Part Collection

Included below is the entire part collection synthesised *in silico* by the St Andrews iGEM team 2020. For more information about the parts, the hyperlink in part number can be accessed which discusses the components further in their respective iGEM registry pages. All parts sequences were edited using SnapGene before addition to the registry.

Part Number	Part Name	Description	Reference
BBa_K3634000	3-Dehydroquinate Synthase (DHQS)	Codon optimised 3-dehydroquinate synthase (DHQS) CDS for use in *E.coli* as part of the shinorine synthesis gene cluster. The original gene for DHQS (Ava_3858) can be taken from the cyanobacteria species Anabaena variabilis ATCC 29413. The enzyme catalyses the conversion of sedoheptulose 7-phosphate, an intermediate of the pentose phosphate pathway (PPP), to (R)-demethyl-4-deoxygadusol.	Minnesota iGEM 2012 - http://parts.igem.org/Part:BBa_K814000
BBa_K3634001	O-Methyltransferase (O-MT)	Codon optimised O-methyltransferase (O-MT) CDS for use in *E.coli* as part of the shinorine synthesis gene cluster. The original gene for O-MT (Ava_3857) can be taken from the cyanobacteria species Anabaena variabilis ATCC 29413. The enzyme catalyses the conversion of (R)/(S)-demethyl-4-deoxygadusol, the product of the first step of the shinorine pathway, to 4-deoxygadusol.	Minnesota iGEM 2012 - http://parts.igem.org/Part:BBa_K814002
BBa_K3634002	ATP-Grasp (ATPG)	Codon optimised adenosine triphosphate (ATP)-grasp (ATPG) CDS for use in *E.coli* as part of the shinorine synthesis gene cluster. The original gene for ATPG (Ava_3856) can be taken from the cyanobacteria species Anabaena variabilis ATCC 29413. The enzyme catalyses the conversion of 4-deoxygadusol (4-DG), produced by O-MT in the previous step of the reaction, to the mycosporine-like amino acid (MAA) mycosporine glycine. ATPG is proposed to phosphorylate 4-DG before conjugate addition occurs at the cyclohexanone ring by the nitrogen of glycine.	Minnesota iGEM 2012 - http://parts.igem.org/Part:BBa_K814001 Balskus, E.P, Walsh, C.T. 2010. The Genetic and Molecular Basis for Sunscreen Biosynthesis in Cyanobacteria. Science. 329 (5999): p1653-1656. DOI:

			10.1126/science.1193637
BBa_K3634003	Nonribosomal Peptide Synthetase (NRPS)	Codon optimised nonribosomal peptide synthetase (NRPS) CDS for use in *E.coli* as the final part of the shinorine synthesis gene cluster. The original gene for NRPS (Ava_3855) can be taken from the cyanobacteria species Anabaena variabilis ATCC 29413. The enzyme catalyses the conversion of mycosporine glycine to the final product shinorine. NRPS has three known domains: an adenylation domain, a thiolation domain and a thioesterase domain. Initially, a serine amino acid is converted into an acyl adenylate species which is then later attacked by a serine residue present on the thiolation domain of NRPS. Adenosine monophosphate is then lost from the intermediate followed by imine formation, thought to occur through an enol ester intermediate and an O-N rearrangement by conjugate addition of the serine nitrogen to the cyclohexenimine ring.	Minnesota iGEM 2012 - http://parts.igem.org/Part:BBa_K814003 Balskus, E.P, Walsh, C.T. 2010. The Genetic and Molecular Basis for Sunscreen Biosynthesis in Cyanobacteria. Science. 329 (5999): p1653-1656. DOI: 10.1126/science.1193637
BBa_K3634004	4-DG Pathway	The following part describes the constitutive expression of the first two enzymes of the shinorine production pathway, optimised for use in *E.coli.* Within the composite part, biobricks BBa_K3634000 (DHQS) and BBa_K3634001 (O-MT) are responsible for converting sedoheptulose 7-phosphate to 4-deoxygadusol. Once produced, 4-deoxygadusol will then be converted by BBa_K3634005 (ATPG and NRPS composite, present on 'plasmid A') to the final product of the pathway, shinorine. The shinorine production pathway is separated in this way as a biosafety mechanism so that UV resistance is not conferred in a bacteria which has lost or gained plasmid A/B alone. As NRPS is determined to be 'rate-limiting' with respect to the pathway, plasmid A will ideally be placed at a higher copy	Balskus, E.P, Walsh, C.T. 2010. The Genetic and Molecular Basis for Sunscreen Biosynthesis in Cyanobacteria. Science. 329 (5999): p1653-1656. DOI: 10.1126/science.1193637

		number to plasmid B to ensure 1:1 stoichiometry of reactants.	
BBa_K3634005	ATPG and NRPS Composite	The following part describes the constitutive expression of the final two enzymes of the shinorine production pathway, optimised for use in *E.coli.* Within the composite part, biobrick BBa_K3634002 (ATPG) and BBa_K3634003 (NRPS) are responsible for converting 4-deoxygadusol (4-DG) to the final product shinorine. Once produced, this mycosporine-like amino acid can absorb UV radiation of wavelength ~333nm. The composite part described will be present on 'plasmid A', separate to the previous enzymes responsible for 4-deoxygadusol production which are maintained on 'plasmid B'. By this arrangement, biosafety of our gene circuit will be ensured such that UV resistance is not conferred in a bacteria which has lost or gained plasmid A/B alone. As NRPS is determined to be 'rate-limiting' with respect to the pathway, plasmid A will ideally be placed at a higher copy number to plasmid B to ensure 1:1 stoichiometry of reactants.	Balskus, E.P, Walsh, C.T. 2010. The Genetic and Molecular Basis for Sunscreen Biosynthesis in Cyanobacteria. Science. 329 (5999): p1653-1656. DOI: 10.1126/science.1193637
BBa_K3634006	ccaS	The membrane-associated histidine kinase ccaS is part of the two-component system (TCS) involved in the eventual transcriptional output of an adjacent phycobilisome-related gene (cpcG2) in response to green light of wavelength 535nm. The system is native to Synechocystis sp. PCC6803 but has been successfully expressed in *E.coli* (Hirose et al. 2008) and has been further used in multichromatic control of gene expression (Tabor et al. 2011). CcaS, alongside the response regulator ccaR, functions as a photoreversible switch between green (535nm) and red (672nm) light by regulation of the output promoter PcpcG2. Within the N-terminal GAF domain of ccaS, the	Hirose Y., Shimada T., Narikawa R., Katayama M., Ikeuchi M. 2008. Cyanobacteriochrome CcaS is the green light receptor that induces the expression of phycobilisome linker protein. PNAS. 105(28): p9528-9533. DOI: 10.1073/pnas.0801826105

		blue phycobilin chromophore phycocyanobilin (PCB) binds to a conserved cysteine residue, imparting reversible photoactivation of signalling activity. Absorption of green light increases the rate of ccaS autophosphorylation, phosphotransfer to ccaR and transcription from PcpcG2. Absorption of red light by ccaS is shown to reverse the process and therefore reduce expression of the output gene. CcaS is also shown to be inactive in the dark.	Tabor J.J., Levskaya A., Voigt C.A. 2011. Multichromatic control of gene expression in Escherichia coli. J Mol Biol. 405(2): p315–324. DOI: 10.1016/j.jmb.2010.10.038
BBa_K3634007	Heme Oxygenase (ho1)	Heme oxygenase (ho1) is the first of two required genes for the conversion of heme into the blue phycobilin phycocyanobilin (PCB), a chromophore required for activation of some two component light-sensing systems (TCS) such as the ccaS/ccaR system in Synechocystis sp. PCC 6803. In the presence of oxygen, heme oxygenase catalyses the opening of the heme ring, releasing iron and generating biliverdin IXα which is then reduced by phycocyanobilin ferredoxin oxidoreductase (pcyA) to produce PCB. PCB can then bind to the appropriate membrane associated histidine kinase of the TCS allowing activation and expression of the output gene in the presence of an activating wavelength of light.	Tabor J.J., Levskaya A., Voigt C.A. 2011. Multichromatic control of gene expression in Escherichia coli. J Mol Biol. 405(2): p315–324. DOI: 10.1016/j.jmb.2010.10.038 UTAustin iGEM 2004 - http://parts.igem.org/Part:BBa_I15008
BBa_K3634008	Phycocyanobilin: Ferredoxin Oxidoreductase (pcyA)	The gene pcyA codes for the functional phycocyanobilin:ferredoxin oxidoreductase protein, the second enzyme required for the conversion of heme to the blue phycobilin phycocyanobilin (PCB); a chromophore required for activation of some two component light-sensing systems (TCS) such as the ccaS/ccaR system in Synechocystis sp. PCC 6803. The protein catalyses the four-electron reduction of biliverdin IXα, previously produced from heme by heme oxygenase, to PCB, which then binds to the appropriate membrane associated	Tabor J.J., Levskaya A., Voigt C.A. 2011. Multichromatic control of gene expression in Escherichia coli. J Mol Biol. 405(2): p315–324. DOI: 10.1016/j.jmb.2010.10.038 UTAustin iGEM 2004 -

		histidine kinase of the TCS allowing activation and expression of the output gene in the presence of an activating wavelength of light.	http://parts.igem.org/Part:BBa_I15009
BBa_K3634009	ccaS + ho1 + pcyA	CcaS, alongside the response regulator ccaR, functions as a photoreversible switch between green (535nm) and red (672nm) light by regulation of the output promoter PcpcG2. Within the N-terminal GAF domain of ccaS, the blue phycobilin chromophore phycocyanobilin (PCB) binds to a conserved cysteine residue, imparting reversible photoactivation of signalling activity. Absorption of green light increases the rate of ccaS autophosphorylation, phosphotransfer to ccaR and transcription from PcpcG2. Absorption of red light by ccaS is shown to reverse the process and therefore reduce expression of the output gene. CcaS is also shown to be inactive in the dark. The required PCB chromophore is produced from heme by the following two enzymes. In the presence of oxygen, heme oxygenase (ho1) catalyses the opening of the heme ring, releasing iron and generating biliverdin IXα. The second enzyme, phycocyanobilin:ferredoxin oxidoreductase (pcyA), then catalyses the four-electron reduction of biliverdin IXα to PCB. PCB then binds to the N-terminal GAF domain of ccaS, allowing for transcriptional output of the gene of interest. BBa_K3634006, BBa_K3634007 and BBa_K3634008 are combined here as a composite part with the associated regulatory regions used by Schmidl et al. (2014). The system they produced, termed CcaSR v2, uses two plasmids for constitutive expression of ccaS and ccaR, and optimised promoter and RBS combinations to maximise PCB production. The design will be recreated in composite BioBrick part	Hirose Y., Shimada T., Narikawa R., Katayama M., Ikeuchi M. 2008. Cyanobacteriochrome CcaS is the green light receptor that induces the expression of phycobilisome linker protein. PNAS. 105(28): p9528-9533. DOI: 10.1073/pnas.0801826105 Tabor JJ., Levskaya A., Voigt CA. 2011. Multichromatic control of gene expression in Escherichia coli. J. Mol. Biol. 405(2): p315-324. DOI: 10.1016/j.jmb.2010.10.038 Schmidl SR., Sheth RU., Wu A., Tabor JJ. 2014. Refactoring and Optimisation of Light-Switchable Escherichia coli Two-Component Systems. ACS Synth. Biol. 3: p820-831. DOI: 10.1021/sb500273n

		form using *E.coli* codon optimised parts to further promote efficiency of the system.	Ong NT., Tabor JJ. 2018. A Miniaturized Escherichia coli Green Light Sensor with High Dynamic Range. ChemBioChem. 19: p1255-1258. DOI: 10.1002/cbic.201800007
BBa_K3634010	lacO- + lacP	The lac operon found in *E.coli* consists of the three lactose metabolising genes lacZ, lacY and lacA which when expressed, allow the bacteria to use the sugar as a source of energy. The initial regulatory mechanisms in the pathway were outlined by Jacob and Monod in 1961, where the topic of inducible and repressible enzyme systems was discussed. In this system, the transcriptional repressor is a protein known as Lac I which binds to DNA at various operator sequences (termed O1, O2 and O3) which exist both upstream and downstream of the transcriptional start site (TSS). Interaction between the Lac I and operator sequences reduces transcription of the downstream lactose metabolising genes unless relieved by the lactose isomer allolactose. In the absence of Lac I, transcription is constitutive and can be further activated by the catabolite activator protein (CAP), with binding site upstream of the promoter sequence. Oehler et al. (1990) mutated each individual operator sequence respectively and then determined the effect of repression by Lac I to which they found mutation in O1 (downstream of the promoter) sufficient to lose almost all total repression. Mutation of O2 and O3 further decreased repression by Lac I	Jacob F., Monod J. 1961. Genetic Regulatory Mechanisms in the Synthesis of Proteins. J. Mol. Biol. 3: p818-356. Gilbert W., Maxam A. 1973. The Nucleotide Sequence of the lac Operator. Proc. Nat. Acad. Sci. USA. 70(12): p3581-3584. Oehler S., Eismann E.R., Krämer H., Müller-Hill B. 1990. The three operators of the lac operon cooperate in repression. EMBO J. 9(v): p973-979.

		70 fold. Here, the St Andrews iGEM team 2020 aimed to utilise these findings to create a regulatory region solely under the control of glucose concentration to allow expression of the toxin ccdB.	
BBa_K3634011	ccdB (BsaI Removed)	The ccdAB toxin-antitoxin (TA) module is a type II TA module where the toxic ccdB protein, poisons the enzyme DNA gyrase, required for negative supercoiling of DNA (Bernard and Couturier, 1992). Through ccdB-gyrase complex formation, DNA cleavage results as well as inhibition of transcription by the formation of RNA polymerase roadblocks. The activity of the unstable ccdA antitoxin separates the ccdB-gyrase complex if present (Vandervelde et al, 2017).	Bernard P., Couturier M. 1992. Cell killing by the F plasmid CcdB protein involves poisoning of DNA-topoisomerase II complexes. J. Mol. Biol. 226: p735–745. Vandervelde A., Drobnak I., Hadži S., Sterckx Y.GJ., Welte T., Greve. H.D., Charlier D., Efremov R., Loris R., Lah J. 2017. Molecular mechanism governing ratio-dependent transcription regulation in the ccdAB operon. NAR. 45(6): p2937-2950. DOI: 10.1093/nar/gkx108
BBa_K3634012	Glucose-Mediated Death Sensor	The 'glucose-mediated death sensor' is a vital part of the St Andrews iGEM 2020 kill switch as when glucose is absent, greater expression of ccdB will overcome intracellular ccdA concentrations causing transcriptional inhibition and DNA cleavage as previously discussed. Expression of ccdB will also relieve the associated ccdAB promoter from ccdA binding	Jacob F., Monod J. 1961. Genetic Regulatory Mechanisms in the Synthesis of Proteins. J. Mol. Biol. 3: p818-356.

		which consequently, will allow for cviJI endonuclease expression to chew up the integrated plasmid constructs and target restriction sites within the genome.	Gilbert W., Maxam A. 1973. The Nucleotide Sequence of the lac Operator. Proc. Nat. Acad. Sci. USA. 70(12): p3581-3584. Oehler S., Eismann E.R., Krämer H., Müller-Hill B. 1990. The three operators of the lac operon cooperate in repression. EMBO J. 9(v): p973-979. Bernard P., Couturier M. 1992. Cell killing by the F plasmid CcdB protein involves poisoning of DNA-topoisomerase II complexes. J. Mol. Biol. 226: p735–745. Vandervelde A., Drobnak I., Hadži S., Sterckx Y.GJ., Welte T., Greve. H.D., Charlier D., Efremov R., Loris R., Lah J. 2017. Molecular mechanism governing ratio-dependent transcription regulation in the ccdAB operon. NAR. 45(6): p2937-2950.

BBa_K3634013	ccdAB promoter + operator	Expression of ccdA and ccdB within the type II TA module is self-regulated by low specificity and affinity of ccdA for individual binding sites of the regulatory region upstream of both ccdA and ccdB genes. This 113bp ccdAB promoter/operator sequence extends into the first ccdA gene and is suspected to have a total of 8 ccdA operator binding sites (Tam & Kline, 1989). The antitoxin binds the operator DNA sites, with some sites overlapping the promoter, functioning as a repressor of ccdAB transcription. The toxin then functions as a co-repressor or de-repressor depending on the molar T:A ratio (Vandervelde et al., 2017).	Tam J.E., Kline B.C. 1989. Control of the ccd operon in plasmid F. J. Bacteriol. 171: p2353–2360. Vandervelde A., Drobnak I., Hadži S., Sterckx Y.GJ., Welte T., Greve. H.D., Charlier D., Efremov R., Loris R., Lah J. 2017. Molecular mechanism governing ratio-dependent transcription regulation in the ccdAB operon. NAR. 45(6): p2937-2950.
BBa_K3634014	ccdAB-Controlled mf-Lon Protease	Expression of ccdA and ccdB within the type II TA module is self-regulated by low specificity and affinity of ccdA for individual binding sites of the regulatory region upstream of both ccdA and ccdB genes. This 113bp ccdAB promoter/operator sequence extends into the first ccdA gene and is suspected to have a total of 8 ccdA operator binding sites (Tam & Kline, 1989). The antitoxin binds the operator DNA sites, with some sites overlapping the promoter, functioning as a repressor of ccdAB transcription. The toxin then functions as a co-repressor or de-repressor depending on the molar T:A ratio (Vandervelde et al., 2017). At low T:A ratio, the operator is repressed however as the ratio is increased, repression is relieved by preferential formation of a V-shaped non-repressing heterohexamer (ccdB-	Tam J.E., Kline B.C. 1989. Control of the ccd operon in plasmid F. J. Bacteriol. 171: p2353–2360. Gur E., Sauer R.T. 2008. Evolution of the ssra degradation tag in mycoplasma: specificity switch to a different protease. PNAS. 105(42): p16113– 16118. Cameron D.E., Collins J.J. 2014. Tunable protein degradation in bacteria. Nat.

		ccdA-ccdB). Specifically, at the moment the molar ratio of T:A > 1, repression is rapidly lost (Vandervelde et al., 2017). Therefore the regulatory region, controlled by intracellular ccdAB concentrations, can be used for precise control of a desired output gene as shown in the native system. Here, the output protein expressed will be mf-Lon protease (BBa_K2333011). mf-Lon protease is evolutionary related to the native Lon protease in *E.coli* and functions similarly in that recognition of a specific degradation tag allows for rapid endogenous breakdown of the protein to which it was attached. The two enzymes differ in their ability to recognise these specific tags; mf-Lon protease cannot recognise degradation tags associated to *E.coli* lon protease and vice versa. By the ability to alter the strength of signals associated with these degradation tags through sequence specific alterations (Collins et al., 2014), intracellular concentrations of proteins can be precisely modulated.	Biotech. 32(12): p1276–1281. Vandervelde A., Drobnak I., Hadži S., Sterckx Y.GJ., Welte T., Greve. H.D., Charlier D., Efremov R., Loris R., Lah J. 2017. Molecular mechanism governing ratio-dependent transcription regulation in the ccdAB operon. NAR. 45(6): p2937-2950.
BBa_K3634015	LacI + mf-Lon Degradation Tag	BBa_K3634015 is a fusion between the lacI gene and the mf-Lon degradation tag, a specific sequence recognised by the mf-Lon protease which allows for rapid endogenous breakdown of the protein to which it is attached. The degradation tag used here is the strongest characterised sequence for the specific protease (BBa_K2333001) and will allow for efficient removal of Lac I from the cell when mf-Lon protease is expressed (BBa_K3634014).	Jacob F., Monod J. 1961. Genetic Regulatory Mechanisms in the Synthesis of Proteins. J. Mol. Biol. 3: p818-356. Oehler S., Eismann E.R., Krämer H., Müller-Hill B. 1990. The three operators of the lac operon cooperate in repression. EMBO J. 9(v): p973-979.
BBa_K3634016	P_{laclq} & LacI Repressor + mf-	Here, alongside a more efficient promoter, lacI is attached to a mf-Lon	Jacob F., Monod J. 1961. Genetic

	Lon Degradation Tag	protease degradation tag to allow for R.CviJI endonuclease expression and cellular destruction when intracellular ccdA concentrations are low.	Regulatory Mechanisms in the Synthesis of Proteins. J. Mol. Biol. 3: p818-356. Calos M.P. 1978. DNA sequence for a low-level promoter of the lac repressor gene and an 'up' promoter mutation. Nature. 274: p762-765. Oehler S., Eismann E.R., Krämer H., Müller-Hill B. 1990. The three operators of the lac operon cooperate in repression. EMBO J. 9(v): p973-979. Glascock C.B., Weickert M.J. 1998. Using chromosomal lacIQ1 to control expression of genes on high-copy-number plasmids in Escherichia coli. Gene. 223(1-2): p221-231.
BBa_K3634017	ccaR	The response regulator ccaR is part of the two-component system (TCS) involved in the eventual transcriptional output of an adjacent phycobilisome-related gene (cpcG2) in response to green light of wavelength 535nm. The system is native to Synechocystis sp.	Hirose Y., Shimada T., Narikawa R., Katayama M., Ikeuchi M. 2008. Cyanobacteriochrome CcaS is the

		PCC6803 but has been successfully expressed in *E.coli* (Hirose et al. 2008) and has been further used in multichromatic control of gene expression (Tabor et al. 2011). CcaR, alongside the membrane-associated histidine kinase ccaS, functions as a photoreversible switch between green (535nm) and red (672nm) light by regulation of the output promoter PcpcG2. Within the N-terminal GAF domain of ccaS, the blue phycobilin chromophore phycocyanobilin (PCB) binds to a conserved cysteine residue, imparting reversible photoactivation of signalling activity. Absorption of green light increases the rate of ccaS autophosphorylation and phosphotransfer to ccaR. Once phosphotransfer has occurred, ccaR binds to an operator site within the sequence of the output promoter PcpcG2. Transcription of the output gene is then activated.	green light receptor that induces the expression of phycobilisome linker protein. PNAS. 105(28): p9528-9533. DOI: 10.1073/pnas.0801826105 Tabor J.J., Levskaya A., Voigt C.A. 2011. Multichromatic control of gene expression in Escherichia coli. J Mol Biol. 405(2): p315–324. DOI: 10.1016/j.jmb.2010.10.038 Schmidl S.R., Sheth R.U., Wu A., Tabor J.J. 2014. Refactoring and Optimization of Light-Switchable Escherichia coli Two-Component Systems. ACS Synth Biol. 3: p820-831. DOI: 10.1021/sb500273n
BBa_K3634018	$P_{L8\text{-}UV5}$	The lac operon found in *E.coli* consists of the three lactose metabolising genes lacZ, lacY and lacA which when expressed, allow the bacteria to use the sugar as a source of energy. The initial regulatory mechanisms in the pathway were outlined by Jacob and Monod in 1961, where the topic of inducible and repressible enzyme	Jacob F., Monod J. 1961. Genetic Regulatory Mechanisms in the Synthesis of Proteins. J. Mol. Biol. 3: p818-356.

		systems was discussed. In this system, the transcriptional repressor is a protein known as Lac I which binds to DNA at various operator sequences (termed O1, O2 and O3) which exist both upstream and downstream of the transcriptional start site (TSS). Interaction between the Lac I and operator sequences reduces transcription of the downstream lactose metabolising genes unless relieved by the lactose isomer allolactose. In the absence of Lac I, transcription is constitutive and can be further activated by the catabolite activator protein (CAP), with binding site upstream of the promoter sequence. Reznikoff et al. (1978) mutated the regulatory region in question at three different sites. Within the CAP binding site, bases -66 (G) and -55 (C) of the wt binding region were substituted with A and T respectively to prevent binding of the CAP protein at low glucose concentrations. The wt -10 promoter sequence was also mutated from TATGTT to TATAAT in order to allow σ factor (RpoD) to bind without relying on further activation by the CAP protein. As a result of these mutations, gene expression mediated by the PL8-UV5 promoter will be independently regulated by intracellular concentrations of the lacI repressor as all CAP-associated regulation has been removed.	Reznikoff W. S., Abelson J. N. 1978. The lac promoter. The operon. 7: p221-243. DOI: 10.1101/0.221-243 Hirschel B.J., Shen V., Schlessinger D. 1980. Lactose Operon Transcription from Wild-Type and L8-UV5 lac Promoters in Escherichia coli Treated with Chloramphenicol. J. Bacteriol. 143(3): p1534-1537.
BBa_K3634019	ccaR-Mediated ccdA Expression System	The response regulator ccaR is part of the two-component system (TCS) involved in the eventual transcriptional output of an adjacent phycobilisome-related gene (cpcG2) in response to green light of wavelength 535nm. The system is native to Synechocystis sp. PCC6803 but has been successfully expressed in *E.coli* (Hirose et al. 2008) and has been further used in	Hirose Y., Shimada T., Narikawa R., Katayama M., Ikeuchi M. 2008. Cyanobacteriochrome CcaS is the green light receptor that induces the expression of

		multichromatic control of gene expression (Tabor et al. 2011). CcaR, alongside the membrane-associated histidine kinase ccaS, functions as a photoreversible switch between green (535nm) and red (672nm) light by regulation of the output promoter PcpcG2. Within the N-terminal GAF domain of ccaS, the blue phycobilin chromophore phycocyanobilin (PCB) binds to a conserved cysteine residue, imparting reversible photoactivation of signalling activity. Absorption of green light increases the rate of ccaS autophosphorylation and phosphotransfer to ccaR. Once phosphotransfer has occurred, ccaR binds to an operator site within the sequence of the output promoter PcpcG2. Transcription of the output gene is then activated. The natural output gene cpcG2 has in the past been replaced with sfgfp in order to quantitatively measure the output of the light-sensing system. Replacing this gene with another output is therefore theoretically feasible. Here, the St Andrews iGEM team of 2020 aims to control the expression of the antitoxin ccdA in response to green light. Under activating conditions, expression of ccdA will be maximised whilst also minimising leaky expression from PcpcG2 by using a truncated version of the promoter (PcpcG2-172), characterised by Schmidl et al. (2014). By regulating expression of ccdA, the dual functionality of the antitoxin can be utilised to prevent ccdB-gyrase formation (where ccdB is previously expressed in BBa_K3634012) and allow for precise transcriptional control of a desired output gene (see	phycobilisome linker protein. PNAS. 105(28): p9528-9533. Tabor J.J., Levskaya A., Voigt C.A. 2011. Multichromatic control of gene expression in Escherichia coli. J Mol Biol. 405(2): p315–324. Schmidl S.R., Sheth R.U., Wu A., Tabor J.J. 2014. Refactoring and Optimization of Light-Switchable Escherichia coli Two-Component Systems. ACS Synth Biol. 3: p820-831. Vandervelde A., Drobnak I., Hadži S., Sterckx Y.GJ., Welte T., Greve. H.D., Charlier D., Efremov R., Loris R., Lah J. 2017. Molecular mechanism governing ratio-dependent transcription regulation in the ccdAB operon. NAR. 45(6): p2937-2950. Ong NT., Tabor JJ. 2018. A Miniaturized Escherichia coli Green Light

		BBa_K3634014) (Vandervelde et al., 2017).	Sensor with High Dynamic Range. ChemBioChem. 19: p1255-1258.
BBa_K3634020	R.CviJI Endonuclease + ssrA Degradation Tag	The restriction endonuclease CviJI (also known as R.CviJI) is taken natively from the Chlorella virus IL-3A, a double-stranded DNA phycodnavirus that infects unicellular, eukaryotic Chlorella-like green algae. As well as being previously expressed in *E.coli* by Skowron et al. (1995) and Swaminathan et al. (1996), the restriction endonuclease is also used commercially and is available via NEB as CviKI-1. The enzyme cuts at RG/CY sites (where R = purines, Y = pyrimidines) in the presence of Mg2+. With the addition of ATP, R.CviJI (now R.CviJI*) cleaves at additional restriction sites RG/CN and YG/CY (where N = any nucleotide) but not YG/CR. Both enzymes cleave DNA frequently and therefore possess a variety of functions such as generating numerous sequence-specific oligonucleotides. The sequence to be used in this part is 278 amino acids in length and does not exhibit additional R.CviJI* activity. The 144-235 amino acid region is also suggested to have a recognition/catalytic domain. R.CviJI will be fused to the ssrA degradation tag AANDENYADAS to prevent plasmid destruction as a result of leaky expression only. Lon protease, native to *E.coli,* will recognise and degrade the fusion construct. The TAG stop codon of the R.CviJI gene was removed and replaced with the AANDENYADAS sequence. TAATAA was then added to the 3' end of the the ssrA to terminate translation.	Skowron PM, Swaminathan N, McMaster K, George D, Van Etten JL, Mead DA. 1995. Cloning and applications of the two/three-base restriction endonuclease R.CviJI from IL-3A virus-infected Chlorella. Gene. 157: p37-41. DOI: 10.1016/0378-1119(94)00564-9. Swaminathan N, Mead DA, McMaster K, George D, Van Etten JL, Skowron PM. 1996. Molecular cloning of the three base restriction endonuclease R.CviJI from eukaryotic Chlorella virus IL-3A. Nucleic Acids Res. 24: p2463-2469. DOI: 10.1093/nar/24.13.2463.
BBa_K3634021	LacI-Controlled CviJI Endonuclease +	As part of the St Andrews iGEM 2020 kill switch mechanism, an enzyme capable of destroying plasmid inserts was of highest priority to prevent	Jacob F., Monod J. 1961. Genetic Regulatory Mechanisms in

	ssrA Degradation Tag	uptake of synthetic genes, whose expression by bacteria in the surrounding environment may have provided a survival advantage. All plasmid insert sequences were subject to commercial enzyme restriction site screening using the SnapGene feature which proposed the CviKI-1/CviJI restriction site to be most common across all sequences. In this case, the small size (4bp) of the restriction site is useful for our project. The restriction endonuclease CviJI moderating the destruction of cellular material will be regulated by an independent lacI-controlled promoter which itself is controlled overall by ccdA concentrations. R.CviJI will be fused to the ssrA degradation tag AANDENYADAS to prevent plasmid destruction as a result of leaky expression only. Lon protease, native to *E.coli*, will recognise and degrade the fusion construct. The TAG stop codon of the R.CviJI gene was removed and replaced with the AANDENYADAS sequence. TAATAA was then added to the 3' end of the the ssrA to terminate translation.	the Synthesis of Proteins. J. Mol. Biol. 3: p818-356. Reznikoff W. S., Abelson J. N. 1978. The lac promoter. The operon. 7: p221-243. DOI: 10.1101/0.221-243 Hirschel B.J., Shen V., Schlessinger D. 1980. Lactose Operon Transcription from Wild-Type and L8-UV5 lac Promoters in Escherichia coli Treated with Chloramphenicol. J. Bacteriol. 143(3): p1534-1537. Skowron PM, Swaminathan N, McMaster K, George D, Van Etten JL, Mead DA. 1995. Cloning and applications of the two/three-base restriction endonuclease R.CviJI from IL-3A virus-infected Chlorella. Gene. 157: p37-41. DOI: 10.1016/0378-1119(94)00564-9.

			Swaminathan N, Mead DA, McMaster K, George D, Van Etten JL, Skowron PM. 1996. Molecular cloning of the three base restriction endonuclease R.CviJI from eukaryotic Chlorella virus IL-3A. Nucleic Acids Res. 24: p2463-2469. DOI: 10.1093/nar/24.13.2463.

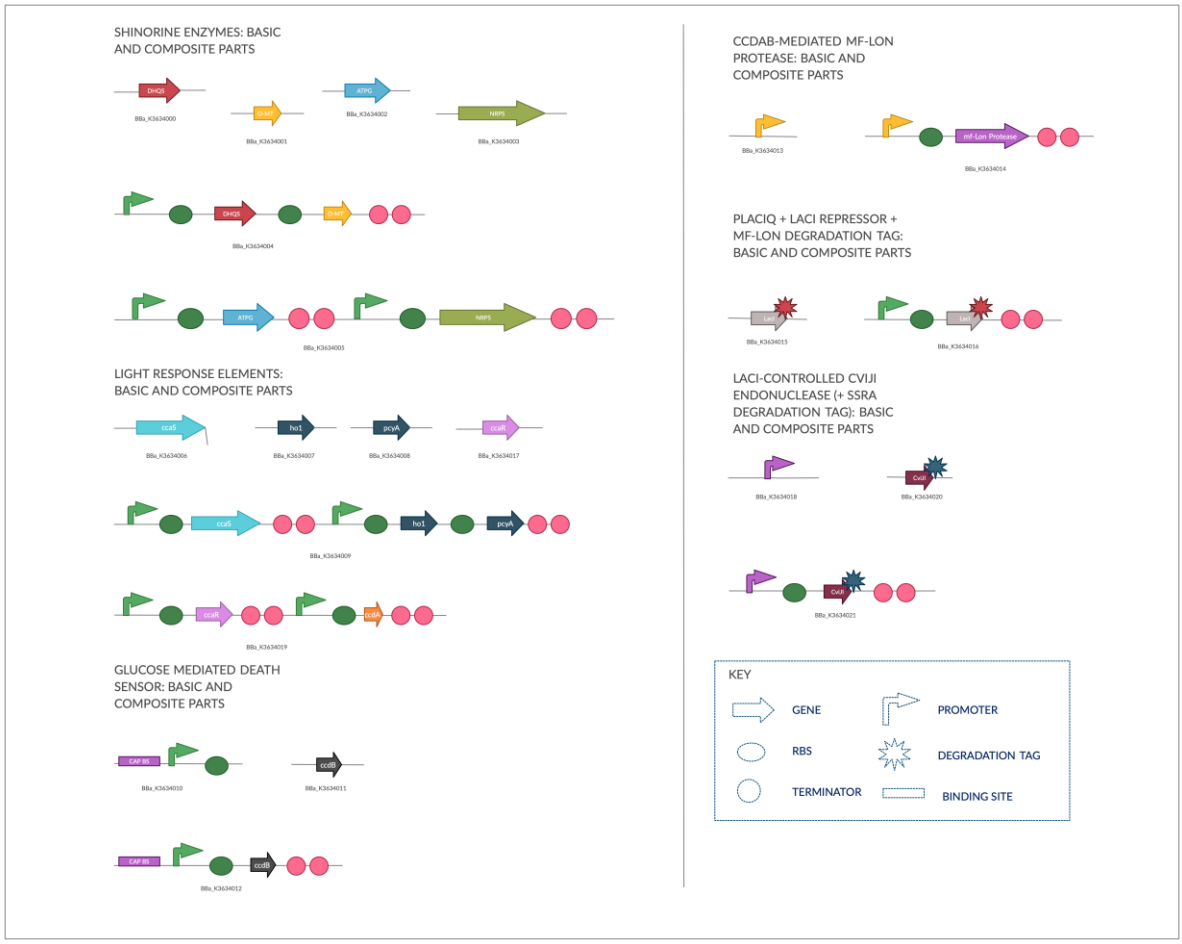

Figure 1. Cartoon visualisation of all parts and composite parts synthesised and used in pSB3B1-DOPH and pSB3E1-AN.