

## The calculation for the co-culture grow curve:

For single-culture system:

$$n[X] = OD_{600} \quad (1.1)$$

$$d[X] = OD_{577} \quad (1.2)$$

$$f[X] = OD_{458} \quad (1.3)$$

where  $[X]$  is the total concentration of the strain in the single-culture system,  $n$  is the absorption coefficient of the strain per unit volume concentration for 600 nm,  $d$  is the absorption coefficient of the strain per unit volume concentration for 577 nm and  $f$  is the absorption coefficient of the strain per unit volume concentration for 458 nm.

As the detailed congruent relationship between the value of OD in each group is not clear. So it is necessary to calculate the relative absorption coefficient in each group, which has a tendency to maintain as a constant as the strains grows. We can use these linear relations for further analysis. (Figure 1)

$$z = d/n \quad (1.4)$$

$$h = f/n \quad (1.5)$$

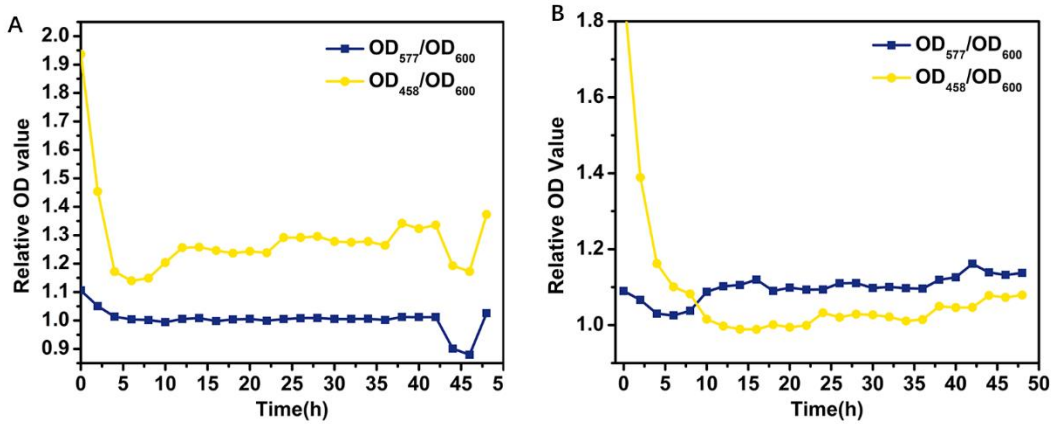


Figure 1 The relative OD value of a the single strain ( $OD_{458}/OD_{600}$  and  $OD_{577}/OD_{600}$ ). (A) The relative OD value of *E. coli* BL21 (DE3) carrying BBA\_K1033917. (B) The relative OD value of *E. coli* BL21 (DE3) carrying BBA\_K1033914.

Therefore,  $z$  and  $h$ , the relative absorption coefficient for 577 nm and 458 nm can be obtained from the data we get.

For co-culture system:

$$n_1[X] + n_2[Y] = OD_{600} \quad (2.1)$$

$$d_1[X] + d_2[Y] = OD_{577} \quad (2.2)$$

$$f_1[X] + f_2[Y] = OD_{458} \quad (2.3)$$

where [X] is the total concentration of the strain in A group, [Y] is the total concentration of the strain in B group in the co-culture system,  $d_1$  is the absorption coefficient of the strain in A group per unit volume concentration for 577 nm,  $d_2$  is the absorption coefficient of the strain in B group per unit volume concentration for 577 nm,  $f_1$  is the absorption coefficient of the strain in A group per unit volume concentration for 458 nm and  $f_2$  is the absorption coefficient of the strain in B group per unit volume concentration for 458 nm. (Table 1)

After several sets of simultaneous equation, the density of strains can be written as:

$$[X] = \frac{h_2 * OD_{600} - OD_{458}}{h_2 - h_1} \quad (2.4)$$

$$= \frac{(z_2 * OD_{600} - OD_{577})}{z_2 - z_1} \quad (2.5)$$

$$[Y] = \frac{OD_{458} - h_1 * OD_{600}}{h_2 - h_1} \quad (2.6)$$

$$= \frac{OD_{577} - z_1 * OD_{600}}{z_2 - z_1} \quad (2.7)$$

where  $z_1$  is the relative absorption coefficient of the strain in A group per unit volume concentration for 577 nm,  $z_2$  is the relative absorption coefficient of the strain in B group per unit volume concentration for 577 nm,  $h_1$  is the relative absorption coefficient of the strain in A group per unit volume concentration for 458 nm and  $h_2$  is the relative absorption coefficient of the strain in B group per unit volume concentration for 458 nm.

	Group	Strain A	Strain B
Codes in Lab	I	BBa_K1033914	none
	II	BBa_K1033917	none
	III	+TND	none
	IV	+PEF	none
	V	+TND	BBa_K1033914
	VI	-TND	BBa_K1033914
	VII	+PEF	BBa_K1033917
	VIII	-PEF	BBa_K1033917
	IX	+TND	+PEF
	X	-TND	+PEF
	XI	-TND	-PEF
	XII	+TND	-PEF

Table 1 The co-culture of the cocultivation growth curve. The strains of A and B are named as the codes of the biobricks carried. “+” means “induced” and “-“ means “non-induced”.