## UVB light inducible uniCAS Repressor (dCas9-UVR8 & COP1-KRAB devices)

You have chosen to repress a gene using a UVB light inducible dCas9-UVR8 & COP1-KRAB device. Therefore you have to order the following plasmids from the iGEM parts registry . After receiving our plasmids, you will have to clone your target sequence into our crRNA plasmid (protocol see below).

| No. | Biobrick | Device | Order |
|---|---|---|---|
| **1** | BBa_K1150029 | CMV:HA-NLS-dCas9-L3-UVR8-NLS:BGH | order |
| **2** | BBa_K1150031 | CMV:NLS-COP1-L3-KRAB-NLS:BGH | order |
| **3** | BBa_K1150022 | CMV:HA-NLS-dCas9-L3-KRAB-NLS:BGH | order |
| **4** | BBa_K1150034 | RNAimer - plasmid (crRNA - plasmid) | order |

Note:
All plasmids are optimized for expression in mammalian systems. The devices are also available containing an SV40 instead of an CMV promotor (have a look at our parts side). This system was tested mainly in CHO-K1 and HEK-293T cells.

**Design of the crRNA plasmid:**

Use our crRNA design tool to design the crRNAs that are needed to target your gene of interest.
It will generate possible target sites and the appropriate oligos. Order the oligos by the company of your choice. We recommend to test several different loci to target your gene of interest because the efficiency of different crRNA-loci can differ.

1. **Oligo annealing:** Anneal forward and reverse oligos to get the desired crRNA. Therefore mix 10 µl of 100 µM forward Oligo, 10 µl of 100 µM reverse Oligo and 80 µl of ddH2O. Heat the solution to 95° C for 5 minutes. Then turn off the heat block and let the solution cool down.
2. **Digest plasmid BBa_K1150034 with Bbs1:** The restriction enzyme Bbs1 should always be stored at -80° C. Mix about 500 ng of BBa_K1150034 with 1 µl of Bbs1, appropriate amount of buffer and fill up to 50 µl with ddH2O. Digest for exactly 3 hours at 37° C. Load the digest on a gel and cut out the DNA band (2900 bp). Purify the gel slice and use DNA for the next step.
3. **Ligate crRNAs (step 1) into Bbs1 cut backbone:** The insert (crRNAs) should be ligated into the backbone in 3 molar insert excess. Therefore use this formular: Required Volume of Insert = 3 x Volume(Backbone) x length(Insert) x concentration (Backbone) / [ length(Backbone) x concentration(Insert) ]. Use about 50 ng of backbone. The length of insert is always 30 basepairs. The length of the backbone is 2900 basepairs. You have to mix the appropriate amount of Backbone and the appropriate amount of Insert with 1 µl of T4 ligase and 2 µl of 10xT4 ligase buffer. Then fill up to 20 µl with ddH2O. This mix should be incubated for 30 minutes at room temperature.

4. **Transform** 3-5 µl of the mix following standard protocol. Pick clones, miniprep the plasmids and sequence it with pSB1C3 reverse sequencing primer (sequence: CGCCTTTGAGTGAGCTGATACCGC).

Now that you have created the desired crRNA plasmids it is possible to use them indiviually or fuse different crRNA loci together into one crRNA plasmid (recommended).

**Design of a multiple target crRNA plasmid:**

It is shown that multiple targeting of one gene of interest increases the efficiency of regulation. If you want to fuse different crRNA loci together into one plasmid use the following protocol:

1. **First of all digest crRNA plasmid** with PstI and SpeI in order to linearize it. Both enzymes cut in the suffix. Using the BioBrick Assembly method, it is possible to assemble multiple crRNA sequences into one plasmid. Therefore mix about 500 ng Backbone with 1 µl enzyme 1 and 1 µl enzyme 2, add an appropriate amount of compatible buffer and fill up to 30-50 µl. Incubate mix at 37° C for 2 hours.
2. **Secondly digest crRNA plasmid** with PstI and XbaI. Using the BioBrick Assembly method, this is your insert (procedure see above).
3. Load digests on a gel and cut out the DNA bands (Backbone 2900 bp, Insert 870 bp). Purify the gel slice and use DNA for the next step.
4. **Ligate** the insert in 3 molar excess into the backbone (formular see paragraph above).
5. **Transform** 3-5 µl of the mix following standard protocol. Pick clones, miniprep the plasmids and sequence it with pSB1C3 forward sequencing primer (sequence: GAGTGCCACCTGACGTCTAAGAAAC) and pSB1C3 reverse sequencing primer (sequence: CGCCTTTGAGTGAGCTGATACCGC).
6. These steps can be repeated various times. Using this method, you can engineer a plasmid with several crRNA targets!

**Experimental design (recommendation for mammalian cell culture):**

For UVB light experiments transfect the following plasmid combinations on two different well plates. One plate will be illuminated with 311 nm at 5 µE for 24 hours. This is the UVB light induced plate. The other plate will be wrapped in aluminum foil. This is the no-induction control. See our light induction project page.

1. **Transfect** BBa_K1150029, BBa_K1150031 (4 fold excess) with all desired crRNA plasmids (seperate crRNA plasmid and/or multiple crRNAs plasmid)
2. **Off target control:** Transfect BBa_K1150029, BBa_K1150031 (4 fold excess)without any crRNA plasmid.
3. **Target efficiency control:** Transfect BBa_K1150022 (4 fold excess) with the same crRNA plasmids as in the first transfection.