**Testing the incorporation of acetylated thymidine**

Reaction Protocol:

1. Prepare MOPS buffers
2. Prepare 7x 1.5mL tubes
3. Prepare Dilutions/Aliquots
    a. Buffer: 10X - check pH of each
    b. Primer: 1μL {200pmol/μL} in 9μL water
    c. **Ac_**dTTP: 2μL {10mM}, no dilution (make ten aliquots of 5μL)
    d. dTTP: 0.5μL {100mM} in 9.5μL water
    e. TdT: 5μL {20U/μL} in 5μL
4. Add components to all "-a" tubes (plus controls) as follows:

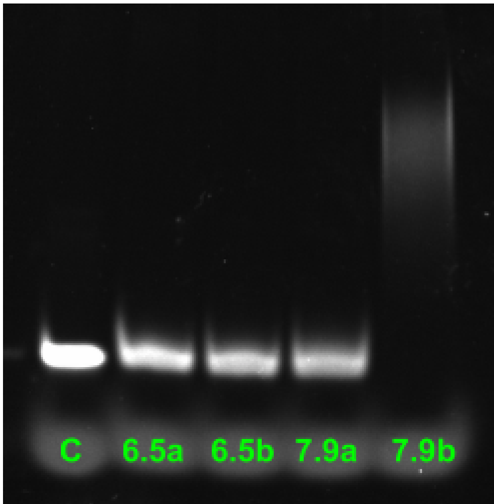
| | **ddH$_2$O** | **buffer** | **CoCl$_2$** | **primer** | **monomer** | | **enzyme** | **EDTA** | **dye** |
|---|---|---|---|---|---|---|---|---|---|
| C | 8μL | 65 1μL | | 1μL | | | | 4μL | 2μL |
| M-65-a | 5μL | 65 1μL | 1μL | 1μL | Ac 1μL | | 1μL | 4μL | 2μL |
| M-65-b | | | | | dT 1μL | | | 4μL | 2μL |
| M-79-a | 5μL | 79 1μL | 1μL | 1μL | Ac 1μL | | 1μL | 4μL | 2μL |
| M-79-b | | | | | dT 1μL | | | 4μL | 2μL |
| ccccc | 5μL | 65 1μL | 1μL | 1μL | dT 2μL | | 1μL | 4μL | 2μL |
| ccccc | | | | | dT 1μL | | | 4μL | 2μL |

5. Get 100mM EDTA ready before adding TdT.
6. React all "-a" tubes (and controls) at 37°.
7. At each "-a" tube's 10min time point, take 5μL and move it into its respective "-b" tube.
8. Add additional unmodified nucleotide to respective "-b" tubes.
9. Before loading into gel, add 2μL 6X DNA Loading Dye

Assay Conditions (for each reaction tube):

| **component** | **moles** | **volume** | **source** |
|---|---|---|---|
| TdT Rxn Buffer | - | 1μL | 10X buffer |
| $CoCl_2$ | 2.5nmol | 1μL | 2.5mM $CoCl_2$ solution |
| primer (5'-FAM-$dT_{15}$) | 20pmol | 1μL | 10x dilution of 1μL from 1μg/μL (200pmol/μL) tube |
| **Ac_**dTTP | 10nmol | 1μL | 10mM solution |
| TdT | 10Units | 1μL | 2x dilution of 1.5μL from 20U/μL |
| water (to complete rxn vol.) | - | 5μL | |
| after reaction: | | | |
| EDTA (to stop reaction) | 0.4μmol | 4μL | 100mM solution |
| DNA Loading Dye | - | 2μL | 6X solution with Bromophenol Blue |

First attempt:




Second attempt: