・ Lab note

6/22~7/24: PCR(Dronpa145N,Dronpa145K)

7/25~7/28: Digested PCR Product of Dronpa 145N,K(6/22) and pCold Vector

7/29~7/31:Ligation (Dronpa145N +pCold Vector),and transformation the ligation product.

8/01: Get three clonies that brite under UV light, pick up these colonies and incubate in iquide culuture

8/02: Miniprep the clones and digest plasmids to confirm we get correct clones

8/03: purified plasmid(Dronpa 145N+pCold)

8/04: transform RFP to Ecoli

8/05: Glycerol stock clones that have plasmid(Dronpa 145N+pcold)

8/06: digested PCR Product of Dronpa 145N,K(7/29) by Ecor I and Pst I

8/07: After miniprep RFP clone, Digested the plasmid (RFP+pSB1C3) by EcoR I and Pst I ,Ligation (pSB1C3+Dronpa 145N,K),transform the ligation product to Ecoli

8/08~8/12: incubated clones (pCold+Dronpa145N) to exprese Dronpa 145N

8/13: digested plasmid (pCold+Dronpa145N) by EcoR I and Xho I

8/14: Coldshock expression of Dronpa 145N, PCR Dronpa145K

8/15: His-tag protein purification of 145N, Restriction enzyme treatment Dronpa145K, Gel Purification Dronpa145K, Ligation 145K+pColod

8/16:

8/17:colonyPCR , DNA extraction(N), colonyPCR , DNA extraction(N)

8/18: SDS-PAGE of 145N, sequence(N)

8/19: Coldshocck expression of Dronpa 145K, colony PCR(K), DNA extraction, sequence

8/20 :His-tag protein purification of 145K

8/21 :SDS-PAGE of 145N and 145K, colony PCR(K)

8/22 :Native-PAGE of 145N and 145K (kenntousuru of 500 nm irradiation time), miniprep pre culture(K)

8/23: miniprep(K)

8/24:

8/25: PCR GFP(E0240)

8/26:

8/27: Electrophoresis N-Nvector+HSP, Restriction enzyme treatment N-Nvector+HSP(XhoI+PstI or SpeI), Electrophoresis

8/28: Restriction enzyme treatment N-Nvector+HSP(SpeI+PstI or SpeI+HindIII), Electrophoresis

8/29: plasmid PCR(K),DNA extraction(K)

8/30 :Native-PAGE of 145N and 145K (of 400 nm irradiation time)

8/31 :Native-PAGE of 145N and 145K, PCR N-Nvector+Luciferase, Electrophoresis

9/01 :Coldshock expression of 145N and 145K, Ligation N-Nvector+luciferaze and pSB1C3+plac, Transformation

9/02 :His-tag protein purification of 145N and 145K, sequence(K), Colony PCR, Restriction enzyme treatment HG(PstI+EcoRI) and plac+145N(PstI+EcoRI), Electrophoresis

9/03 :Native-PAGE of 145N and 145K, colonyPCR(K), Miniprep (L), Electrophoresis, PCR Luciferase(K325210), Electrophoresis

9/04 :Observe of 145N and 145K in E.coli using the Confocal laser scanning microscope, plasmidPCR(BBa_B0012),DNAextraction(Bba_B0012)

9/05: sequence(BBa_B0012)

9/06 :Measure of 145N and 145K fluorescence intensity

9/07 :PCR,luciferase, after digested ,insert pcold and transformation, Miniprep (L10, L5, PC), Electrophoresis, Restriction enzyme treatment L10, L5, PC(SpeI+HindIII and XhoI+PstI)

9/08 :minprep and check insert, transformation(Bba_B0012), Electrophoresis Lp,, Restriction enzyme treatment Lp(EcoRI+PstI), Electrophoresis

9/09: miniprep(Bba_B0012), Cytoclasis Lp, Miniprep HSP, Electrophoresis, Protein purification L10, L5

9/10: DNA Extraction(Bba_B0012), Miniprep plac-145N, Electrophoresis

9/11: Protein purification L10, L5, L0

9/12: Miniprep plac-145N, Electrophoresis,

9/13: Electrophoresis plac-145N, Gel purification

9/14:

9/15 :Observe of 145N and 145K in E.coli using the Confocal laser scanning microscope, Cell destruction L10, L5, L0

9/15 :Measure of 145N and 145K fluorescence intensity

9/16 :Native-PAGE of 145N and 145K, Functional test L10, L5, L0

9/17 :Observe of 145N and 145K in E.coli using the Confocal laser scanning microscope

9/18 :SDS-PAGE of 145N and 145K