TdT Characterization Experiment

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1 Procedure Purpose

Charecterize the rate of Terminal Deoxynucleotidyl Transferase extension of ssDNA with varying nucleotide:DNA ratios.

2 Overview

An array of samples will be tested with these conditions (dTTP:DNA ratio (moles) by time of reaction):

		1	2	3	4	5	6	7	8	9	10	11	12
		30s	45s	1min	2min	3min	5min	7min	10min	15min	20min	30min	Controls
A	2												
B	3												
C	5												
D	7												
E	10												
F	15												
G	20												
H	40												H12

Samples are addressed by their 96Well plate location (eg. H12, as marked)
They will be stopped with EDTA and sent to PAN. The most promising samples will be run on a gel for better resolution.
For PAN analysis, we will be sending in Terminal Deoxynucleotidyl Transferase extended samples with a concentration of 2ng/μL.

3 Safety Information

1. **Terminal Deoxynucleotidyl Transferase** is toxic if inhaled. May cause cancer. Toxic to aquatic life with long lasting effects. Avoid breathing dust/fume/gas/mist/vapours/spray. Use personal protective equipment as required. If Inhaled: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Dispose of contents/container in accordance with local/regional/national/international regulations.[?]
2. **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.[?]
3. Working in a communal lab space is dangerous. Do not assume your fellow workers cleaned up sufficiently.

4 Dilutions

1. Dilute 19.57μL of BSStart (100uM) in 480.43μL. Votex Well.
2. Dilute 10mM dTTP Stock to 100μL of .1mM (making at least 197μL)
3. Dilute 10mM dTTP Stock to 100μL of 1mM (making at least 28.181μL)
4. Dilute 0.9597μL of 90-mer (AGTATTCCTGTCCCCGCTCGGTATCGTGATGCTCGTGCTTATGCGAACGTGCAGATGACGTTTGGCGCGTATTTAGATGCGCTAGACGTT), 2.898μL of 15Mer (ATGGACATGGACTAC) and 2.793μL of BS-Comp-8 (AACGCACA) (both from 100uM stock) into 1993.349μL Nuclease-Free water. Vortex well before use, this is the DNA Control Solution.

5 Procedure

5.1 Prepare Plate

In to each well, pipette 1.5uL of 10X Terminal Deoxynucleotidyl Transferase buffer and 1.5uL of $CoCl_2$. Alternatively, if possible (reagents allow), use a multichannel pipette to pipette in 3μL of a 1:1 $CoCl_2$:10X TdT Buffer mixture into each cell.
For each row, create the following dilutions of dTTP from dilutes prepared before:

Row	dTTP Dilute (.1mM)	dTTP Dilute (1mM)	Water
A	9.39μL		110.6μL
B	14.1μL		105.9μL
C	23.5μL		96.5μL
D	32.9μL		87.1μL
E	47μL		73μL
F	70.5μL		49.5μL
G		9.3936μL	110.606μL
H		18.787μL	101.213μL

For each column **except for 12** pipette 2uL of Terminal Deoxynucleotidyl Transferase into each well.
For each well in each row, pipette 5uL of the corresponding dilute.

5.2 Begin Expiriment

For each time, begin by pipetting in 5uL of the DNA dilute prepared earlier. Wait until the allotted time for that column has elapsed and cease the reaction with 5uL of EDTA.

5.3 Controls

No Terminal Deoxynucleotidyl Transferase should be added to the controls. H12 must be left empty.

5.4 Sending to PAN

Pipette 10uL out of each well and store in another well plate for a future gel run. Pipette in 20uL of DNA Control Solution into each well and submit to PAN. (This gives us roughly 60ng of DNA per lane to run on a gel later)

6 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.