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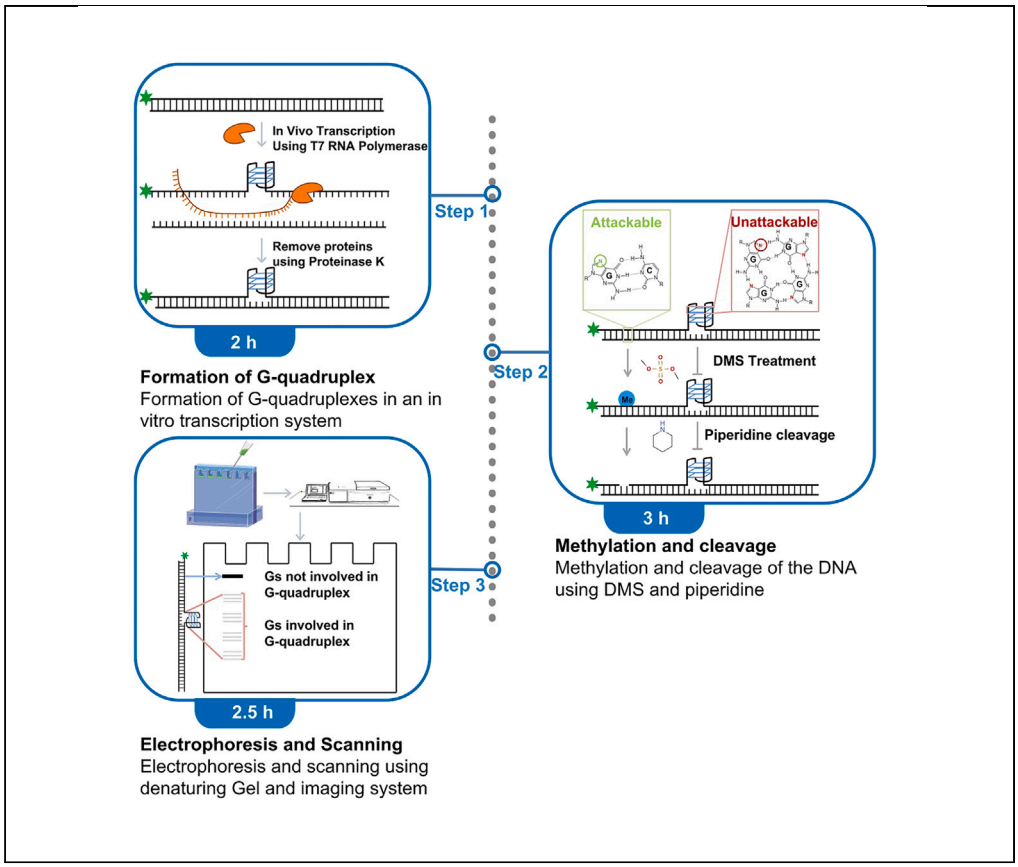

G-quadruplexes are four-stranded nucleic acid structures that play vital roles in regulating gene expression, maintaining genomic stability, and supporting various biological processes. This protocol details their formation from double-stranded DNA via *in vitro* transcription. It includes steps for selecting suitable DNA templates, assembling necessary components (such as RNA polymerase, nucleotides, and buffers), setting optimal incubation conditions, and performing dimethyl sulfate (DMS) footprinting to analyze the structures.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Steps for methylation and cleavage of DNA using DMS and piperidine

Instructions for assembling *in vitro* transcription components

Guidance on optimizing G-quadruplex formation

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Protocol for forming G-quadruplexes from double-stranded DNA during transcription

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SUMMARY

G-quadruplexes are four-stranded nucleic acid structures that play vital roles in regulating gene expression, maintaining genomic stability, and supporting various biological processes. This protocol details their formation from double-stranded DNA via *in vitro* transcription. It includes steps for selecting suitable DNA templates, assembling necessary components (such as RNA polymerase, nucleotides, and buffers), setting optimal incubation conditions, and performing dimethyl sulfate (DMS) footprinting to analyze the structures.

For complete details on the use and execution of this protocol, please refer to Zhang et al.[1] and Gong et al.[2]

BEFORE YOU BEGIN

G-quadruplexes are four-stranded secondary structures formed from nucleic acid sequences rich in guanine (G).[3] These structures arise through the assembly of guanine tetrads, which play crucial roles in various cellular processes, such as transcription, replication, genomic stability, and even the progression of cancer and related diseases. Notably, a large number of putative G-quadruplex sequences (PQSs) are located near transcription start sites (TSSs) within the human genome, indicating that G-quadruplexes might significantly influence the regulation of transcription.[4]

Research indicates that G-quadruplexes can modulate gene expression during transcription by influencing the activity of RNA polymerase. Their capability to form stable secondary structures allows them to affect the binding of transcription factors and other regulatory proteins, thereby impacting the initiation and overall expression levels of genes.[5] Thus, investigating the formation of G-quadruplexes during transcription is essential for understanding the specific mechanisms by which they regulate gene expression. The purpose of this manuscript is to present the preparation of an *in vitro* transcription system, along with techniques for detecting G-quadruplex formation using gel electrophoresis and DMS footprinting.

In our manuscript, we reference Zhang et al. and Gong et al. to provide essential context for our protocol on G-quadruplex formation during transcription.[1,2] Zhang et al. investigate the formation of a DNA:RNA hybrid G-quadruplex (HQ) during transcription of DNA containing G-tracts, demonstrating its regulatory role in transcription by elucidating a structural cascade involving R-loop formation and G-tract interactions.[1] Gong et al. explore how single-nucleotide variations (SNVs) can

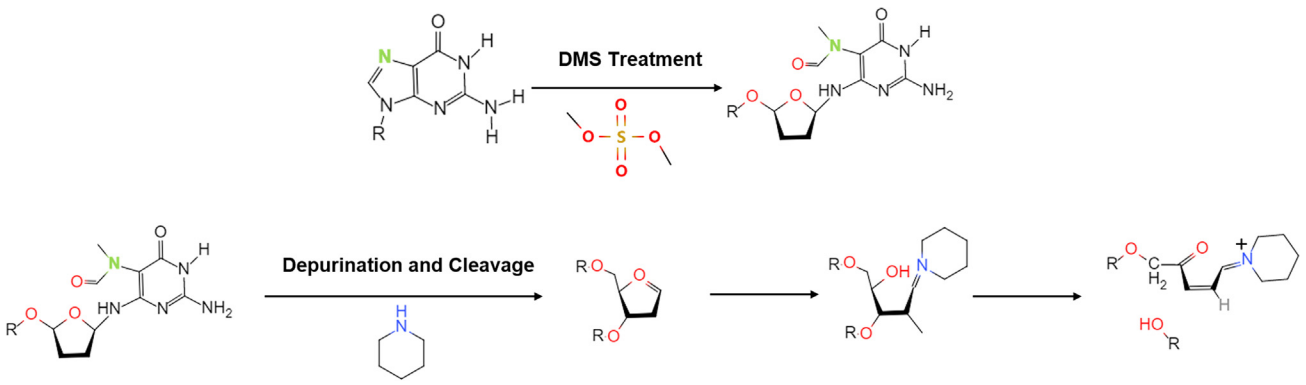


Figure 1. The suggested mechanism for the methylation of DMS and the following cleavage reaction, modified from Onel et al.[7]

alter G-quadruplex structures in the human genome, emphasizing their impact on gene expression and their potential as therapeutic targets.[2] These studies support our methodology and objectives by enhancing the understanding of G-quadruplexes in transcription processes and their significance in gene regulation.

There are various methods for identifying G-quadruplexes, including Electrophoretic Mobility Shift Assay (EMSA), Dimethyl Sulfate (DMS) footprinting, DNA polymerase stop assay, Circular Dichroism (CD) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy. Among these methods, only DMS footprinting and NMR spectroscopy can effectively identify the specific guanine involved in the formation of G-quadruplexes, which is of significant importance for studying the dynamic changes of structures. DMS footprinting allows researchers to distinguish between protected and unprotected bases, thereby accurately identifying the specific architecture of G-quadruplexes. NMR spectroscopy, on the other hand, provides atomic-level structural information about G-quadruplexes, enabling a deeper understanding of their complex three-dimensional structures. However, NMR experiments typically require a long time for data acquisition and processing, and standard high-field NMR equipment is expensive, with relatively high operational and maintenance costs. This can somewhat limit its widespread application in research. In contrast, DMS footprinting has a relatively simpler experimental procedure. Researchers only need to react the sample with DMS and then perform the corresponding electrophoresis analysis to reveal the bases involved in G-quadruplex formation, and this method is less demanding in terms of sample concentration. This makes DMS footprinting a highly effective and efficient tool for studying G-quadruplex formation and dynamic changes.

DMS (dimethyl sulfate) footprinting is a method originally devised for the chemical sequencing of DNA, consisting of three main steps (Figure 1).[6,7] First, DMS targets the N7 atom of guanine, leading to its methylation. In the second step, at a temperature of 90°C, piperidine displaces the methylated base from the sugar ring, resulting in depurination at the modified site. Finally, in an alkaline solution, the DNA chain is cleaved where piperidine has modified it. The resulting DNA fragments are resolved using denaturing polyacrylamide gel electrophoresis, producing a distinctive banding pattern that indicates the positions of methylation.

In both double-stranded and single-stranded DNA, the N7 position of guanine does not participate in hydrogen bonding, making it highly susceptible to DMS attack. However, in G-quadruplex structures, the N7 position of guanine engages in hydrogen bonding, conferring resistance to DMS modification. By exploiting this distinction, researchers can apply the same treatment conditions to both single-stranded DNA and G-quadruplexes. Following electrophoresis, they can compare the sequences of the protected bases, unveiling critical insights into the formation of G-quadruplex structures.[8]

Table 1. PCR mixture 1

Reagent	Final concentration	Amount
10× $(NH_4)_2SO_4$ Taq buffer	1×	5 μL
$MgCl_2$ (25 Mm)	1.5 mM	3 μL
dNTP (2.5 mM)	0.2 mM	4 μL
Overlap-up primer (1 μM) (446-W- Overlap-up primer or 446-GM-Overlap-up primer)	0.02 μM	1 μL
Overlap-dn primer (1 μM) (446-W-Overlap-dn primer or 446-GM-Overlap-dn primer)	0.02 μM	1 μL
Taq DNA polymerase (5 U/μL)	0.02 U/μL	0.2 μL
Total	N/A	**50 μL**

The protocol presented in this manuscript aims to advance research on the formation of G-quadruplexes during transcription and their impact on transcriptional levels. Additionally, it is designed to investigate how drugs affect the stability and structure of G-quadruplexes, as well as the associated changes in transcriptional activity. This will provide valuable tools for researchers in this field, fostering a deeper understanding of the intricate interactions between G-quadruplexes, transcription factors, and small molecules.

Prepare double-stranded DNA

Timing: 5 h

1. Prepare PCR Mixture 1, Table 1.
2. Initiate the first step of overlap PCR using the following cycling conditions. The main purpose of this step is to generate a long double-stranded DNA molecule from two short single-stranded DNAs, which will then serve as the template for the subsequent PCR reaction.
   a. 94°C for 3 min (1) pre-Denaturation: to remove any secondary structures that may be present on the DNAs.
   b. 94°C for 1 min (2) Denaturation: to remove any secondary structures that may be present on the DNAs.
   c. 55°C for 30 s (3) Annealing: to allow the overlaps of DNA to bind to each other. The annealing temperature is determined based on the Tm values of the overlapping regions of the two single-stranded DNAs: the Overlap-up primers (446-W-Overlap-up primer or 446-GM-Overlap-up primer) and the Overlap-dn primers (446-W-Overlap-dn primer or 446-GM-Overlap-dn primer), as well as the results from preliminary experiments.
   d. 72°C for 30 s (4) Extension: to extend to produce complete double-stranded DNA.
   e. Repeat steps (2) to (4) for a total of 4 cycles. Cycling: to repeat the cycles several times to ensure complete extension.
3. Run the PCR products on a 12% non-denaturing gel. If a single band of 168 bp appears, it indicates that this step of the PCR has achieved the expected results.

Table 2. PCR mixture 2

Reagent	Final concentration	Amount
10× $(NH_4)_2SO_4$ Taq buffer	1×	5 μL
$MgCl_2$ (25 Mm)	1.5 mM	3 μL
dNTP (2.5 mM)	0.2 mM	4 μL
Fam-sense-primer (10 μM)	0.2 μM	1 μL
Antisense-primer (10 μM)	0.2 μM	1 μL
Template		1 μL
Taq DNA polymerase (5 U/μL)	0.02 U/μL	0.2 μL
Total	N/A	**50 μL**

Table 3. dsDNA buffer

Reagent	Final concentration	Amount
$LiAsO_2(CH3)_2$, pH 7.9 (100 mM)	10 mM	5 μL
LiCl (500 mM)	50 mM	5 μL
dsDNA 2 μM	1 μM	25 μL
Total	N/A	**50 μL**

4. Excise the target band using a blade, then crush it and add 10 mM Tris-HCl buffer, along with twice the volume of buffer relative to the gel volume, to ensure optimal dissolution and maximize recovery of the desired DNA fragments.
5. Incubate at 55°C for 1 h, then centrifuge to collect the supernatant as the template.
6. Prepare PCR Mixture 2, Table 2.
7. Perform the following cycling conditions for the extension.
   a. 94°C for 3 min (1),
   b. 94°C for 1 min (2),
   c. 62°C for 30 s (3),
   d. 72°C for 30 s (4),
   e. Repeat steps (2) to (4) for a total of 29 cycles,
   f. 72°C for 5 min (5).
8. Purification of PCR Products.
   a. Use the Universal DNA Purification Kit to purify the dsDNA.
9. Reannealing of dsDNA.
   a. Dilute the DNA solution to 2 μM/μL, if the amount of DNA is not enough, increase the volume of PCR Mixture 2. Prepare dsDNA buffer, Table 3.
   b. Place the reaction mixture in a thermal cycler and heat it to 95°C for 5 min. Gradually cool the mixture to 25°C at a rate of 0.03°C/S.

Prepare the competitor DNA

Timing: 1 h

10. Prepare the following mixture Table 4.
11. Place the reaction mixture in a thermal cycler and heat it to 95°C for 5 min. Gradually cool the mixture to 25°C at a rate of 0.03°C/S.

Prepare 10× transcription buffer

Timing: 1 h

12. Prepare the solution according to the following formulation Table 5.
13. Store the prepared solution at −20°C and thaw it in advance before use.

Prepare 10× TBE buffer

Timing: 1 h

Table 4. Competitor DNA

Reagent	Final concentration	Amount
$LiAsO_2(CH3)_2$, pH 7.9 (100 mM)	10 mM	5 μL
LiCl (500 mM)	50 mM	5 μL
Competitor DNA1 (400 μM)	100 μM	12.5 μL
Competitor DNA2 (400 μM)	100 μM	12.5 μL
Total	N/A	**50 μL**

Table 5. 10× Transcription buffer

Reagent	Final concentration	Amount
Tris–HCl, pH 7.9 (1M)	400 mM	400 μL
$MgCl_2$ (4 M)	80 mM	20 μL
Spermidine (500 mM)	20 mM	40 μL
DTT (1 M)	100 mM	100 μL
Total	N/A	**1 mL**

14. Weigh the following components:
    a. 108 g of Tris base.
    b. 55 g of boric acid.
    c. 7.5 g of EDTA-2Na.
15. In a 1 L volumetric flask or a large beaker, add the 108 g of Tris base, the 55 g of boric acid and 7.5 g of EDTA-2Na.
16. Add high-quality distilled water to the mixture until the total volume reaches 1 L.
17. Stir the solution until all components are completely dissolved.
18. Check the pH of the solution and adjust it to pH 8.0 if needed. Use a pH meter to adjust the pH; any model can be used. If the pH is greater than 8, 1M HCl can be used for correction.
19. Transfer the prepared 10× TBE buffer to a clean, labeled storage bottle and store it at room temperature or in a refrigerator for future use.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
T7 RNA polymerase	Thermo Fisher Scientific	Cat# EP0111
NTP set	Thermo Fisher Scientific	Cat# R0481
Pyrophosphatase	Thermo Fisher Scientific	Cat# EF0221
Proteinase K	Takara	Cat# 9034
Taq DNA polymerase	Thermo Fisher Scientific	Cat# EP0402
dNTP	Thermo Fisher Scientific	Cat# R72501
PEG 200	Sigma-Aldrich	Cat# P3015
KCl	Amresco	Cat# 0395
$MgCl_2$	Amresco	Cat# 0288
Urea	Amresco	Cat# 0568
Acryl/Bis 40% solution (19:1)	Sangon Biotech	Cat# B546012
Tris base	Amresco	Cat# 0497
Boric acid	Amresco	Cat# BS141
EDTA-2Na	Amresco	Cat# 0105
LiCl	Sigma-Aldrich	Cat# 203637
Dithiothreitol (DDT)	Amresco	Cat# 0281
Spermidine	Sigma-Aldrich	Cat# S2626
DMS	Sigma-Aldrich	Cat# 320293
Cacodylic acid	Sigma-Aldrich	Cat# 271837
Salmon sperm DNA	Solarbio	Cat# D8030
β-mercaptoethanol	Sigma-Aldrich	Cat# 516732
Phenol/chloroform/isoamyl alcohol (25:24:1, PH > 7.8)	Solarbio	Cat# P1012
Ammonium acetate	Amresco	Cat# 0103
Glycogen	Amresco	Cat# D0812
Piperidine	Sigma-Aldrich	Cat# 411027
Deionized formamide	Sangon Biotech	Cat# A600211

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
446-W-overlap-up primer	Sangon Biotech	GGCTTCGGAGTCCCCTGCTAATACGACTCACTATAGAGCA CTGGACTGAGAGTTCGCTGTGCCACGGGGAGGGGAGGGG GGTTTGC
446-W-overlap-dn primer	Sangon Biotech	CCAGCCTGCGGCGAGTGTAGGATCACATGGAGTGATCG AACGCATCATAGCTCCGACTTTACCAACAGTACTCGGAA TAGCAGCAAACCCCCCTCCCCTCCCCGTGGC
446-GM-overlap-up primer	Sangon Biotech	GGCTTCGGAGTCCCCTGCTAATACGACTCACTATAG AGCACTGGACTGAGAGTTCGCTGTGCCACGGGGAGG CGAGGGGGGTTTGC
446-GM-overlap-dn primer	Sangon Biotech	CCAGCCTGCGGCGAGTGTAGGATCACATGGAGTG ATCGAACGCATCATAGCTCCGACTTTACCAACAGTA CTCGGAATAGCAGCAAACCCCCCTCGCCTCCCCGTGGC
Fam-sense-primer	Sangon Biotech	Fam-GGCTTCGGAGTCCCCTG
Antisense-primer	Sangon Biotech	CCAGCCTGCGGCGAGTG
Competitor DNA1	Sangon Biotech	GAAATTAATACGACTCACTATA
Competitor DNA2	Sangon Biotech	TATAGTGAGTCGTATTAATTTC
Other		
Typhoon FLA 9500	GE Healthcare	29-0040-80
Universal DNA purification kit	TIANGEN	DP214
Software		
Image J	National Institutes of Health	https://imagej.nih.gov/ij/ or https://fiji.sc/

STEP-BY-STEP METHOD DETAILS

Induce the formation of G-quadruplex structures during the transcription

Timing: 2 h

1. Prepare the Transcription Reaction Mix, Table 6.

   Alternatives: Use the non-transcribed sample as a control, which should not include T7 RNA polymerase and inorganic pyrophosphatase.

2. Incubate the transcription reaction at 37°C for 1 h. Transcription time is crucial for the formation of G-quadruplexes; typically, 1 h is sufficient, and extending the time does not enhance the quantity of G-quadruplexes formed. 37°C is the optimal temperature for T7 RNA polymerase reactions, conducive to the formation of G-quadruplexes. Although G-quadruplexes can form at 25°C, it takes significantly longer to achieve the same results as at 37°C.
3. After the 1-h incubation, add Stop buffer, Table 7 to the transcription reaction, and incubate at 37°C for 30 min.

Table 6. Transcription reaction mix

Reagent	Final concentration	Amount
10× Transcription Buffer	1×	10 μL
PEG 200	40% (w/v)	36 μL
KCl (500 mM)	50 mM	10 μL
NTP Mix (25 mM)	1 mM	4 μL
Template DNA (1 μM)	0.1 μM	10 μL
T7 RNA Polymerase (20 U/μL)	2 U/μL	10 μL
Inorganic Pyrophosphatase(0.1 U/μL)	0.004 U/μL	4 μL
Total	N/A	**100 μL**

Table 7. Stop buffer

Reagent	Final concentration	Amount
Tris-HCl, pH 7.9 (100 mM)	10 mM	10 μL
PEG 200	40% (w/v)	36 μL
KCl (500 mM)	50 mM	10 μL
competitor DNA (100 μM)	2.5 μM	2.5 μL
Proteinase K	1 μg/μL	10 μL
Total	N/A	**100 μL**

Note: Sequences capable of forming G-quadruplexes can be referenced in these studies.[1,9] They are categorized into three types: DQ (DNA G-quadruplex), represented by the sequence GGGGGAGGGGGGGAGGG[1]; HQ (DNA-RNA Hybrid G-quadruplex), represented by the sequence GGGGGAGGGGGGG[1]; and GVBQ (Guanine-vacancy–bearing G-quadruplex), represented by the sequence GGAGGGTCGGGTGGG.[9]

Increasing the concentration of NTPs can enhance transcription efficiency and increase the amount of G-quadruplex, but it is necessary to simultaneously raise the concentration of Mg^{2+}. Typically, the concentration of Mg^{2+} should be at least 2 mM higher than the total concentration of the four NTPs.

Methylation and cleavage of DNA using DMS and piperidine

Timing: 3 h

4. DNA Methylation.
   a. To the completed transcription and post-processing samples, add 4 μL of 10% DMS solution.
   b. Incubate at 37°C for 6 min.
   c. Immediately following the DMS reaction, add a total volume of 75 μL of the termination solution prepared earlier.
   d. Extract the reacted samples with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1).
   e. Centrifuge at 12,000 g for 10 min and carefully collect the upper aqueous layer.
5. Precipitation.
   a. To the upper aqueous layer, add 1/3 volume of 10 M ammonium acetate (pH 5.2), 2 μL of glycogen, and 3 volumes of anhydrous ethanol.
   b. Place the mixture at −70°C for 30 h.
   c. Centrifuge at 12,000 g for 30 min at 4°C, carefully remove the supernatant.
   d. Add cold 70% ethanol to the pellet and centrifuge at 12,000 g for 10 min at 4°C.
   e. Repeat the previous step (Step 6d) once more to wash the pellet thoroughly.
   f. Allow the pellet to dry in a 50°C oven.
6. Piperidine cleavage.
   a. Prepare a solution of 10% piperidine for the subsequent step.
   b. Resuspend the dried pellet in 100 μL of 10% piperidine solution.
   c. Heat in a water bath at 90°C for 30 min.
   d. Allow the heated sample to cool on ice, then add 100 μL of water.
   e. Extract with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1).
   f. Centrifuge at 12,000 g for 10 min and collect the upper aqueous layer.
7. Precipitation and dissolution.
   a. To the upper aqueous layer, add 1/3 volume of 10 M ammonium acetate (pH 5.2), 2 μL of glycogen, and 3 volumes of anhydrous ethanol.
   b. Place the mixture at −70°C for 30 min.
   c. Centrifuge at 12,000 g for 30 min at 4°C, carefully remove the supernatant.
   d. Add cold 70% ethanol to the pellet and centrifuge at 12,000 g for 10 min at 4°C.
   e. Repeat the previous step (Step 6d) once more to wash the pellet thoroughly.

Table 8. 10% urea polyacrylamide gel

Reagent	Amount
Urea	16.8 g
10× TBE buffer	4 mL
Acryl/Bis 40% Solution (19:1)	12.6 mL
H_2O	13.5 mL
TEMED	150 μL
20% APS	30 μL
Total	**40 mL**

f. Allow the pellet to dry in a 50°C oven.
g. Dissolve the final pellet in 80% deionized formamide. Heat at 95°C for 5 min, then cool on ice.

Note: Maintain appropriate safety precautions while handling DMS, piperidine, and other chemicals. Use personal protective equipment (PPE) as necessary.

Detect G-quadruplex formation using denaturing gel electrophoresis

Timing: 2.5 h

8. Prepare 10% Urea Polyacrylamide Gel, Table 8.
   a. Prepare 40 mL of gel solution for a denaturing acrylamide gel with dimensions of 20 cm × 22 cm x 1 mm.
   b. In a clean container, dissolve 16.8 g of urea in 13.5 mL of distilled water until fully dissolved. Add 12.6 mL of Acryl/Bis 40% Solution (19:1) and 4 mL of 10× TBE buffer to the urea solution.
   c. Immediately add the appropriate amount of 150 μL APS (Ammonium Persulfate) and 30 μL TEMED (N,N,N′,N′-Tetramethylethylenediamine) to initiate polymerization.
   d. Pour the mixture into a gel casting tray, insert the comb for wells, and allow the gel to polymerize at 25°C for 30–60 min.
9. Pre-electrophoresis.
   a. Set up the electrophoresis apparatus.
   b. Place the polymerized gel still attached to the glass plates into the electrophoresis tank and fill it with the 1× TBE buffer.
   c. Connect the apparatus to the power supply and conduct pre-electrophoresis at 450 V for 30 min.
10. Electrophoresis.
   a. Load Samples into the Wells.
   b. Connect the apparatus to the power supply and set the voltage to 450 V. Run the electrophoresis for 1 h or until the desired separation has occurred.
11. Visualize the Gel.
   a. After complete electrophoresis, carefully remove the gel with the attached glass plates from the apparatus.
   b. Proceed directly to the visualization step without peeling off the gel. Place the gel with glass plates into the imaging system (e.g., Typhoon 9400, Typhoon Trio+, Typhoon FLA 9500) following the manufacturer's instructions (https://rega.kuleuven.be/bac/economou/files/pdf/manuals/typhoon-fla9500-biomolecular-imager.pdf) for visualization.
   c. Analyze the results using image J.

Note: During the procedure, it is essential to wear gloves and a mask to prevent exposure to the hazards associated with Acryl/Bis, APS, and TEMED. If accidental contact occurs, it is necessary to immediately rinse the affected area with plenty of water.

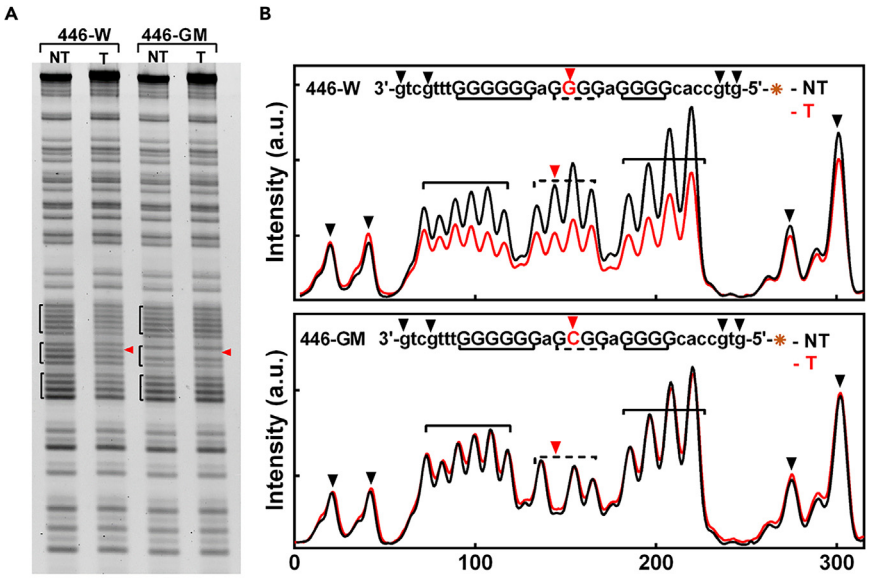


Figure 2. Formation and analysis of G-quadruplex DNA. In this experiment, two DNA samples were compared: 446-W and 446-GM

The 446-W sample is capable of forming G-quadruplex structures, while the 446-GM sample cannot.

(A) Denaturing gel electrophoresis: Following separation on a denaturing gel, the 446-W sample showed distinct bands characteristic of G-quadruplex formation, whereas the 446-GM sample did not display such bands, indicating its inability to form G-quadruplex structures.

(B) Band quantification: Band intensity for each sample was quantified using Image J.

> *Note:* The electrophoresis process generates heat, which is beneficial for denaturing gels but could lead to damage. Regularly monitor the temperature of the electrophoresis system, and if it becomes too hot, pause the run to allow it to cool down before resuming.

EXPECTED OUTCOMES

Expected outcomes: Generally, regions on the DNA that contain guanine (G) are expected to produce strong cleavage bands (Figure 2). However, in cases where the DNA forms G-quadruplex structures during transcription, the cleavage within these G-quadruplex regions will be noticeably weaker compared to the control group.

LIMITATIONS

DMS footprinting is a valuable tool for detecting the presence of G-quadruplex structures, but it is unsuitable for providing precise quantitative data regarding their abundance or stability in specific samples. Therefore, if needed, researchers should utilize complementary methods to obtain quantitative insights into G-quadruplex formation. To perform quantitative analysis of G-quadruplexes, one can refer to ref. 5, which utilizes Electrophoretic Mobility Shift Assay (EMSA) to detect the ratio of double-strands that form G-quadruplexes.

TROUBLESHOOTING

Problem 1

No fluorescent signal was detected on the gel when visualize the gel using imaging systems.

Potential solution

- Check Detection Channels: Ensure that the correct detection channels are being used for the specific fluorescent dye or marker attached to the DNA. Review the excitation and emission wavelengths for the dye and adjust the settings on the imaging system accordingly.

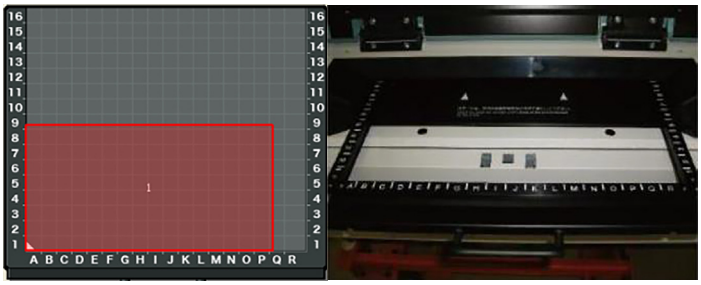
- Adjust Electrophoresis Time: If the electrophoresis run time was excessively long, it may have caused the DNA bands to migrate off the gel. Reduce the run time and verify that bands remain well within the gel matrix during electrophoresis.
- Monitor Band Migration: Consider running a small test gel with a known sample to observe the migration patterns. This can help confirm whether bands are remaining visible on the gel.

Problem 2

The fluorescent signal appears blurry visualize the gel using imaging systems.

Potential solution

- Focus Settings: When scanning gels with the Typhoon 9400 and Typhoon Trio+ to achieve optimal sensitivity, low-fluorescence glass plates should be used, ensuring the bottom plate is 3 mm thick. The scanner control software includes two focal plane parameters. Selecting the +3 mm parameter focuses the optical lens system 3 mm above the glass plate to enhance signal clarity.
- Proper Height for Gel Placement: Utilize the specialized platform available on the Typhoon FLA imaging system to ensure that the glass slide with the gel is positioned at the correct height. Proper positioning can significantly enhance the quality of the detected signal. The image below illustrates the target imaging area of the Typhoon FLA 9500 along with its fluorescence imaging scanning platform.



- Optimize Scanning Parameters: Consider adjusting other scanning parameters, such as resolution and sensitivity, to further refine the output image. Experimenting with these settings can yield a clearer signal. Using the Typhoon FLA 9500 as an example, the following settings were employed: fluorescence mode was selected, the appropriate fluorescence channel was chosen, an excitation wavelength of 473 nm was utilized, an LPB (510LP) filter was applied, the PMT voltage was initially set to 450 V, and a sensitivity of 100 μm was selected.
- Clean the Imaging Surface: Ensure that the imaging surfaces are clean and free from any dust or smudges that could interfere with the clarity of the image.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hanqing Chen (chenhq@ccmu.edu.cn).

Technical contact

Technical questions on executing this protocol should be directed to and will be answered by the technical contact, Jiayu Zhang (zhangjy86@ihep.ac.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This paper does not report the original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

J.Z. and J.G. designed the project and discussed the results with all other authors. J.Z. conducted the related material preparation, structural characterization, and biological experiments. J.Z. prepared the first version of the manuscript. J.C. and H.C. revised the manuscript and supervised the project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Zhang, J.Y., Zheng, K.W., Xiao, S., Hao, Y.H., and Tan, Z. (2014). Mechanism and manipulation of DNA:RNA hybrid G-quadruplex formation in transcription of G-rich DNA. J. Am. Chem. Soc. *136*, 1381–1390. https://doi.org/10.1021/ja4085572.

2. Gong, J.Y., Wen, C.J., Tang, M.L., Duan, R.F., Chen, J.N., Zhang, J.Y., Zheng, K.W., He, Y.d., Hao, Y.H., Yu, Q., et al. (2021). G-quadruplex structural variations in human genome associated with single-nucleotide variations and their impact on gene activity. Proc. Natl. Acad. Sci. USA *118*, e2013230118. https://doi.org/10.1073/pnas.2013230118.

3. Varshney, D., Spiegel, J., Zyner, K., Tannahill, D., and Balasubramanian, S. (2020). The regulation and functions of DNA and RNA G-quadruplexes. Nat. Rev. Mol. Cell Bio. *21*, 459–474. https://doi.org/10.1038/s41580-020-0236-x.

4. Xiao, S., Zhang, J.Y., Zheng, K.W., Hao, Y.H., and Tan, Z. (2013). Bioinformatic analysis reveals an evolutional selection for DNA:RNA hybrid G-quadruplex structures as putative transcription regulatory elements in warm-blooded animals. Nucleic Acids Res. *41*, 10379–10390. https://doi.org/10.1093/nar/gkt781.

5. Zhang, C., Liu, H.H., Zheng, K.W., Hao, Y.H., and Tan, Z. (2013). DNA G-quadruplex formation in response to remote downstream transcription activity: long-range sensing and signal transducing in DNA double helix. Nucleic Acids Res. *41*, 7144–7152. https://doi.org/10.1093/nar/gkt443.

6. Umeyama, T., and Ito, T. (2017). DMS-Seq for In Vivo Genome-wide Mapping of Protein-DNA Interactions and Nucleosome Centers. Cell Rep. *21*, 289–300. https://doi.org/10.1016/j.celrep.2017.09.035.

7. Onel, B., Wu, G., Sun, D., Lin, C., and Yang, D. (2019). Electrophoretic Mobility Shift Assay and Dimethyl Sulfate Footprinting for Characterization of G-Quadruplexes and G-Quadruplex-Protein Complexes. Methods Mol. Biol. *2035*, 201–222. https://doi.org/10.1007/978-1-4939-9666-7_11.

8. Sun, D., and Hurley, L.H. (2010). Biochemical techniques for the characterization of G-quadruplex structures: EMSA, DMS footprinting, and DNA polymerase stop assay. Methods Mol. Biol. *608*, 65–79. https://doi.org/10.1007/978-1-59745-363-9_5.

9. Li, X.M., Zheng, K.W., Zhang, J.Y., Liu, H.H., He, Y.D., Yuan, B.F., Hao, Y.h., and Tan, Z. (2015). Guanine-vacancy-bearing G-quadruplexes responsive to guanine derivatives. Proc. Natl. Acad. Sci. USA *112*, 14581–14586. https://doi.org/10.1073/pnas.1516925112.