**CRISPR. Adapted from:** Y. Li *et al.*, “Metabolic engineering of Escherichia coli using CRISPR–Cas9 meditated genome editing,” *Metab. Eng.*, vol. 31, pp. 13–21, Sep. 2015, doi: 10.1016/J.YMBEN.2015.06.006.

Note: Two different sgRNA sequence used in different experiments:

* sgRNA1: 5’ - promoter – ATTCCGCGTAAGTATCGCGG– scaffold – terminator 3’
* sgRNA2: 5’ - promoter – CGTACAAAAGTACGTGAGGA– scaffold – terminator 3’

Day 1:

1. Start an overnight culture (37°C) by inoculating LB medium from a single colony.

Day 2:

1. Prepare competent cells following your favourite protocol.
2. Transform *E. coli* cells with plasmid pREDCas9 and plate the cells at 30°C in LB supplemented with 50 µg/mL spectinomycin.

Day 3:

1. Start an overnight culture at 30°C in LB/Spec from a single colony.

Day 4:

1. Next morning refresh the culture with LB/Spec and grow the cells until OD600 reaches 0.1.
2. Add IPTG to a final concentration of 2mM and grow the cells to an OD600 =0.6 (recombination proteins are being expressed at this point).
3. Frost cells on ice for 20 minutes and electrocompetent cells are then prepared by washing bacteria with ice-cold milliQ water after spinning aliquots 10 min at 5000 rpm in a 4°C centrifuge. (Heat shock transformation also could be performed)
4. After 2 washes, resuspend cells in the residual milliQ water and electroporated with pEC-CRISPR2-BC.
5. After zapping cells, add 950μl of fresh LB without antibiotics and resuspend cells. Incubate cells 1h at 30°C.
6. Then, spread 100μL of bacterial culture on LB plates supplemented with Spec (50 µg/mL) /Amp (100 µg/mL).

Day 5:

1. Check colonies for barcode presence by colony-PCR
2. Inoculate a positive clone in 2mL of LB/Spec/Ara (30mM) (sgRNA targeting pUC origin in pEC-CRISPR2-BC is expressed)
3. Grow for 4-6h
4. Plate on LB/Spec

Day 6:

1. Check some colonies for Ampicillin resistance by restreaking them on LB/Amp

Day 7:

1. Take a sensitive clone and restreak it on LB plates at 37°C.

Day 8:

1. Check again by colony PCR the presence of the barcode and restreak on LB/Spec and LB/Amp plates to double check plasmid curing.