8.10

1.Plasmid preparation

Dissolve freeze-dried pResponse-RFP, pActivator without sgRNA, pActivator with sgRNA. plasmids in ddH20.

8.11

1.Overnight culture

Inoculate 5 mL of LB medium with antibiotics with a single colony of freshly grown E. coli harbouring either pResponse-RFP, pActivator without sgRNA or pActivator with sgRNA.
Incubate at 37℃ with shaking (250rpm) overnight.

8.12

1.Plasmid Isolation

Overnight culture of E. coli DH5α harboring plasmid pResponse-RFP, pActivator without sgRNA or pActivator with sgRNA were subjected to plasmid isolation following the Plasmid Isolation Protocol. Final elution was done with 30µL of pre-warmed MilliQ.

name	Concentration (ng/ul)
pResponse-RFP,	76.7
pActivator without sgRNA	149.1
pActivator with sgRNA	145.4

2.Bacteria recovery and stock preparation

Dilute each overnight culture 1:100 in 5ml LB with antibiotic and grow 3-4 hours at 37 C with shaking (250rpm). Streak bacteria in exponential stage in LB plate and make glycerol stock accordingly.

3.Tansformation

Transform pResponse-RFP into BL21(DE3).
Co-transform pResponse-RFP and pActivator without sgRNA into BL21(DE3).
Co-transform pResponse-RFP and pActivator with sgRNA into BL21(DE3).

Streak the bacteria culture on LB plate with corresponding antibiotics. For cotransformation, tetracycline was added to LB plate with kanamycin and chloramphenicol.
Incubate overnight at 37°C.

8.13

1.Testing-overnight culture

As expected, only dcas9 with sgRNA showed red colonies on LB plate with tetracycline.

So, we picked out several colonies for three types of transformants, (a)pResponse-RFP, (b)pResponse-RFP plus pActivator without sgRNA, (c)pResponse-RFP plus pActivator with sgRNA, and inoculate each of them in 5 mL of LB medium with antibiotics overnight at 37℃.

8.14

1.Testing

Sadly, we only had one successful culture for a, b, c. Inoculate 1% overnight bacterial culture in Erlenmeyer flask and incubate for 2h to reach early exponential stage.

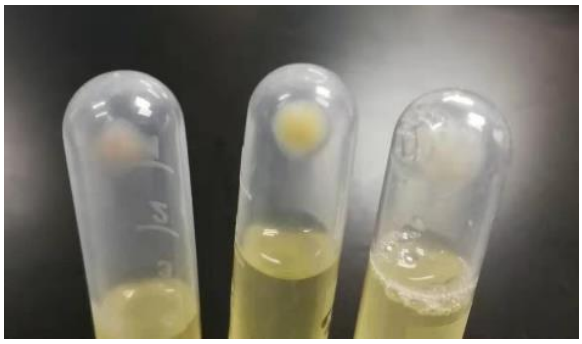
Add tetracycline to final concentration of 100 ng/μL.

Incubate overnight at 30℃.

8.15

1.Testing

After centrifuging a, b and c at 6500rpm for 1min.



	单转reportor	dcas9+reportor	dcas9-sgRNA+reporto	PBS
	61	83	125	34
	63	82	118	37
	60	77	123	37
平均	61.3	80.6	122	36
绝对RFP荧光	25.3	44.6	86	

8.20

1.Testing-overnight culture

This time, we picked out two confirmed colonies for three types of transformants, (a)pResponse-RFP, (b)pResponse-RFP plus pActivator without sgRNA, (c)pResponse-RFP

plus pActivator with sgRNA, and inoculate each of them in 5 mL of LB medium with antibiotics overnight at 37℃.

8.21

1.Testing

Inoculate 1% overnight bacterial culture in Erlenmeyer flask and incubate for 2h to reach early exponential stage. Add tetracycline to final concentration of 100 ng/μL.
Incubate overnight at 30℃.

8.22

1.Testing

Below are preliminary data for observation and microplate reader.

		a		b		c	
荧光	诱导前	534	523	486	481	494	504
	诱导后	2224	2024	3469	3481	9696	9696
OD	诱导前	0.089	0.088	0.085	0.085	0.087	0.083
	诱导后	0.414	0.543	0.579	0.614	0.431	0.440
Florescence/OD600	诱导前	6010	5943	5728	5653	5711	6040
	诱导后	5378	3729	5997	5670	22498	22050