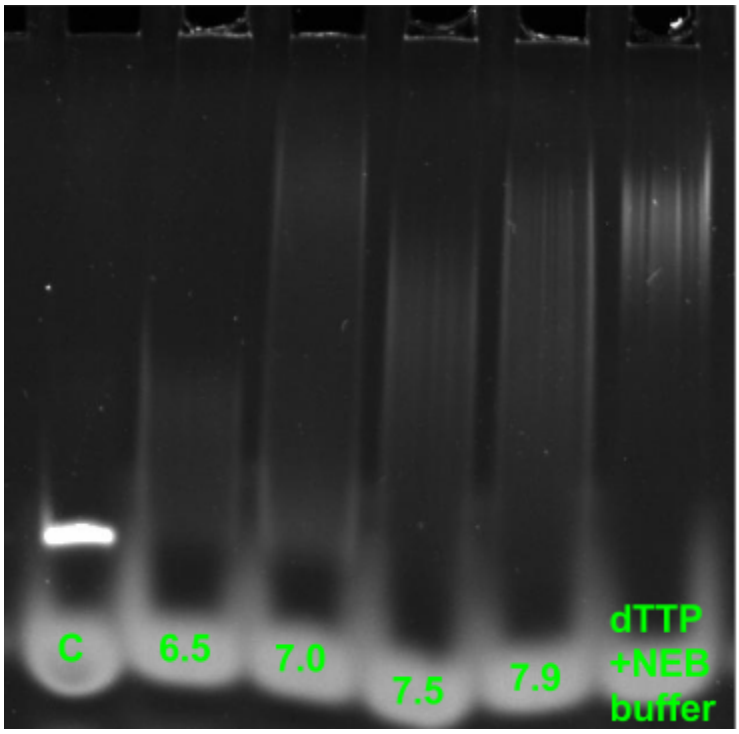
Testing the effect of pH on acetyl hydrolysis

Reaction Protocol:

1. Prepare six 1.5mL tubes and label them: C, 6.5, 7.0, 7.5, 7.9, NEB
2. Prepare four MOPS 10X buffers (10mL), one at each pH to be tested.
3. Prepare Dilutions/Aliquots
    a. Primer: 0.5µL {200pmol/µL} in 4.5µL water
    b. **Ac_**dTTP: 4µL {10mM}, no dilution
4. Add the following to all tubes (control only gets primer, buffer, and water):
    a. 1µL Rxn Buffer (from 10X of respective pH)
        i. add instead 1µL of NEB's TdT buffer to the NEB tube
    b. 1µL $CoCl_2$ (from 2.5mM stock)
    c. 1µL Primer dilution
    d. 1µL **Ac_**dTTP aliquot
    e. 5µL water
5. Warm up raw ingredients in tubes before adding TdT (optional)
6. Get 100mM EDTA ready (for stopping the reaction)
7. Prepare TdT aliquot (at fridge, then move to incubator room for Step 8)
    a. 1.5µL from stock (20U/µL) into 1.5µL sterile H_2O
8. Add 1µL from TdT dilution to the three rxn tubes and start timing immediately
    a. alternatively, use 0.5µL each from stock TdT
9. Leave all four tubes in 37° incubator for exactly 10 minutes
10. At each tube's timepoint, remove from incubator and add 4µL EDTA
11. 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})	5pmol	1µL	20x dilution of 0.5µL 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


C
6.5
7.0
7.5
7.9
dTTP
+NEB
buffer