

Derivation of formula to calculate loss of stable RNA and mRNA

Definitions:

- S_n = amount of stable RNA in sample n
- M_n = amount of mRNA in sample n
- S_n' = amount of stable RNA per cell in sample n
- M_n' = amount of mRNA per cell in sample n
- C_n = number of cells in sample n
- f_s = factor relating S_1' to S_2' ($S_1' * f_s = S_2'$)
- f_m = factor relating M_1' to M_2' ($M_1' * f_m = M_2'$)
- $R = M_1' / S_1'$

1. Equal RNA is loaded (more or less), giving:

- 1a. $S_1 + M_1 = S_2 + M_2$
- 1b. $(S_1' * C_1) + (M_1' * C_1) = (S_2' * C_2) + (M_2' * C_2)$

2. The ratio of total fluorescent intensity in the experimental condition (condition 2) and that of the control condition (condition 1) is:

- 2a. $M_2/M_1 = (S_1 + M_1 - S_2) / M_1$
 $= (S_1' C_1 + M_1' C_1 - S_1' f_s C_2) / (M_1' C_1)$ [definition]
 $= (S_1' + M_1' - S_1' f_s C_2/C_1) / M_1'$
 $= (1 + R - f_s C_2/C_1) / R$
- 2b. $C_2/C_1 = (S_1' + M_1') / (S_2' + M_2')$ [1b]
 $= (S_1' + M_1') / (S_1' f_s + M_1' f_m)$
 $= (1 + R) / (f_s + R f_m)$
- 2c. $M_2/M_1 = (f_s + R f_m + R f_s + R^2 f_m - f_s - R f_s) / (R f_s + R^2 f_m)$
 $= (f_m + R f_m) / (f_s + R f_m)$

3. R is very small. 2% in *E. coli* growing under normal conditions, 5% for *E. coli* growing very slowly (1.5 hr doubling)

$$3a. M_2/M_1 \approx f_m / f_s \quad [2c, R \approx 0]$$

4. f_s can be calculated, given M_2/M_1 and f_m

M_2/M_1 is measurable, as the ratio of total signal in the experimental condition to the total signal in the control condition (no normalization).

f_m is measurable as the same ratio but after normalization

$$4a. f_s = f_m [(1 + R) / (M_2/M_1) - R] \quad [2c]$$

$$4b. \approx f_m / (M_2/M_1) \quad [3a]$$

The calculated value of f_s is not very sensitive to R . If R is as high as 50% (which would be pretty remarkable), then the error in the calculation of f_s is only:

$$(1 - M_2/M_1) / (2 M_2/M_1) = 13\% \text{ for the most extreme case of } M_2/M_1 = 1.34$$