# High through-put CRISPRi screen

The protocol described here was used in Cui et al., Nat. Comm. 2018. The growth conditions provided here are just an example. Screens can be performed in any condition as long as cells grow for a sufficient number of generations in order to obtain strong fold changes in the abundance of guide RNAs. We recommend a minimum of 12 generations.

1. Thaw a tube of LC-E75 cells carrying the psgRNA library (LC-E75 is as strain with pTet\_dCas9 in the chromosome).
2. Dilute the cells (1:100) into 100 ml LB without antibiotics. Check the OD600 value (it should be between 0.001 and 0.01).
3. Grow the cells at 37°C with shaking until the OD600 reaches around 0.2. Take 50 ml of culture as the Time 0 sample.
4. add aTc to a final concentration of 1 uM to induce dCas9 expression.
5. Perform the following step 3 times:
   1. Keep the cells growing at 37°C until the OD600 reaches around 2. Take 5 ml of culture and perform plasmid extraction immediately. Take 1 ml of the culture and dilute into 100 ml of LB with aTc (1 uM) to grow the cells for more generations.

Note:

* It might seem convenient to spin cells and freeze pellets in order to perform the miniprep for all time points all at once. We recommend to avoid this, as we have noticed that the recovered plasmid DNA yields illumina libraries of poor quality.