April 29th

LR - (1/3 of cloning procedure)

our plates in incubator too long

→ use previous group's plates

they did LR to insert gene of interest

expression. gene & promoter lined up

→ will express proteins

implies we used gateway (LR)

↓

putting together 2 entry vectors

↓

have 1 thing
- either gene or promoter

destination vector has everything else

↓

antibiotic backbone

ours have CCDB (removed during LR)

Qiagen (for miniprep)

we digested them to make sure plasmid has everything it needs

ran a gel & compared

tagBFP - blue
mKate - red
TRET - inducible promoter

↳ induced by dox & rtta

CMV - constitutive promoter from a virus

Golden Gate

PCR.

amplify both halves of gene separately
so we can put them back together

it's not recombination, it's cutting w/ 2S enzymes
- it's cut to side of recognition site
- we use BSAI a lot
- still produces 4 base overhang

cool things it can do → pushes rxn till done
- cut it's recognition site out
- you can choose the overhangs
- no scars

backbone = Golden Gate donor (standard)
entry vector
- LacCd
- when cell grows on +Gal } selection marker
  it turns blue