day 1 and Day 2.*

Complete Wash Buffer	
Component	**Amount per sample**
Wash Buffer	100 uL
60 mM Spermidine	0.8 uL

Complete Antibody Buffer	
Component	**Amount per sample**
Antibody Buffer	83 uL
1% Digitonin	5 uL
1 % NP40	1 uL
60 mM Spermidine	0.8 uL
10% BSA	10 uL

1. Incubate Antibody 1

1.1. Count the cells and check the cell viability (> 85%) by Trypan Blue staining. Aliquot 200,000 cells to a 1.5 mL tube per sample.

1.2. Centrifuge the cells at 600 x *g* for 3 minutes at 4°C. Remove and discard the supernatant.

1.3. Resuspend the cells with 100 uL Complete Wash Buffer. Centrifuge cells at 600 x *g* for 3 minutes at 4°C. Remove and discard the supernatant.

1.4. Resuspend the cells with 94 uL of Complete Antibody Buffer and 6 uL of Antibody 1. Incubate the tube on an orbital shaker at 20 RPM overnight at 4°C.

DAY 2

Prepare fresh buffers according to the tables below. Keep the buffers on ice.

Complete Dig-150 Buffer	
Component	**Amount per sample**
Dig-150 Buffer (10x)	210 uL
1% Digitonin	105 uL
60 mM Spermidine	17.5 uL
10% BSA (from Day 1)	210 uL
1 % NP40	21 uL
Nuclease-free ddH_2O	1537 uL

Complete Dig-300 Buffer	
Component	**Amount per sample**
Dig-300 Buffer (10x)	110 uL
1% Digitonin	55 uL
60 mM Spermidine	9.2 uL
1 % NP40	11 uL
10% BSA (from Day 1)	110 uL
Nuclease-free ddH_2O	805 uL

2. Incubate Antibody 2

2.1. Centrifuge cells at 600 x *g* for 5 minutes at 4°C. Remove and discard the supernatant. Avoid disturbing the cells.

2.2. Resuspend the cells with 200 uL Complete Dig-150 Buffer by gentle pipetting. Incubate at room temperature for 10 minutes.

Note: *Gently mix the cells once with a pipette at 5 minute time point.*

2.3. Repeat the wash steps 2.1 - 2.2 twice (three washes in total).

2.4. Resuspend the cells with 99 uL Complete Dig-150 Buffer and 1 uL Antibody 2 by gentle pipetting. Transfer to a new 1.5 mL tube. Incubate the tube on an orbital shaker at 20 RPM for 1 hour at room temperature.

3. Incubate Antibody 3

3.1. Repeat wash steps 2.1 - 2.2 three times in total.

3.2. Resuspend the cells with 99 uL Complete Dig-150 Buffer and 1 uL Antibody 3 by gentle pipetting. Transfer to a new 1.5 mL tube. Incubate the tube on an orbital shaker at 20 RPM for 1 hour at room temperature.

4. Incubate pA-Tn5 transposome

4.1. Repeat wash steps 2.1 - 2.2 three times in total.

4.2. Resuspend the cells with 98 uL Complete Dig-300 Buffer and 2 uL Assembled pA-Tn5 Transposome by gentle pipetting. Transfer to a new 1.5 mL tube. Incubate the tube on an orbital shaker at 20 RPM for 1 hour at room temperature .

5. Tagmentation of target DNA

5.1. Centrifuge the cells at 600 x *g* for 5 minutes at 4°C. Remove and discard the supernatant. Avoid disturbing the cells.

5.2. Resuspend the cells with 300 uL Complete Dig-300 Buffer by gentle pipetting. Incubate at room temperature for 10 minutes.

Note: *Gently mix the cells once with a pipette at 5 minute time point.*

5.3. Repeat wash step 5.1 - 5.2 twice (three washes in total).

5.4. Resuspend the cells with 49.5 uL Complete Dig-300 Buffer and 0.5 uL Tagmentation Buffer by gentle pipetting. Transfer to a new 1.5 mL tube. Incubate at 37°C for 1 hour.

Note: *Gently mix the cells once with a pipette at 30 minute time point.*

6. Release of DNA

6.1. Add 6 uL Stop Buffer and 1 uL of Neutralize Buffer by gentle pipetting. Incubate at 55°C for 1 hour.

Note: *Mix Stop Buffer just before using. Gently mix the cells with a pipette at 20 and 40 minute time points.*

6.2. Add 110 uL DNA Beads and vortex to mix. Incubate at room temperature for 10 minutes.

6.3. Separate the beads by placing the tube on a magnetic rack for 4 minutes. Remove and discard the supernatant. Wash the beads twice with 200 uL 80% ethanol while keeping the tube on magnetic rack without disturbing the beads. Air dry at 37°C for 3 min to completely remove the residual ethanol.

6.4. Resuspend the beads with 20 uL Nuclease-free ddH_2O and transfer the bead suspension to a new PCR tube.

7. Library amplification

7.1. Add 1 uL P5 PCR Primer (20 uM), 1 uL P7 PCR Primer (20 uM), and 22 uL PCR Mix (2x) to the PCR tube by gentle pipetting.

7.2. PCR Amplification using the following PCR program:

Cycle number	Temperature	Time
1X (initial heating)	72°C	5 minutes
1X (denaturation)	98°C	30 seconds
14 ~ 17X	98°C	10 seconds
	60°C	30 seconds
	72°C	60 seconds
1X	72°C	5 minutes
1X	4°C	Hold

DAY 3

8. Enrichment of library fragments

8.1. Resuspend and transfer the beads to a new 1.5 mL tube. Add an extra 27 uL fresh DNA Beads to PCR product. Mix thoroughly and incubate at room temperature for 10 minutes.

8.2. Place the tube on a magnetic rack for 4 minutes. Transfer the supernatant to a new 1.5 mL tube.

8.3. Add 17 uL fresh DNA Beads to the isolated supernatant. Mix and incubate at room temperature for 10 minutes.

8.4. Place the tube on a magnetic rack for 4 minutes. Remove and discard the supernatant. Wash the beads twice with 200 uL 80% ethanol while keeping the beads on magnetic without disturbing the beads.

8.5. Allow the beads to air dry completely. Add 10-20 uL Nuclease-free ddH_2O to resuspend the beads and elute the DNA.

8.6. Use the magnetic rack to separate the beads. Transfer the supernatant (library) to a fresh tube.

9. Library quantification

9.1. Take 1 uL library for DNA concentration measurement with a Qubit™ Fluorometer.

- If the total DNA amount is ≥ 20 ng, the library is ready for sequencing.
- If the total DNA amount is < 20 ng, PCR amplify the library with 2-4 more cycles in step 7.

Kit performance

A G4 CUT&Tag Library for 200,000 Hela cells was prepared with this kit according to the kit protocol. The library was sequenced on an Illumina Sequencer. The typical distribution of G4 peaks in the genome is displayed in Fig. 1. G4 peaks in CUT&Tag-seq and ChIP-seq correlate well as shown in Fig.2.

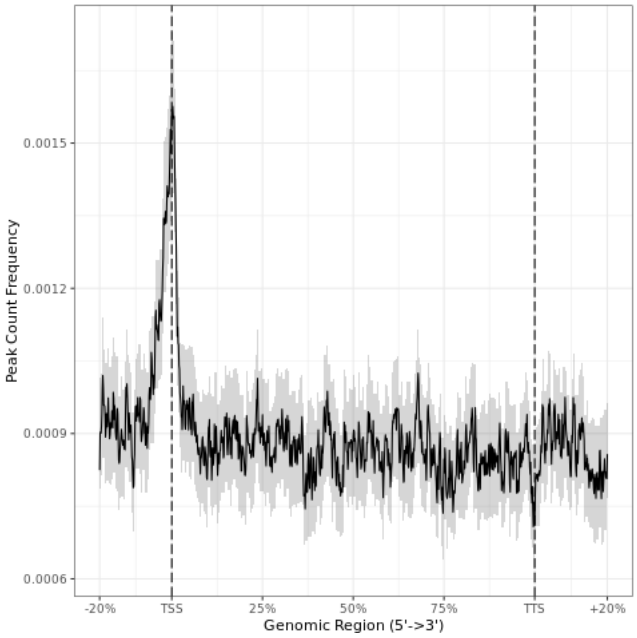

Fig. 1. The distribution of G4 peaks in the genome, showing characteristic enrichment at promoter.

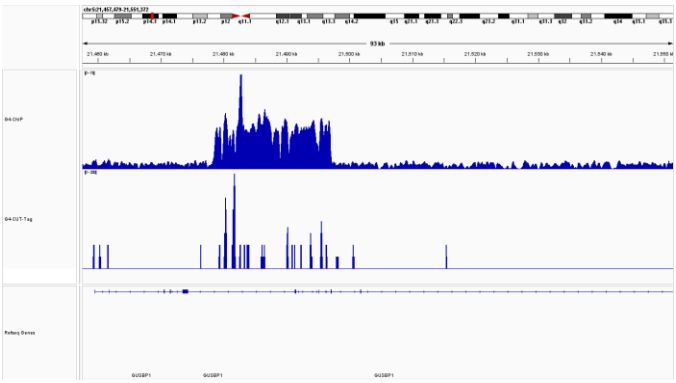

Fig. 2. The IGV view of G4 ChIP (upper) and G4 CUT&Tag (lower) tracks

Troubleshooting

Problems	Possible reasons	Solutions
Low signal-to-background ratios in low-depth sequencing	Often caused by unhealthy cells or other non-ideal input material.	1. Pre-treat cells with DNase or sort viable cells. 2. Deeper sequencing.
Low library amplification	1. Special sequence or structure difficult for PCR. 2. Residual ethanol in Step 6.3 that inhibited PCR.	1. Increase PCR cycles. 2. Add Nuclease-free ddH_2O to elute the DNA after the ethanol has completely evaporated.

Appendixes

1. P5 and P7 PCR primer sequences (5'→3')

P5, Index 1	AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTC
P5, Index 2	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGTCGTCGGCAGCGTC
P5, Index 3	AATGATACGGCGACCACCGAGATCTACACACTGCATATCGTCGGCAGCGTC
P5, Index 4	AATGATACGGCGACCACCGAGATCTACACAAGGAGTATCGTCGGCAGCGTC
P7, Index 1	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGG
P7, Index 2	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGG
P7, Index 3	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGG

Note: *The sample multiplexing indexes are yellow highlighted and marked in green and red. A P5 can be paired with a P7 PCR primer in any index combination for sample multiplexing in sequencer.*

2. Library fragment schematic sequence

5'-
AATGATACGGCGACCACCGAGATCTACACIIIIIIIITCGTCGGCAGCGTC
AGATGTGTATAAGAGACAG-NNNNNN-
CTGTCTCTTATACACATCTCCGAGCCCACGAGACIIIIIIIIATCTCGTATG
CCGTCTTCTGCTTG-3'

IIIIIIII: P5 Index (8 bases)
IIIIIIII: P7 Index (8 bases)
NNNNNN: Target DNA sequence