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):

	30s	45s	1min	2min	3min	5min	7min	10min	15min	20min	30min	Controls
2												
3												
5												
7												
10												
15												
20												
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 6ng/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 28.18μL)

5 Procedure

5.1 Prepare Plate

In to each well, pipette 3uL of Terminal Deoxynucleotidyl Transferase buffer.
For each row, create the following dilutions of dTTP from dilutes prepared before:

Row	dTTP Dilute (.1mM)	dTTP Dilute (1mM)	Water
A	9.394μL		110.6μL
B	14.09μL		105.9μL
C	23.48μL		96.52μL
D	32.88μL		87.12μL
E	46.97μL		73.03μL
F	70.45μL		49.55μL
G		9.394μL	110.6μL
H		18.79μL	101.2μL

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 20μL of Nuclease-Free Water 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.