Second device assembly		
	BBa_K119010:p.(TetR+luxR)+luxR/p.(luxR+AHL)+cI*/p.lacZ+aiiA→Prk415 **BBa_K11901: p.(TetR+luxR)+luxR/p.(luxR+AHL)+cI*/p.lacZ*+aiiA→Prk415**	
STEPS	**DETAILS**	**PROBLEMS**
Primer design	**Our oligos were design specifically, so we could change GFP from Chiba biopart an put CI there** **Also, this oligos were bearing sites for restriction digests.** **EcoRI-LuxR-XbaI-CI-HindIII-aiia-PstI**	
Extract DNA from the registry	**Get alredy assembled biobricks we will use**	
Ask Chiba to send their biopart		**But there were no DNA**
Ask MIT for CI biopart		**We waited some time**
PCR	**Amplified bioparts and modified Chiba biopart** **Using specific oligonucleotides, getting 3 parts (LuxR and aiia from Chiba) and CI from MIT**	
Chek	**Check our PCR product with electrophoresis on agarose gel**	
Cloning into pJET	**Blunting PCR product. pJET vector is already blunted** **Ligation:Using rapid ligation protocol, T4 DNA ligase and blunt-end ligation**	
Transformation	**Transformation: E. Coli - DH5α by heat-shock**	
Check	**Extraxt plasmid for selected colonies** **Restriction digest of all colony extractions** **PCR with specific primers for our device** **Check the Restriction products and PCR on agarose gel**	
Extraction	**Plasmid extraction from confirmed colonies**	
Purification	**Doble restriction digest of plasmids to extract our cloned device** **Gel band purification of restricted bioparts**	
Assembly of the complete device	**Ligation of the three individual devices** **Sticky end ligations**	**We just be able to ligate two of three parts** **One oligo got bound somewhere in aiia biopart.**
Cloning into pBBR1MCS-5	**Restriction Digest of pRK415 vector** **Ligation: pRK415 + first bioparts** **Ligation: prk415 + ligation of two bioparts**	**We had a lot of troubles trying to clone our bioparts in PRK**
Transformation	**Transformation: E. Coli - DH5α by heat-shock**	
Extraction	**Extract plasmid for selected colonies (Xgal control)**	**Very few white colonies.**
Check	**Restriction digest of all colony extractions** **PCR with specific primers for our device** **Check the Restriction products and PCR on agarose gel**	**Many false positives with Xgal control**
Alternatives **(Not realized)**	**Ligate all the bioparts in one, using a rapid ligation protocol, keeping the sites for pJet oligos** **Hybrid plasmid: a plasmid made by the joint of two different plasmids, pJet and PRK with DNA ligation using shrimp alkaline phosphatase**	**Not done**
Transformation of the final receptor cell	**Transformation of E. Coli Yoh- with the asembled device**	**Not done**
Check	**Extraxt plasmid for selected colonies** **Restriction digest of all colony extractions** **PCR with specific primers for our device** **Check the Restriction products and PCR on agarose gel** **Add sufix and prefix** **Clonate the device in a standard plasmid** **Get sequence of our final construction**	**Not done**