TdT Characterization Experiment

Written by **Michael Uttmark**

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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 Materials

- "BS_Start" Oligonucleotide (Sequence: 5'-AGTTACCATGACCGTGTGCG-3')
- "BS_Comp_8" Oligonucleotide (Sequence: 5'-AACGCACA-3')
- 15Mer (ATGGACATGGACTAC)
- 90-mer (AGTATTCCTGTCCCCGCTCGGTATCGTGATGCTCGTGCTTATGCGAACGTGCAGATGACGTTTGGCG
- Terminal Deoxynucleotidyl Transferase (20 U/μL)
- dTTP (100 mM)
- NEB Terminal Transferase Buffer (10X)
- CoCl2 (10x)
- RNAse Free Water

5 Dilutions

1. Dilute 39.13μL of BSStart (100uM) in 960.87μL of nuclease-free water for a 24 ng/uL (3.914 uM) working stock. Vortex well.
2. Dilute 5 μL 100mM dTTP Stock in 495 μL water to 500μL of 1mM (making at least 56.347μL). Vortex well.
3. Dilute 50 uL 1mM dTTP Stock in 450μL water to 500μL of .1mM (making at least 394μL)
4. Dilute 4.32 uL of 90-mer (AGTATTCCTGTCCCCGCTCGGTATCGTGATGCTCGTGCTTATGCGAACGTGCAGATGACGTTTGGCGCGTATTTAGATGCGCTAGACGTT), 13.04 uL of 15Mer (ATGGACATGGACTAC) and 12.56 of BS-Comp-8 (AACGCACA) (both from 100uM stock) into 3970.08 μL Nuclease-Free water in a 15 mL tube. Vortex well before use. This is the DNA Control Solution.
5. Prepare a trough of a mixture of 300μL 10x Buffer and 300μL 10x $CoCl_2$

6 Procedure

6.1 Prepare Plate

In to each well, pipette 3μL using a multichannel pipette from the 1:1 $CoCl_2$:10X TdT Buffer mixture into each well except H12.
For each row, create the following dilutions of dTTP from dilutes prepared before:

Row	dTTP Dilute (.1mM)	dTTP Dilute (1mM)	Water
A	18.8μL		221.2μL
B	28.2μL		211.8μL
C	47μL		193μL
D	65.7μL		174μL
E	93.9μL		146μL
F		14.087μL	225.913μL
G		18.782μL	221.218μL
H		37.565μL	202.435μL

For each well in each row, pipette 10μL of the corresponding dTTP dilute (except H12).
For each column **except for 12** pipette 4μL of Terminal Deoxynucleotidyl Transferase into each well except H12.

6.2 Begin Experiment

For each reaction in order of decreasing incubation time, begin by pipetting in 10μL of the DNA dilute prepared earlier. Write down the time each column was started and stopped when time permits. Wait until the allotted time for that column has elapsed and cease the reaction with 10μL of EDTA.

6.3 Controls

No Terminal Deoxynucleotidyl Transferase should be added to the controls in column 12. H12 must be left empty.

6.4 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)

7 Stop Procedure

Ensure that all reagents have been returned to their proper storage locations and clean up the work area. Save all samples in the -20 freezer for possible future use.