IBEHS 2P03: Health Solutions Design Projects II

M2: Detecting Heavy Metals and Contaminants in Drinking Water

Group 21

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Our group has chosen to address the variability of water quality, specifically within Ontario. Recently, heavy metal contaminants, mercury, lead, nickel, and cadmium, were identified in concerningly large concentrations within Lake Ontario [1]. In addition, polychlorinated biphenyl-laden oils (PCBs) have been confirmed to be leaking from the contained site into the fractured rock beneath the surface in Smithville, Ontario [2]. To ensure the public remains aware of their water quality, our group is proposing an in situ device, in the form of a prokaryotic vector delivered to plant cells, to be placed within a house plant. When the plant is watered, different fluorescent signals will indicate the presence and severity of water toxicity.

Device Overview	
Inputs: Water Contaminants	**Outputs: Fluorescent Signals**
Mercury	Green fluorescent protein (GFP)
Lead	amilCP (blue chromoprotein)
Nickel	amilGFP (yellow chromoprotein)
Cadmium	Red fluorescent protein (RFP)
Polychlorinated biphenyl	

Part Names and Role	Part Description/Rationale	Part Link
Host: Agrobacterium tumefaciens	A common pathogenic bacteria will be used to protect and contain the genes of interest, to be delivered to the user's house plant [3]. A bacteria culture can be purchased from Carolina Biological Supply Company [4].	https://www.carolina.com/bacteria/agrobacterium-tumefaciens-living-pathogen-tube/154825.pr
Plasmid: Tumour-inducing (Ti) plasmid from Agrobacterium tumefaciens: pBBR1MCS	The Ti plasmid has become an industry standard for developing transgenic plants, by using a nonpathogenic version of the plasmid, inserting, and replicating the plasmid within the Agrobacterium tumefaciens bacterial cells [5]. All below-described parts can be introduced into the Ti plasmid, proliferated within A. tumefaciens, and delivered into the host plant. The Ti plasmid provides the structural framework for sensing and regulatory genes of interest. For the specified plasmid, the following restriction enzymes may be used for PCR amplification: KpnI, ApaI, XhoI, HincII, SalI, ClaI, HindIII, EcoRV, EcoRI, SmaI, BamHI, SpeI, XbaI, SacII, SacI [6, 7]. The plasmid can be acquired from the addgene vendor	https://www.addgene.org/85168/?gclid=CjwKCAiAj-_xBRBjEiwAmRbqYtZU5H5sRBBTqhpwWsfC_YQM5o73xal5IQRRX6C-AAjpouf1j5tu4RoCFpsQAvD_BwE

	[6].	
B0030, K1513002: Mercury-binding CDS and GFP reporter CDS	This part contains a ribosome-binding site (RBS), a merR codon-determining sequence (CDS), and a GFP CDS. The part would constitutively express merR, a transcriptional factor associated with the mer operon. When mercury (Hg^{2+}) ions are present, merR is activated and binds to the promoter PmerT of the mer operon. When $mercury^{+}$ ions are not present, merR represses self transcription and mer operon transcription [8,9]. These two parts are related to our problem as the part detects the presence of mercury. If mercury is present, a fluorescent GFP will be outputted to indicate that there is a contaminant in the water. The 2 parts are located on the iGEM registry, however, the parts are not readily available. Thus, the gene will be formulated in the lab, using genetic sequencing details given on iGEM's registry. Further processing is not needed.	http://parts.igem.org/Part:BBa_K1513001
J23100, B0034, I721002, B0010: Promoter, RBS, lead-binding CDS, termination sequence	This part contains a promoter, RBS, lead-binding protein CDS, and termination sequence, in that order. Promoter, J23100, is a strong promoter designed to enable lead detection. When lead (Pb^{2+}) enters the cell, it will couple with the lead-binding protein, forming a dimer [10]. This part will detect if lead is present; if present, the protein could signal a downstream sequence to output a fluorescent signal, indicating a contaminant in the water. The 2 parts are located on the iGEM registry, however, the parts are not readily available. Thus, the gene will be formulated in the lab, using genetic sequencing details given on iGEM's registry. Further processing is not needed.	http://parts.igem.org/Part:BBa_K3161001
K346002: PmerT promoter (mercury-responsive)	This part is a promoter from the mer operon, a mercury-sensing operon. This part serves as the binding region to which the merR regulatory protein binds, as described earlier. When mercury ions are not present, merR regulatory protein binds this promoter sequence, preventing RNA polymerase recruitment. When mercury ions are present, the PmerT promoter is structurally changed, such that merR transcription occurs [11]. Based on the logic gate, mercury-sensing sequence could activate or inhibit the expression of various outputs when mercury is present. Currently, this part is in stock on iGEM and could be obtained from iGEM. This promoter could be useful in the repression or activation of a fluorescent output (such as GFP) in order to indicate mercury presence in water. Further processing is not needed.	http://parts.igem.org/Part:BBa_K346002
K519010: SmtA CDS (cadmium-, zinc-,	This is a coding part that produces a metallothionein that binds to heavy metals like cadmium, zinc, and copper. It can also be	http://parts.igem.org/Part:BBa_K519010

copper-responsive)	fused with other proteins such as GFP, allowing the present metals to be identified via fluorescent signal [12]. If the zinc is transported from host plant cells to the host A. tumefaciens, competitive uptake of zinc, a plant micronutrient, could hinder the plant's development. Thus, a cadmium-specific binder would be preferred over this multispecific part. The part is currently in stock on the iGEM website and could be accessed. Further processing is not needed.	
K549001: Ni(II) dependent rcnA-promoter and GFP-reporter CDS	The rcnA promoter is repressed by an RcnR-regulator. In the presence of nickel (Ni^{2+}) ions, RcnR binds to the ions and to the rcnA promoter, activating transcription. Therefore, the promoter is no longer repressed and downstream coding regions for GFP will be expressed. This part could be useful because it will sense the presence of a nickel, a heavy metal, in the water. This promoter does have weaker sensitivity to cobalt, another toxin and cancer-causing agent in humans. Thus, this part may come with added benefits of detecting large amounts of cobalt in water samples. The part is located on the iGEM registry but not readily available.. Thus, the gene will be formulated in the lab, using genetic sequencing details given on iGEM's registry. Further processing is not needed.	http://parts.igem.org/Part:BBa_K549001
K592009: amilCP, blue chromoprotein CDS	A coding part that produces a blue/purple chromoprotein [13]. This could be used as a visual indicator (output/reporter) when certain metals are detected in the water. This part is currently attainable from the iGEM site. This could interact with other coding parts such as SmtA. Further processing is not needed.	http://parts.igem.org/wiki/index.php/Part:BBa_K592009
K592010: amilGFP, yellow chromoprotein CDS	A coding part that produces a protein that displays a yellow colour [14]. This would be used as a reporter for when specific contaminants are in the water. This could be expressed by interacting with other coding parts (ex. SmtA) or promoters (ex. PmerT) based on contaminants present. It is currently in stock on iGEM. Further processing is not needed.	http://parts.igem.org/wiki/index.php/Part:BBa_K592010
E1010: Red fluorescent protein CDS	A coding part that produces a red protein that could be used as a reporter when specific contaminants are present [15]. This could be expressed by interacting with other coding parts (ex. SmtA) or promoters (ex. PmerT) based on contaminants present. This part is currently attainable from the iGEM site. Further processing is not needed.	http://parts.igem.org/wiki/index.php/Part:BBa_E1010
J33201: ArsR repressor site, RBS, and ArsR CDS	Although not one of highlighted toxins in overview, arsenic is a toxin, and thus detecting its presence would be beneficial. This part contains a repressor site, RBS, and ArsR CDS. ArsR is a self-regulating protein. When arsenic, in the form of arsenate or	http://parts.igem.org/Part:BBa_J33201:Experience

	arsenite, is not present, ArsR binds its repressor site, preventing transcription of ArsR. Downstream sequences will not be expressed, and there will be a minute amount of ArsR protein present. When arsenic is present, ArsR is transcribed normally and downstream sequences, like fluorescent reporters, are expressed to identify water contamination. This part is available on iGEM [16]. Further processing is not needed.	
K1413021: bphR2 CDS (PCB sensor)	A CDS of gene, bphR2, that encodes for a PCB-sensing protein [17]. In absence of PCB, bphR2 protein binds bphR2 operator, repressing self-transcription. In the presence of PCB, bphR2 protein binds to a different site on a bphR1 gene (not included in the selected sequence), activating transcription of a degradation enzyme for PCB, eventually leading to PCB conversion to acetyl coA [18]. The self-regulating aspect of the bphR2 gene can be used to sense presence of PCB, and when combined, with a downstream fluorescent reporter, will alert to presence of PCB in the water. Because the related bphR1 gene encodes for a degradation enzyme that creates a source of acetyl coA to be used in cell metabolism, caution will need to be taken to ensure pre-existing cell metabolic machinery does not negatively interact with the bphR2 gene and its recognition of PCB, before the fluorescent signal can be reported. This part can be ordered from the iGEM registry. No further processing is needed.	http://parts.igem.org/Part:BBa_K1413021

Sources

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