24-08-19

OBJECTIVE: To clone the selected transcriptional regulators (see list below, copied from 17-08-19, and see also PCR primer design entry 18-08-19) into pBAD18.

Today – aim to check PCR products from 23-08-19, set up KpnI-XbaI digests of PCR products and vector, and then purify and set up overnight ligations.

PCR #	Gene	Expected size (bp)
1	*leuO*	945
2	*fnr*	753
3	*fur*	447
4	*crp*	633

Made 6 agar plates (LB/amp100) to plate out ligations after cloning. (See recipe from 05-07-19.)

Made a small 1.0% agarose gel (TAE) and ran yesterday's PCRs on an agarose gel: 5 µl each PCR reaction + 2 µl loading dye, with the Promega 100bp DNA ladder (https://www.promega.co.uk/products/cloning-and-dna-markers/molecular-weight-markers/100bp-dna-ladder/?catNum=G2101), at 100V, until the dye front reached the end of the gel. Original file from gel-doc saved at User/home/datafiles/DNA-gels/24-08-19.jpg

PCR4 produced a band of the expected size (~600 bp). Stored at -20°C in Freezer 3 until ready to proceed with cloning.

Need to repeat PCRs 1-3. For template DNA, will try colony PCR again – try a different annealing temperature or perhaps a gradient PCR?

Streaked *E. coli* K-12 strain MG1655 on LB agar for tomorrow, incubated O/N 37°C – will use as a template for colony PCRs. If this doesn't work, will try purifying genomic DNA and using that as a template.