First device assembly	BBa_K119009 - p.(cI + RcnR) + rcnA on pBBR1MCS-5	
STEPS	**DETAILS**	**PROBLEMS**
Primer design	To assemble our designed device: p.(cI + RcnR) + rcnA	
Genomic DNA extraction	From k12 strain	
PCR	Add the restriction sites and cI promoter to the natural part	Delay on primeres delivery
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 - DH5a 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 BBa_k119009 biopart	
Cloning into pBBR1MCS-5	Restriction Digest of pBBR1MCS-5 vector Ligation: pBBR1MCS-5 + BBa_K119009 biobrick	
Transformation	Transformation: E. Coli - DH5α by heat-shock	
Extraction	Extract plasmid for selected colonies (Xgal control)	
Check	Restriction digest of all colony extractions PCR with specific primers for our device Check the Restriction products and PCR on agarose gel	
Purification	Plasmid extraction from confirmed colonies Gel band purification of pBBR1MCS-5 + BBa_K119009	
Transformation of the final receptor cell	Transformation of E. Coli Yoh- with the asembled device	
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	