# Polyamine Environment Experiment

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## 1 Procedure Purpose

Aim to improve the efficiency of the backspace method by seeing if a polyamine environment affects Exonuclease T cleavage in DNA strands.

## 2 Overview

This experiment is intended to determine how a polyamine environment affect Exonuclease T activity. We are introducing this environment through the addition of spermine, spermidine, and putrescine.
At physiological levels, the presence of these polyamines causes DNA to condense through site-specific, structural, and electrostatic interactions. The increased stability may protect against overcutting by Exonuclease T.This protocol builds on previous experiments undertaken using Exonuclease T. [**?**] [**?**] [**?**] [**?**]

## 3 Safety Information

1. **SYBR Gold** has no data available addressing the mutagenicity or toxicity of SYBR® Gold nucleic acid gel stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.[**?**]
2. When diluting the polyamines, work in fume hood when possible, especially when you're doing those dilutions from really concentrated stock.
3. Working in a communal lab space is dangerous. Do not assume your fellow workers cleaned up sufficiently.

## 4 Materials

- "BS_Start_pos5" Oligonucleotide (Sequence: 5'-AGTTACCATGACCGTGTGCGTTTTT-3')
- "BS_Start_pos" Oligonucleotide (Sequence: 5'-AGTTACCATGACCGTGTGCGT-3')
- "BS_Start" Oligonucleotide (Sequence: 5'-AGTTACCATGACCGTGTGCG-3')
- "BS_Comp_6" Oligonucleotide (Sequence: 5'-AACGCA-3')
- Spermine (1g)
- Spermidine (1g)
- Putrescine (1g)
- Exonuclease T (5 U/$\mu$L)

- SYBR Gold (10,000X)
- NEBuffer 4 (10X)
- TBE Buffer (1X)
- Gel Loading Buffer II (2X)
- Two Novex TBE Gels (20%)
- IDT 10/60 Ladder
- RNAse Free Water
- RNAse Free PCR tubes
- RNAse Free pipette tips
- Two thermocyclers

# 5 Dilutions

1. Dilute each of the oligonucleotides listed in 'Materials' to a 100uM liquid stock in RNAse free water. Thoroughly vortex and store modified oligos at -20C after use.
2. Dilute each of the complementary oligonucleotides listed (except and BS_Comp_6) to 10uM working stock by putting 5 uL of the 100 uM stock in 45 uL RNAse free water.
3. Dilute BS_Comp_6 to 10uM working stock by putting 15 uL of the 100 uM stock in 135 uL RNAse free water.
4. Dilute 1g of spermine to 5M working stock by adding .574 mL of RNAse free water into the bottle.
5. Dilute 5M spermine to 5mM working stock by adding 1$\mu$L of the 5M stock to 999$\mu$L of RNAse free water.
6. Dilute 5mM spermine to 100uM working stock by adding 10 $\mu$L of 5mM stock to 490$\mu$L of RNAse free water.
7. Dilute 100uM spermine to 50uM working stock by adding 50 $\mu$L of 100uM stock to 50$\mu$L of RNAse free water.
8. Dilute 100uM spermine to 10uM working stock by adding 10 $\mu$L of 100uM stock to 90$\mu$L of RNAse free water.
9. Dilute 1g of spermidine to 5M working stock by adding 1.38 mL of RNAse free water into the bottle.
10. Dilute 5M spermidine to 5mM working stock by adding 1$\mu$L of the 5M stock to 999$\mu$L of RNAse free water.
11. Dilute 5mM spermidine to 100uM working stock by adding 10 $\mu$L of 5mM stock to 490$\mu$L of RNAse free water.
12. Dilute 100uM spermidine to 50uM working stock by adding 50 $\mu$L of 100uM stock to 50$\mu$L of RNAse free water.
13. Dilute 100uM spermidine to 10uM working stock by adding 10 $\mu$L of 100uM stock to 90$\mu$L of RNAse free water.
14. Dilute 1g of putrescine to 5M working stock by adding 2.27 mL of RNAse free water into the bottle.
15. Dilute 5M putrescine to 5mM working stock by adding 1$\mu$L of the 5M stock to 999$\mu$L of RNAse free water.

16. Dilute 5mM putrescine to 100uM working stock by adding 10 $\mu$L of 5mM stock to 490$\mu$L of RNAse free water.
17. Dilute 100uM putrescine to 50uM working stock by adding 50 $\mu$L of 100uM stock to 50$\mu$L of RNAse free water.
18. Dilute 100uM putrescine to 10uM working stock by adding 10 $\mu$L of 100uM stock to 90$\mu$L of RNAse free water.

# 6 Procedure

## 6.1 Sample Explanations

Consult the following list for a functional explanation of each sample tube:

1. S0.5, S2.5, S12.5 refer to samples that will have 0.5, 2.5, and 12.5 micromolar concentrations, respectively, of spermine to test whether the presence of that polyamine will increase the efficiency of Exonuclease T halting at the correct position.
2. SD0.5, SD2.5, SD12.5 refer to samples that will have .5, 2.5, and 12.5 micromolar concentrations, respectively, of spermidine to test whether the presence of that polyamine will increase the efficiency of Exonuclease T halting at the correct position.
3. P0.5, P2.5, P12.5 refer to samples that will have 0.5, 2.5, and 12.5 micromolar concentrations, respectively, of putrescine to test whether the presence of that polyamine will increase the efficiency of Exonuclease T halting at the correct position.
4. Replicates will be prepared for all the same solutions. The tubes will be labeled exactly the same but with an "R" at the end.
5. P refers to a positive control (the 21-bp starting DNA strand) bound to a DNA complementary strand (BS_Comp_6), N refers to a negative control (the 20-bp unextended DNA strand) bound to a complementary strand (BS_Comp_6).
6. The D control (for DNA) contains BS_Start_pos5, BS_Comp_6, and ExoNuclease T with no polyamines.
7. The E control contains only the extended starting strand, "BS_Start_pos5," as a length comparison and to ensure there is no manufacturer defect with the starting strand.

## 6.2 Quantitative Control Preparation

1. Label PCR Tubes according to Table 1.

| | PCR Tube Labeling | | |
|---|---|---|---|
| Spermine Samples | S0.5 | S2.5 | S12.5 |
| Spermidine Samples | SD0.5 | SD2.5 | SD12.5 |
| Putrescine Samples | P0.5 | P2.5 | P12.5 |
| Positive Controls | P | P | |
| Negative Controls | N | D | |
| Quantitative Controls | E | | |

2. Into tube 'E', add 2.09 $\mu$L of 10 uM 'BS_Start_pos5' oligonucleotide and 22.91 $\mu$L of water. Vortex.

## 6.3 Complementary Strand Annealing

1. Pipette 2.92$\mu$L nuclease-free water into PCR Tubes S0.5, S2.5, SD0.5, SD2.5, P0.5, P2.5.
2. Pipette 1.42$\mu$L nuclease-free water into PCR Tubes S12.5, SD12.5, P12.5.
3. Pipette 3.92 $\mu$L nuclease-free water into PCR Tube D.

4. Pipette 5.52 $\mu$L nuclease-free water into PCR Tubes P and N.
5. Pipette 1$\mu$L of 10uM spermine into PCR Tube S0.5.
6. Pipette 1$\mu$L of 10uM spermidine into PCR Tube SD0.5.
7. Pipette 1$\mu$L of 10uM putrescine into PCR Tube P0.5.
8. Pipette 1$\mu$L of 50uM spermine into PCR Tube S2.5.
9. Pipette 1$\mu$L of 50uM spermidine into PCR Tube SD2.5.
10. Pipette 1$\mu$L of 50uM putrescine into PCR Tube P2.5.
11. Pipette 2.5$\mu$L of 100uM spermine into PCR Tube S12.5.
12. Pipette 2.5$\mu$L of 100uM spermidine into PCR Tube SD12.5.
13. Pipette 2.5$\mu$L of 100uM putrescine into PCR Tube P12.5.
14. Pipette in 2$\mu$L of 10X NEBuffer 4 into PCR Tubes P, N, D, S0.5, S2.5, S12.5, SD0.5, SD2.5, SD12.5, P0.5, P2.5, P12.5.
15. Pipette in 3.16$\mu$L of 10$\mu$M 'BS_Start_pos5' 25mer working stock into PCR Tubes D, S0.5, S2.5, S12.5, SD0.5, SD2.5, SD12.5, P0.5, P2.5, and P12.5.
16. Pipette in 3.16$\mu$L of 10$\mu$M 'BS_Start_pos' 21mer working stock into PCR tube P.
17. Pipette in 3.16$\mu$L of 10$\mu$M 'BS_Start' 20mer working stock into PCR tube N.
18. Pipette in 9.32$\mu$L of 10$\mu$M of "BS_Comp_6" working stock into PCR tubes P, N, D, S0.5, S2.5, S12.5, SD0.5, SD2.5, SD12.5, P0.5, P2.5, and P12.5.
19. Put all test samples and positive and negative controls into the thermocycler.
20. Set thermocycler to hold at 94C for two minutes, then lower the temperature to 5C forever at a rate of 0.5°C per second.

## 6.4 Exonuclease T Incubation

1. Keeping the samples **in the thermocycler**, pipette 1.6 $\mu$L Exonuclease T into each test sample tube (S0.5, S2.5, S12.5, SD0.5, SD2.5, SD12.5, P0.5, P2.5, P12.5) and D.
2. Incubate for 1 hour at 5°C.
3. Pipette 5$\mu$L of RNAse-free 500 mM EDTA into each test sample, positive control, and negative control tube.

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OPTIONAL STOP POINT

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## 6.5 XCell Surelock Setup and Pre-Run

1. Remove two 20% polyacrylamide gels from pouches and rinse with deionized water.
2. Peel off tape on bottom of 20% polyacrylamide gels and remove combs.
3. Lower the Buffer Core (the piece that holds the gels) into the Lower Buffer Chamber so that the negative electrode fits into the opening in the gold plate.
4. Insert the Gel Tension Wedge into the XCell Surelock behind the buffer core. Make sure it is in its 'unlocked' position, which allows the wedge to slip into the unit.

5. Insert gel cassettes into the lower buffer chamber. The shorter "well" side of the cassette faces into the buffer core. The slot on the back must face outward. If only one gel is being run, insert a buffer dam in the place of a gel cassette.
6. Pull forward on the Gel Tension Lever toward the buffer core until the gel cassettes are snug against the buffer core. This puts it in the 'locked' position.
7. Fill the Upper Buffer Chamber (between the gels) with 1x TBE running buffer. Ensure it is not leaking.
8. Fill the Lower Buffer Chamber completely with running buffer by pouring 1x TBE next to the Gel Tension Wedge.
9. Wash each gel well with 12 $\mu$L running buffer.
10. Place the gel cover on the apparatus in the correct orientation. Connect the electrodes to the power source, and pre-run the gel for 30 minutes at 200V.

## 6.6 Sample Preparation for Gel Running

| Well number | Sample |
|---|---|
| 1 | *misloaded* 10/60 Ladder |
| 2 | *misloaded* N |
| 3 | S0.5 |
| 4 | S2.5 |
| 5 | S12.5 |
| 6 | P |
| 7 | SD0.5 |
| 8 | SD2.5 |
| 9 | SD12.5 |
| 10 | D |
| 11 | P0.5 |
| 12 | P2.5 |
| 13 | P12.5 |
| 14 | P |
| 15 | *not loaded* E |

Figure 1: Gel 1

## 6.7 Gel Loading and Running

1. Obtain a sizable piece of parafilm. Pipette 5 $\mu$L of 2x Gel Loading Buffer II in two 5 x 3 grids of droplets, and label each droplet with the corresponding sample.
2. For ladder wells, mix 4 $\mu$L of 1X TBE Buffer and 1 $\mu$L of 10/60 ladder into the loading dye droplet.
3. For the remaining gel wells, mix 5$\mu$L of the appropriate sample with the 5$\mu$L of Gel Loading Buffer II already on the parafilm, mix well, and then load 5$\mu$L of the mix directly into the appropriate gel well on the first gel, and 10$\mu$Linto the second gel.
4. Run the gels at 200V until the dark blue dye is three quarters of the way to the bottom of the gel. If the dark blue dye is not visible, run the gel for two hours.

| Well number | Sample |
|---|---|
| 1 | 10/60 Ladder |
| 2 | N |
| 3 | S0.5R |
| 4 | S2.5R |
| 5 | S12.5R |
| 6 | P |
| 7 | DR |
| 8 | SD0.5R |
| 9 | SD2.5R |
| 10 | SD12.5R |
| 11 | P |
| 12 | E |
| 13 | P0.5R |
| 14 | P2.5R |
| 15 | P12.5R |

Figure 2: Gel 2

### 6.8 Staining and Viewing Gel

1. While the gel runs, prepare 1X SYBR Gold Staining Solution with TBE as dilute.
2. Once gel has finished running, submerge gel in SYBR Gold solution for 15 minutes.
3. Rinse the gel with DI water before viewing.
4. Review gel with gel viewer. Save results.
5. Post pictures to Slack.

## 7 Stop Procedure

Store the DNA 10 uM stocks in the -20 freezer immediately after use. Ensure that the SYBR Gold has been returned to the -20 freezer, and clean up the work area. Save all samples for possible future use. Return polyamines to the cold room. Do not dispose of serial dilutions unless through a licensed chemical professional.

### 7.1 Analysis

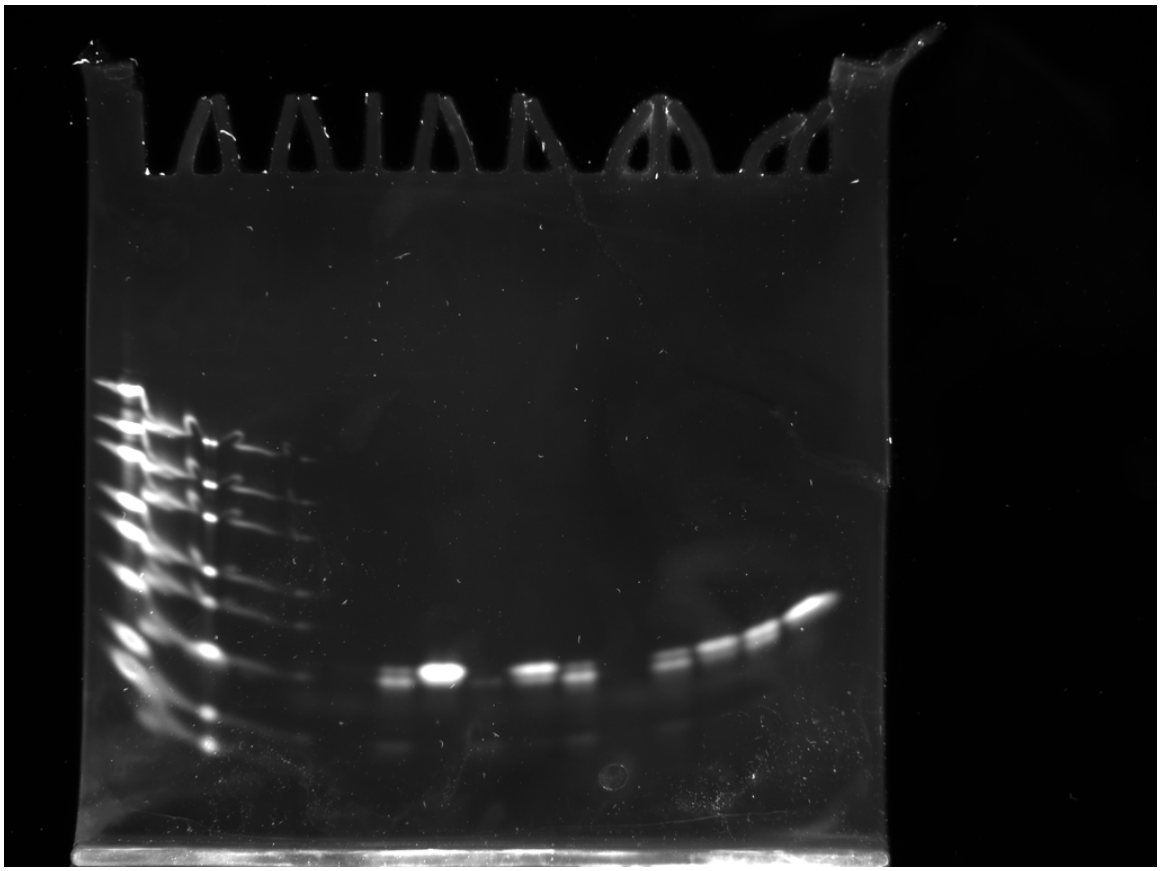

Figure 3: Gel 1

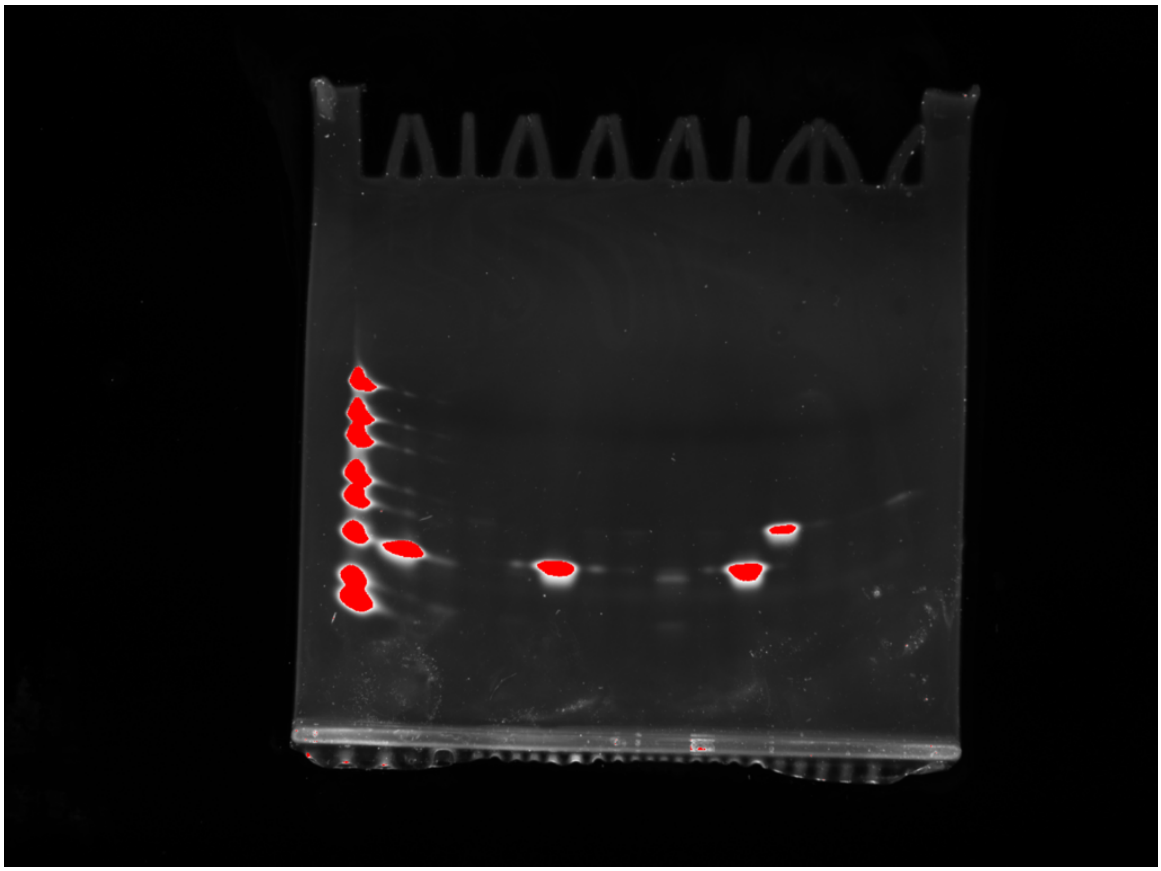

Figure 4: Gel 2