Tunable Transcription Factor Based on CRISPR to Repress Hsp90

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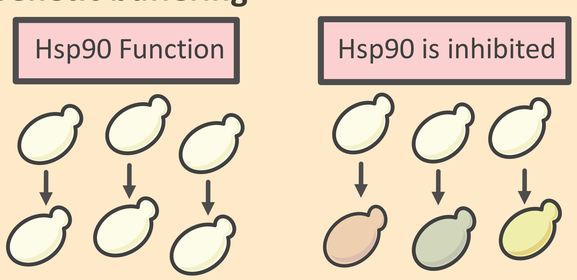
Heat shock protein can promote protein folding or complex assembly. I focus on the Heat shock protein 90 (Hsp90) because Hsp90 involves in the genetic buffering mechanism.

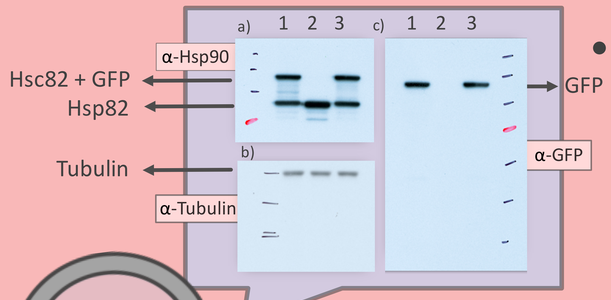
Hsp90 can suppress (buffer) the effects of genetic variations, and thus it allows variations to accumulate without phenotypic consequences. However, when Hsp90 is inhibited, the hidden variations would reveal their effects. Based on this feature, we are able to observe the phenotype variation by inhibiting the Hsp90. The previous system to repress the Hsp90 level in our lab is using tet promoter, but I found out the doxycycline is very unstable. The cells, which were spotted separately on different days, grew different on the same batch of doxycycline plates. Therefore, I tried to construct another system to solve this problem. In my project, I developed the transcriptional regulation by CRISPR-based system to repress Hsp90. This system used mutation Cas9 which is endonuclease deficient (called dCas9).Furthermore, dCas9 acts together with guide RNAs to target the specific regions. Through the engineering of guide RNAs, dCas9 can bind different positions of HSP90 promoter, which blocking progression of RNA polymerase and hence silence expression of the targeted gene. By doing so, I tried to repress Hsp90-GFP expression in the yeast cells and test fluorescence per cell by flow cytometer to analysis the degree of repression among different dCas9 binding sites.

Genetic buffering



- **Buffer the effects of variation** genetic variation but without phenotypic consequence
- **Hidden genes are released** phenotypic variance between strains increases

• Western Blot to check Hsp90 fused with GFP



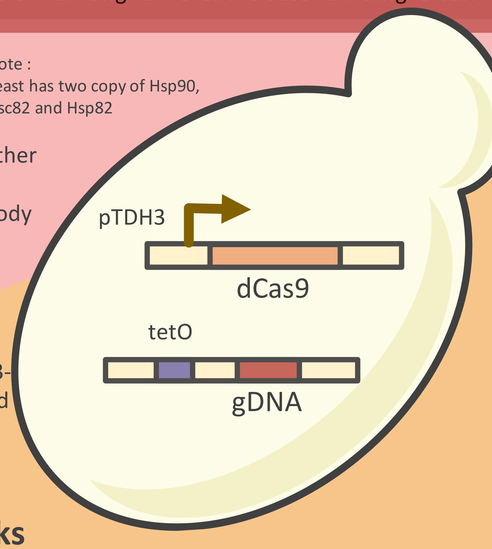
Before I transformed the crisprTFs, I did Western Blot to check whether Hsc82 is really fused with GFP.

a) Identify Hsp90 including Hsc82-GFP and Hsp82 by anti-Hsp90 antibody
b) Identify tubulin with anti-tubulin antibody for internal control
c) Identify GFP with anti-GFP antibody

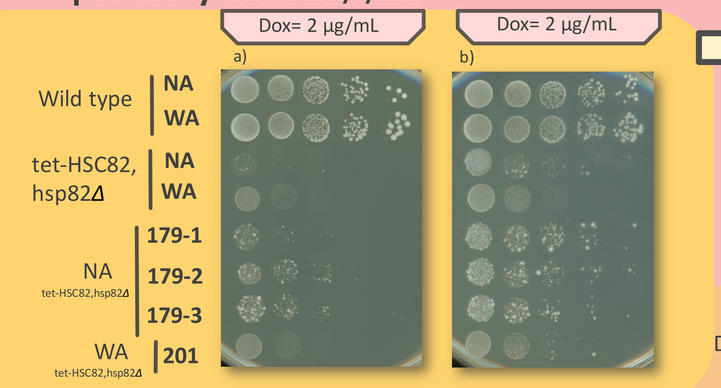
Note :
Yeast has two copy of Hsp90, Hsc82 and Hsp82

• Experiment Goal

First, I transformed constitutive pRS303H-pTDH3-dCas9 cut by ApaI into yeast. Then, I transformed specific gDNA with tet operator into the cells containing dCas9. Finally, I constructed the crisprTFs into our lab strain.

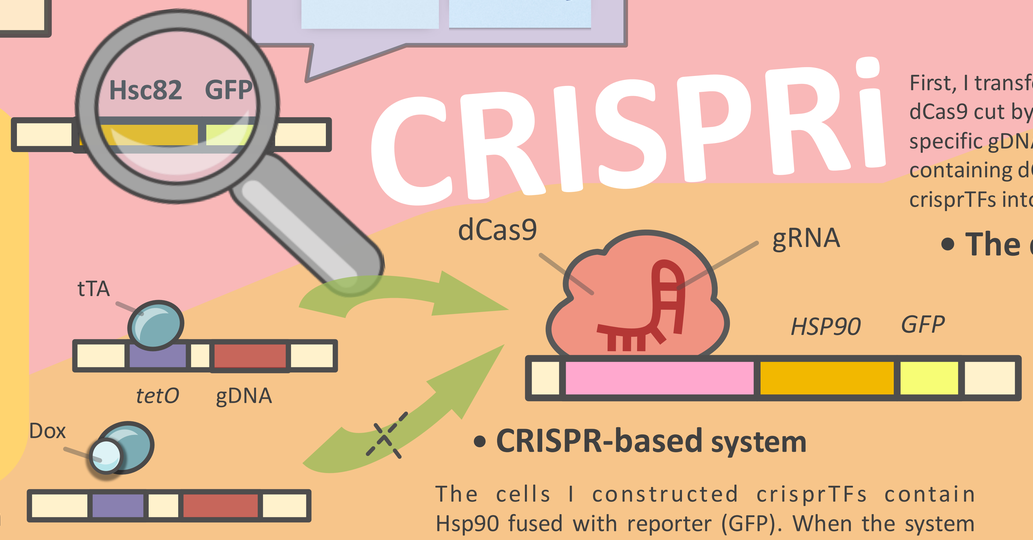


• Spot assay test for doxycycline



• Tet-off system to test crisprTFs function

The strain I used contains tetracycline-controlled transactivator (tTA). In the absence of doxycycline (Dox), the tTA will bind tetracycline operator sequences (tetO) and the activation domain promotes gDNA expression. In the presence of Dox, Dox will block tTA to bind the tet operator and result in reduced gDNA expression. Based on this feature, I was able to test whether the Hsp90 level is controlled by gRNA expression via Dox treatment.

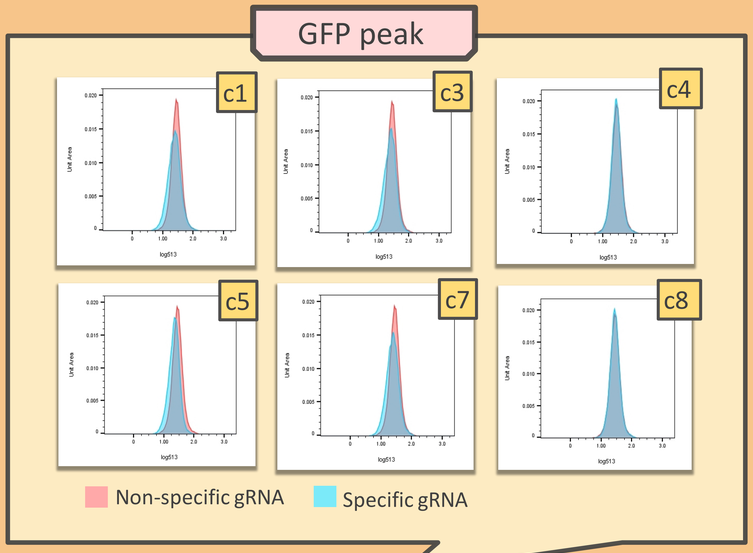


Note:
Dox is the analog of tetracycline

• CRISPR-based system

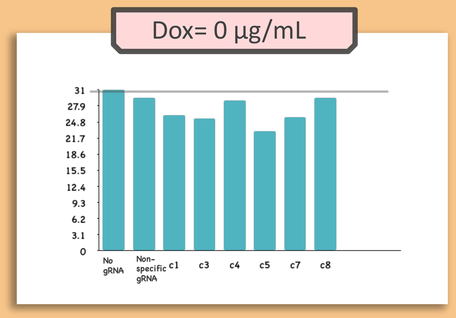
The cells I constructed crisprTFs contain Hsp90 fused with reporter (GFP). When the system work, dCas9 would be guided by specific gRNAs to suppress the expression of HSP90.

• The distribution of GFP peaks

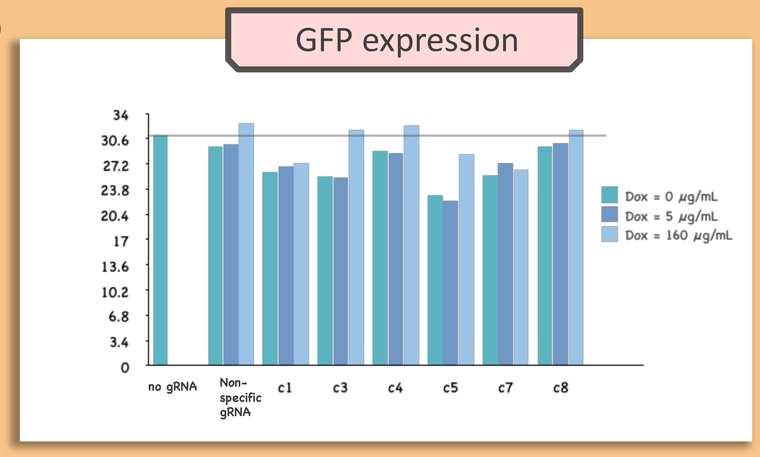


•Regulation of GFP expression by crisprTFs based on the individual gRNAs

a)

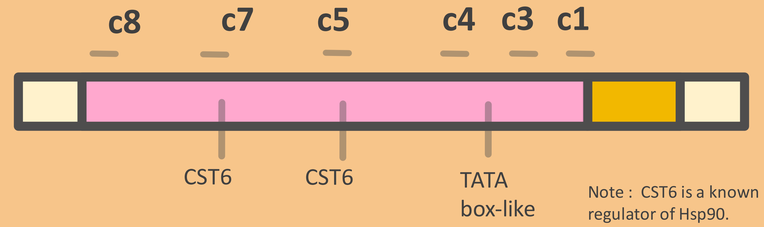


b)



a) The results show that targeting crisprTFs to sequences associated TF binding site (c5 and c7) resulted in lower *GFP* expression than the others. Moreover, crisprTFs binding to upstream of TATA box-like sequences (c1 and c3) also resulted in lower GFP expression than control.

b) Using tet off system to test the effects of crisprTFs work. The cells were cultured in the doxycycline treatment for 22 hours.



Note : CST6 is a known regulator of Hsp90.

• The design of gRNA sites

These six different gRNA binding sites that are separated between 100 to 200 bp.

Reference :
Fahim Farzadfard etal., *ACS Synth. Biol.* 2013, 2, 604-613