DNA-Binding-Domain (DBD) system

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Gibson assembly of pSB1C3+DBDCaff

The DBD-Caff construct was assembled with a linearized high copy plasmid pSB1C3, using *NEBuilder HiFi DNA Assembly Reaction*. Two strains of *E. coli*, DH5α and Top10, were transformed according to the *Transformation of chemically competent E. coli cells* protocol with the assembled plasmid. The transformed cells were plated on chloramphenicol plates. Colony PCR according to the *Colony PCR* protocol and an agarose gel according to *Agarose Gel Electrophoresis* protocol of those PCR products was performed the following day to screen the colonies and confirm if the assemblies had been carried out correctly for the two strains.

A miniprep according to the *GeneJET Plasmid Miniprep Kit, K0502* protocol was then performed the next day on the overnight cultures. In the second to last step 15 uL of elution buffer was added instead of 50 uL. The DNA concentration was measured by Nanodrop (Thermo Fisher) and the product was sent for sequencing with the *Eurofins Mix2seq kit* to determine whether the sequence had any mutations.

Gibson assembly of pSB3K3+pCadBA

The backbone pSB3K3 was linearized and divided into two samples. One sample was run on a gel and then extracted from the gel according to Thermo Fisher's *GeneJET Gel Extraction Kit* protocol (see protocol on their website). The other sample was treated with DpnI according to Thermo Fisher's *DpnI* protocol (see protocol on their website) and then underwent PCR purification according to *GeneJET PCR Purification Kit, K0701*. In the last step of the PCR purification 15 uL of the elution buffer was added to the tube and there was a two minutes incubation before centrifugation.

Both of these samples were assembled with the pCadBA promoter sequence using the *NEBuilder HiFi DNA Assembly Reaction* protocol. The samples were incubated for 60 minutes at 50 degrees in both protocols. The *E. coli* strain DH5α was transformed with both types of assembled plasmid and plated on

kanamycin plates. The next day colony PCR was performed according to the *Colony PCR* protocol on a total of 10 colonies, 2 red colonies and 8 white, which were all from the DpnI treated sample. An agarose gel was done according to the *Agarose Gel Electrophoresis* protocol with the colony PCR products to confirm the fragment insertions by size. Based on the results on the gel, a miniprep according to the *GeneJET Plasmid Miniprep kit, K0502* was done the following day on the overnight cultures of the 8 white colonies. The DNA concentration was measured by Nanodrop (Thermo Fisher) and the product was sent for sequencing with the *Eurofins Mix2seq kit* to screen the inserted fragment for mutations.

Double transformation

The two harvested plasmids *i.e* pSB1C3+DBD-Caff and pSB3K3+pCadBA were used to double transform competent *E. coli* DH5α cells according to the *Transformation of chemically competent E. coli cells* protocol. Generally a total of 10 ng DNA is used for the transformation according to the protocol, in this case that is 5 ng of each plasmid. But just in case, we also decided to try 10 ng of each plasmid, transformation with a total of 20 ng DNA to ensure successful transformation. The cells were plated on double antibiotic plates of chloramphenicol and kanamycin.

Subcloning and double transformation

The plasmids pSB3K3+DBD-Caff and pSB1C3+pCadBA were assembled by digestion and ligation, see *Subcloning: digestion and ligation protocol*. To obtain pSB3K3+DBD-Caff from pSB1C3+DBD-Caff, the backbone pSB3K3+mRFP was used. After E/P digestion, ligation and transformation, the colonies that were not red (to exclude pSB3K3+mRFP) were chosen for colony PCR according to the *Colony PCR* protocol. To obtain pSB1C3+pCadBA from pSB3K3+pCadBA, the backbone pSB1C3+AmilCP was used. After E/P digestion, ligation and transformation, the colonies that were not blue (to exclude pSB3K3+AmilCP) were chosen for colony PCR. The colony PCR products were run on an agarose gel according to the *Agarose Gel Electrophoresis* protocol to confirm the size of the integrated fragments (Figure 1).

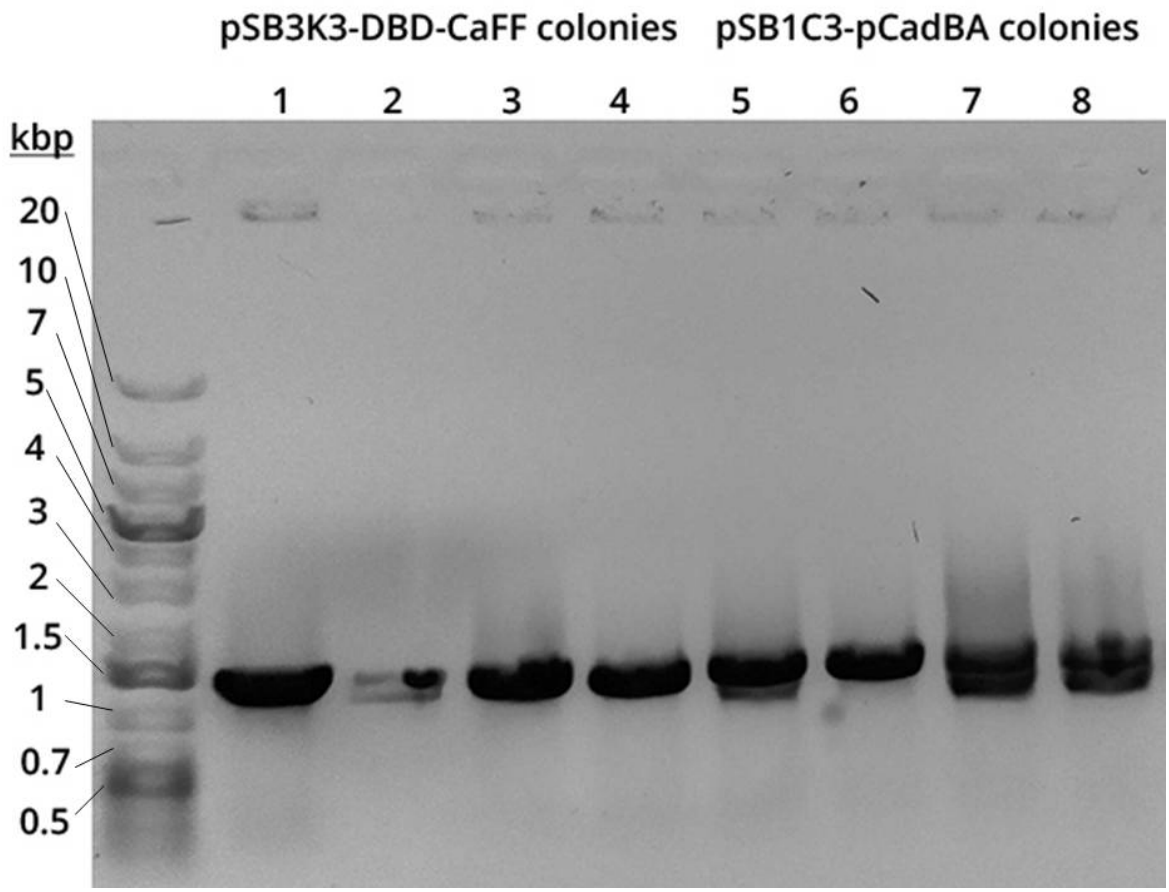

Figure 1. Gel of colony PCR products of the eight colonies (1-8). Wells 1-4 is the subcloned pSB3K3-DBD-CaFF and well 5-8 pSB1C3-pCadBA. Using standard primers VF2 and VR, the expected amplification product for both plasmids was around 1200 bp.

The following day miniprep according to *GeneJET Plasmid Miniprep Kit, K0502* protocol was done on the overnight cultures of these colonies. Again, in the second to last step 15 uL of elution buffer as added instead of 50 uL. The DNA concentration was measured by Nanodrop (Thermo Fisher) and the product was sent for sequencing with the Eurofins Mix2seq kit to test the inserted sequence for mutations. The plasmids with an intact sequence were used for double transformation of DH5α cells according to the *Transformation of chemically competent E. coli cells* protocol that were plated on chloramphenicol+kanamycin plates.

Caffeine assay

The caffeine assays were carried out according to the *Caffeine Assay* protocol with the initial constructs, pSB1C3+DBD-Caff and pSB3K3+pCadBA and then on the subcloned constructs. As negative controls, double transformed cells without caffeine were used. No positive controls were used since no other system reacting to caffeine was available. The samples were the double transformed *E. coli* with DBD-Caff and pCadBA and *E. coli* transformed with just pCadBA without DBD-Caff as the negative control. The caffeine dilutions used in the experiment were made from pure caffeine powder dissolved in ddH2O. The expression of mRFP was measured at an absorbance of 584nm, the excitation wavelength. To measure the growth of the culture, instead of using the usual 600nm absorbance, 700nm was used because mRFP expression interferes with the measurements at 600nm (Hecht et al 2016).

The proof that the system is inducible under presence of caffeine is shown in Proof of concept.

References

Hecht, A., Endy, D., Salit, M., & Munson, M. S. (2016). When wavelengths collide: bias in cell abundance measurements due to expressed fluorescent proteins. *ACS synthetic biology*, *5*(9), 1024-1027.