Fluorometry: Characterisation of the Theophylline Riboswitches

Day 1

Preparation

1. Grow bacteria (see below) in 8 ml LB broth with ampicillin and incubate at 37°C overnight.

Venus spectrum

- Pr-RBS-Ch-V-dt
- Cre-dt

Time course

- Pr-t_2-Ch-V-dt
- Pr-t_1-Ch-V-dt
- Cre-dt

2. Transfer 2 ml of each overnight culture into 50 ml of fresh LB broth.

3. Incubate the culture at 37°C to mid-log phase (OD_{600} = 0.55) (use LB broth with ampicillin as blank)

Venus Spectrum

Aim: To determine and confirm the excitation wavelength of the venus reporter protein that produces the maximum fluorescent intensity.

Materials and Methods:

1. Place the cultures on ice, and make a 10 fold serial dilution (1X, 10X, 100X) of the culture with the transformed bacteria.

2. Use the culture with bacteria containing Cre-dt as a blank for the fluorometer.

3. Use the fluorometer to determine the spectrum of the venus fluorescent protein in the culture containing Pr-RBS-Ch-V-dt (constitutively “on”).

Time course experiment

Aim: To determine the translational activity of the theophylline riboswitches over time. This will give an indication of the timeframe in which to perform further riboswitch characterisation experiments.

Materials and Methods:

1. Aliquot 900 µl of the Pr-t_2-Ch-V-dt culture into a 1.5 ml eppendorf tube and keep on ice.

2. Add 100 µl of 2.2 mM theophylline to the bacterial culture transformed with Pr-t_2-Ch-V-dt (to get a final theophylline concentration of 2 mM).

3. Excite the 2 mM theophylline-containing culture at the wavelength determined from the venus fluorescent protein spectrum until a plateau in the fluorescence is reached.

4. Perform step 1-3 again with the culture with bacteria transformed with Pr-t_1-Ch-V-dt and Cre+dt (negative control).

<u>Day 2</u>

Preparation

1. Grow bacteria transformed with:

- Pr-t_2-Ch-V-dt
- Pr-t_1-Ch-V-dt
- Pr-t_2V-dt
- Pr-t_1V-dt
- Pr-RBS-Ch-V-dt
- Cre-dt

in 8 ml LB broth with ampicillin and incubate at 37°C overnight.

2. Transfer 2 ml of each overnight culture into 50 ml of fresh LB broth with ampicillin.

3. Incubate the culture at 37°C to mid-log phase (OD_{600} = 0.55) (use ampicillin LB broth as blank).

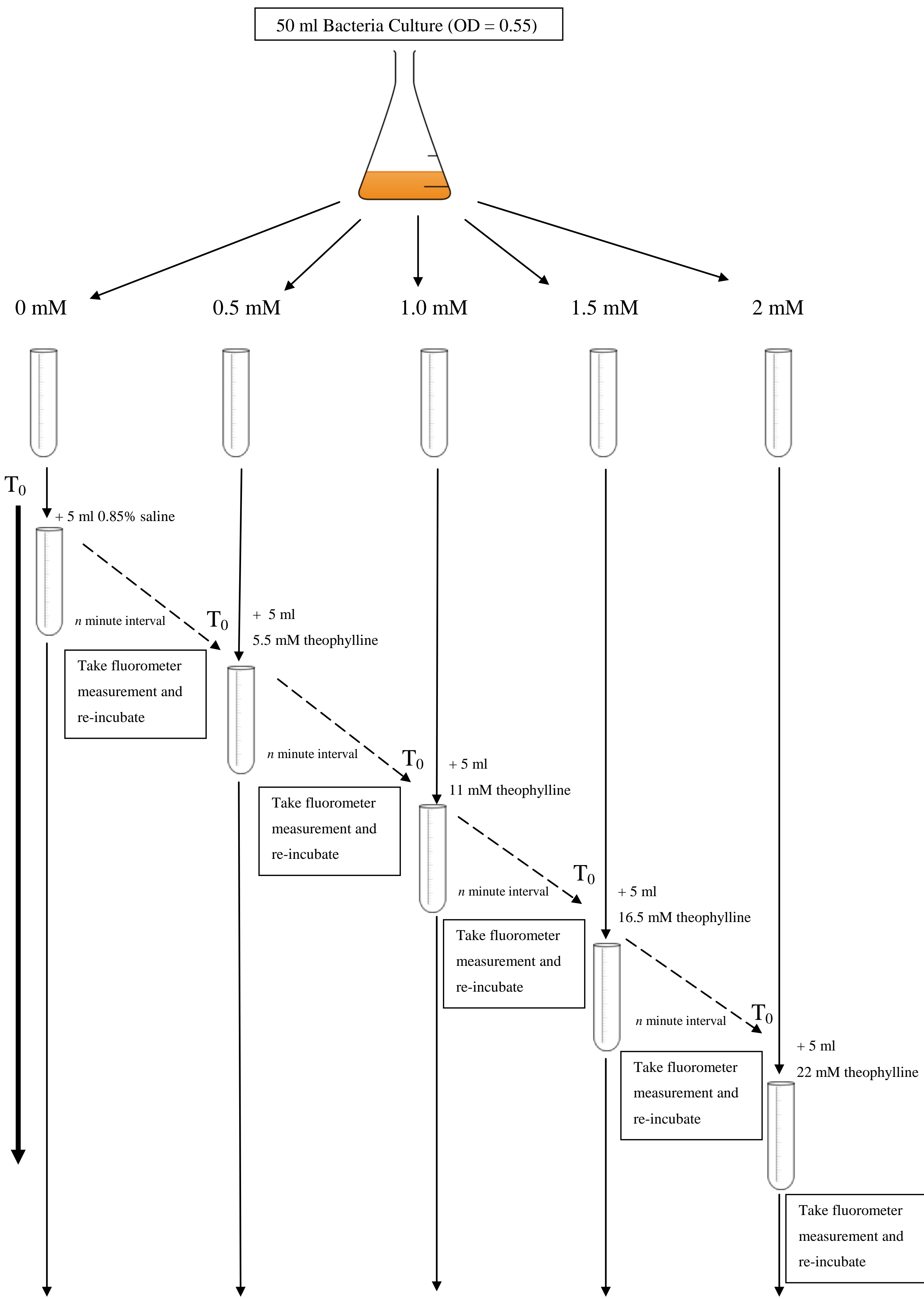
4. Place on cultures on ice.

Characterisation of the theophylline riboswitches

Aim: To characterise the translational response of the theophylline riboswitches, over time, under different concentrations of theophylline.

1. Add 5 ml of 0.85% saline, 5.5 mM, 11 mM, 16.5 mM and 22 mM theophylline to the 50 ml culture (see Appendix 1).

2. Take fluorometer measurements for each theophylline concentration at set time increments (See Figure 1) and re-incubate afterwards at 37°C until the next measurement.


50 ml Bacteria Culture (OD = 0.55)
0 mM
0.5 mM
1.0 mM
1.5 mM
2 mM
T_0
+ 5 ml 0.85% saline
n minute interval
Take fluorometer measurement and re-incubate
T_0
+ 5 ml
5.5 mM theophylline
n minute interval
Take fluorometer measurement and re-incubate
T_0
+ 5 ml
11 mM theophylline
n minute interval
Take fluorometer measurement and re-incubate
T_0
+ 5 ml
16.5 mM theophylline
n minute interval
Take fluorometer measurement and re-incubate
T_0
+ 5 ml
22 mM theophylline
Take fluorometer measurement and re-incubate

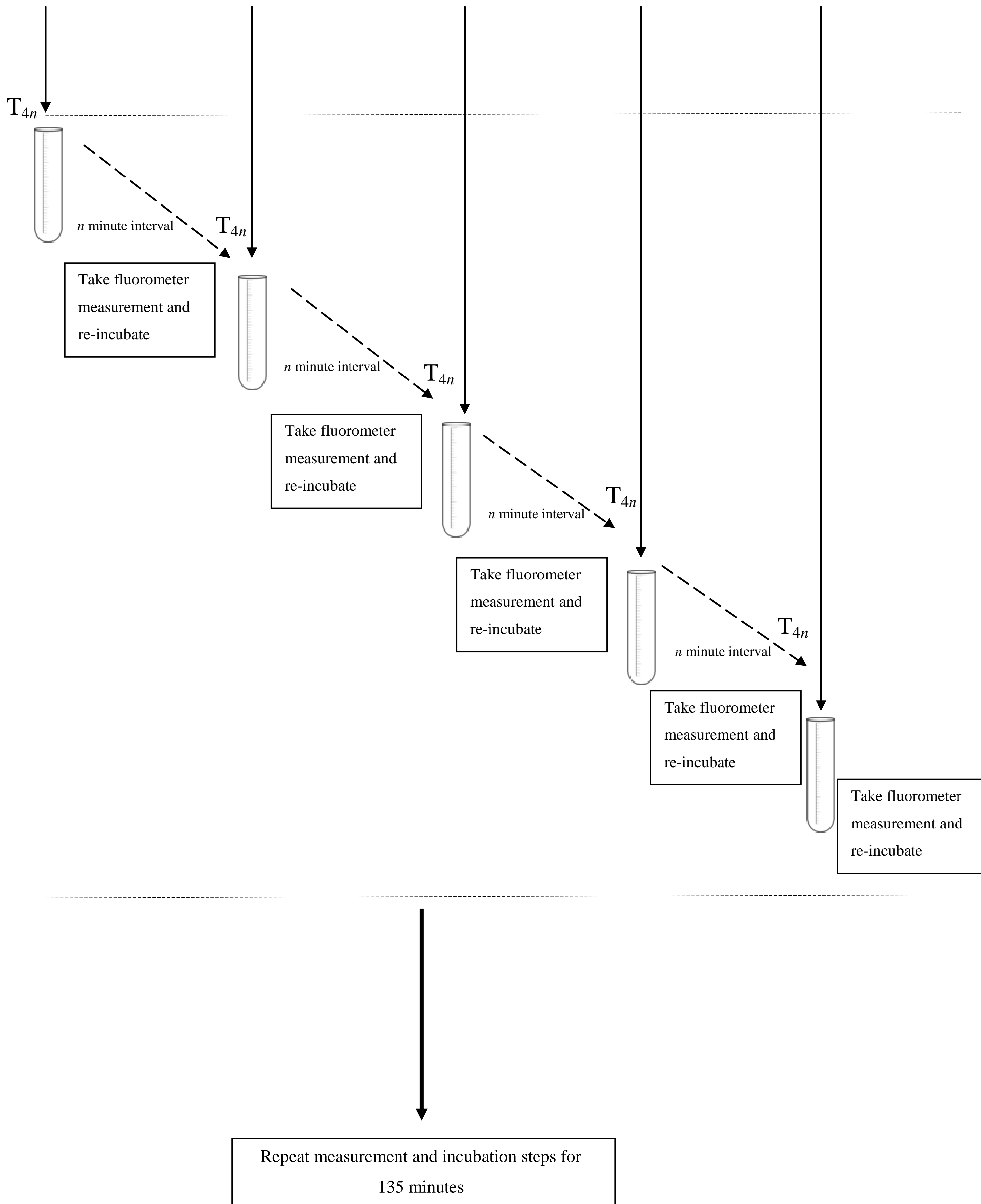


Fig 1: Schematic diagram of the materials and methods for the characterisation of the theophylline riboswitches

Appendix 1

Stock solution: 25 mM theophylline (2.25 g theophylline powder + 500ml water)

Table1: The dilution of 2.2mM theophylline stock solution into the different working theophylline concentrations.

Final concentration (mM)	Working concentration (mM)	Volume of water (ml)	Volume of 25 mM stock (ml)
0.00	0.00	50.00*	0.00
0.50	5.50	39.00	11.00
1.00	11.00	28.00	22.00
1.50	16.50	17.00	33.00
2.00	22.00	6.00	44.00

* 0.85% saline was used (instead of water) for 0 mM theophylline to prevent cell osmolysis