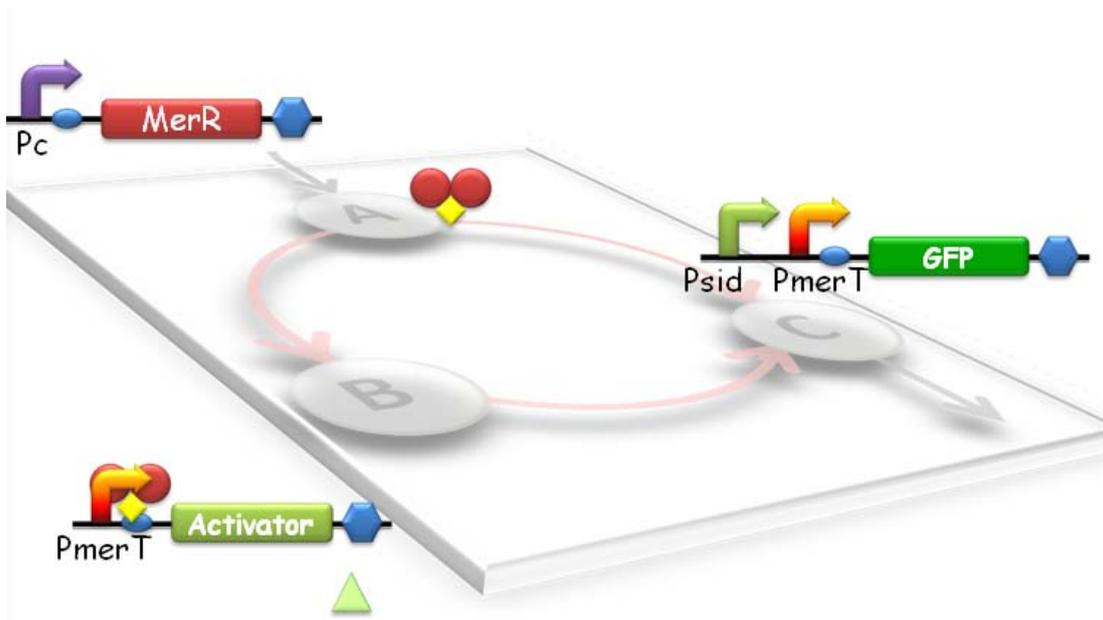


## Kit Operation

Here is a general view of how our heavy metal decontamination biokit functions. Our biokit consists of two parts: the bioreporter and the bioabsorbent. We can firstly determine the heavy metal pollution level by the bioreporter, and then clean the contaminated water exploiting the bioabsorbent.

When heavy metal emerges, a tri-node response will be switched on in our biosensor (Fig.1). Different strains with parameter variations between the nodes will have different response threshold to heavy metal ions, such as mercury.



**Fig 1. Structure of Tri-node response system. According to the results of modeling, we designed the specific genetic circuit to realize the linear response function. Node A is a generator of MerR. For Node B, the gene is the activator which can activate the psid promoter. Node C is GFP whose expression was driven by Psid (activated by activator) and PmerT (activated by MerR).**

Since the transfer function of bioreporter response represents linear now. The working range of the bioreporter will be expanded, and the error rating will be reduced. This type of bioreporter will be excellent for in lab accurate measurement or heavy metal pollution assessment.

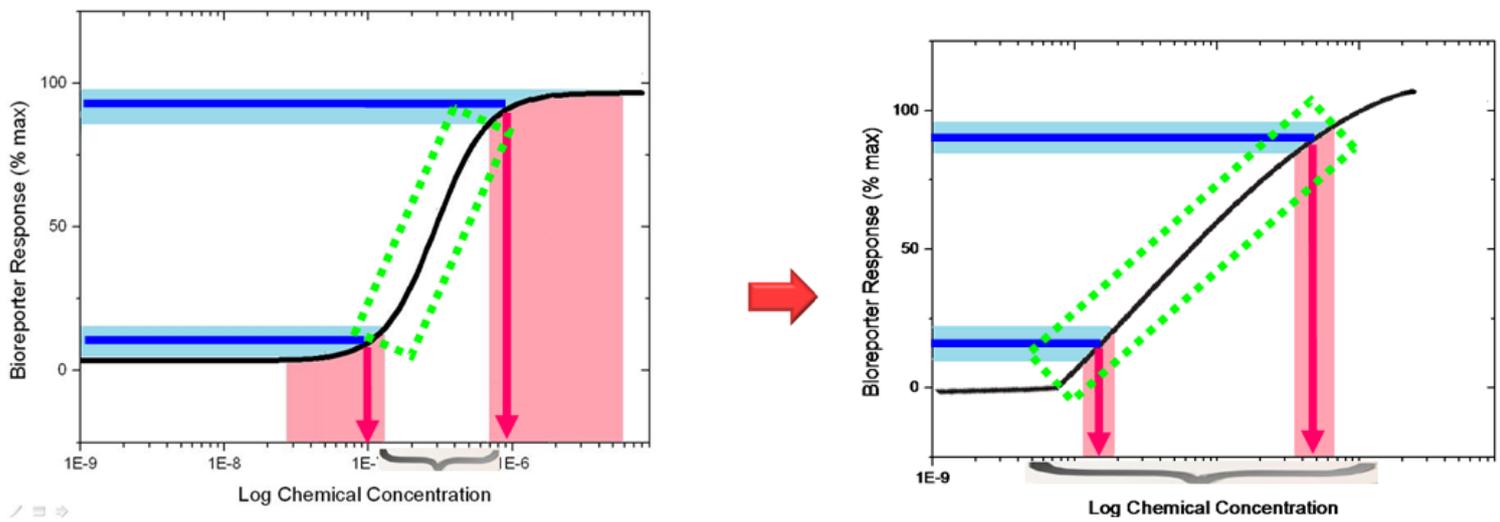


Fig 2. Our bioreporter with tri-node response system will possess a linear transfer function (right) rather than the natural hill function (left).

Before wiki freezing, data collecting of the final result (linear transfer function transformed from hill function) are still under progressing. Primary result demonstrates that it works as expected, which we will show at Jamboree.

To determine the pollution level in field, we developed an assay easy to understand and accessible to the general public, called traffic light bioassay. By using bacteria strains with different sensitivity threshold to mercury, the heavy metal concentration can be easily determined by the number of reporter strains reacting to a sample, which is representative to different heavy metal concentration range (Fig.3).

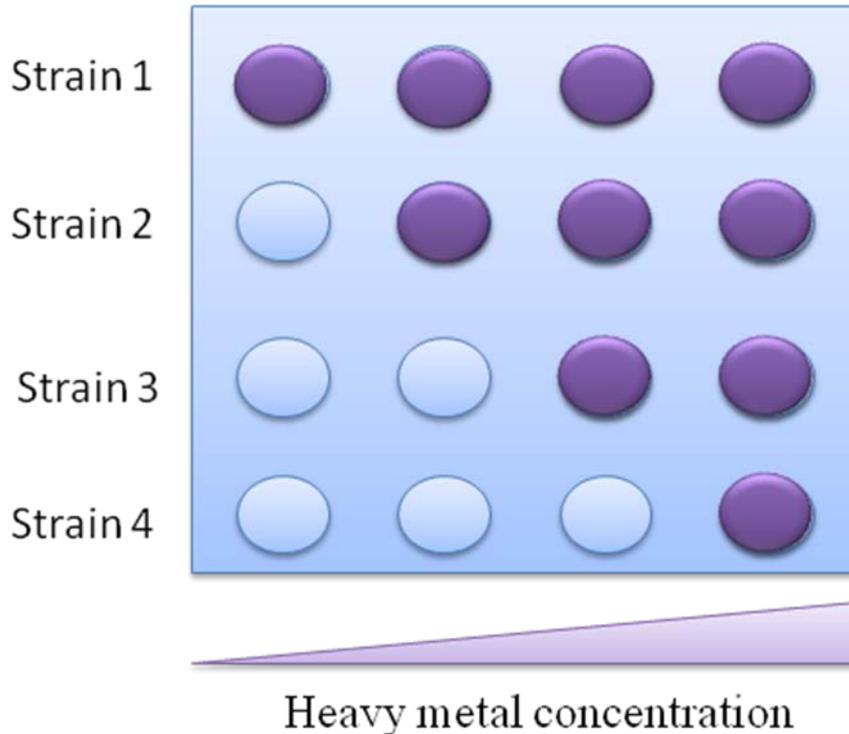
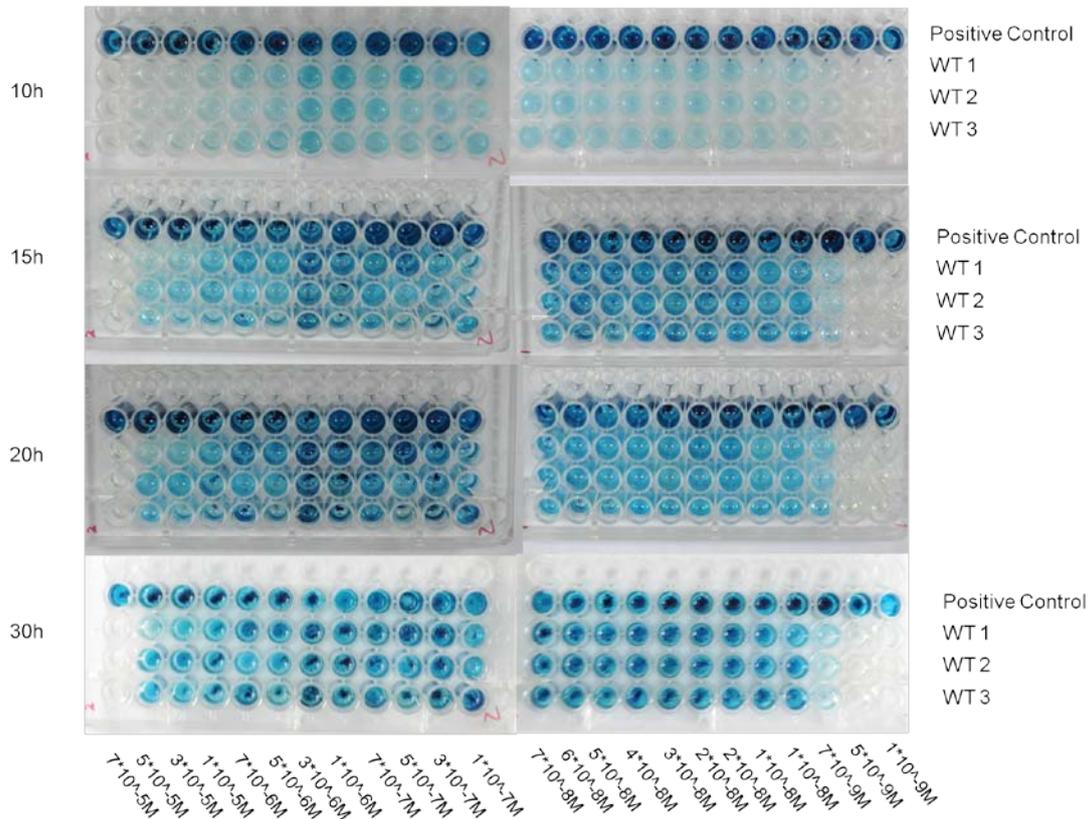


Fig 3. The schematic sketch of how the biosensor functions.

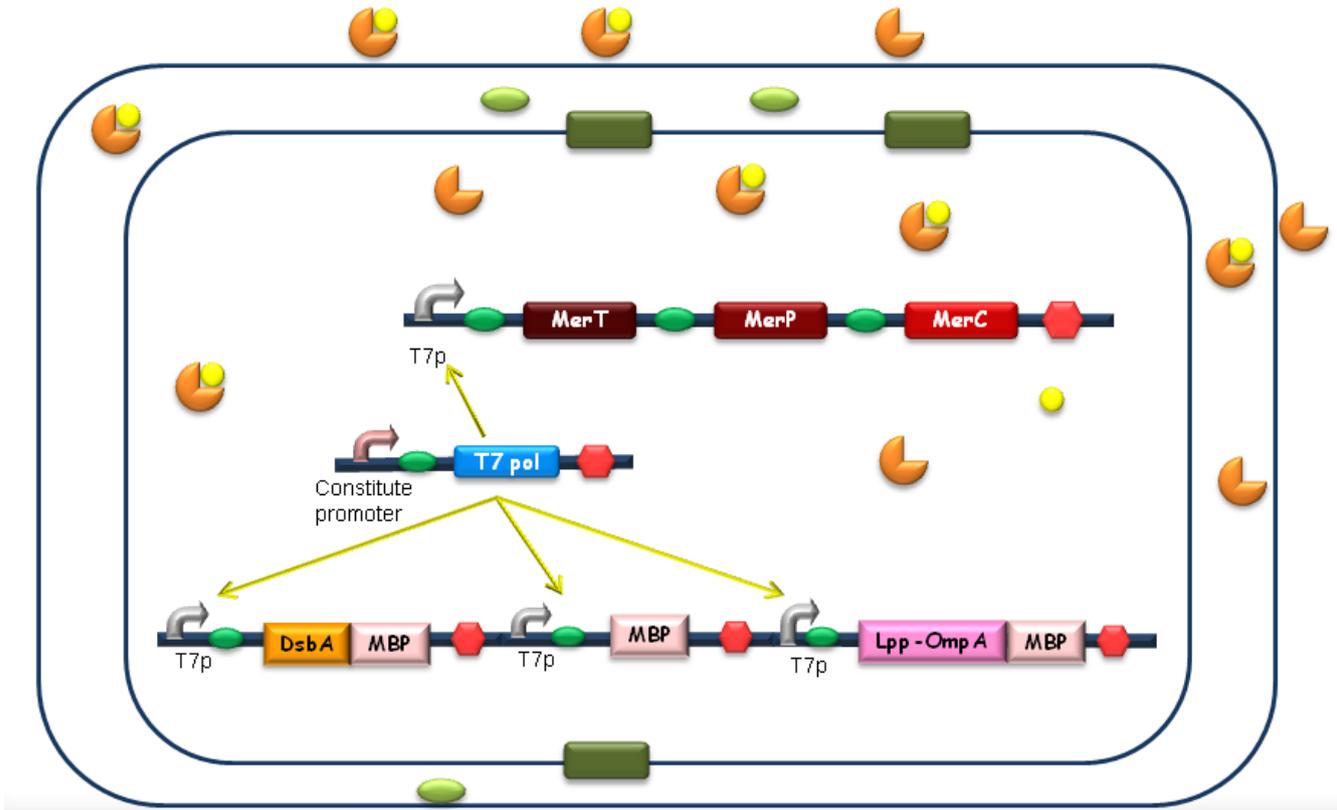


**Fig 4. The primary result of traffic light bioassay, which was performed in the 96-well plate. The response of whole-cell bioreporter incubated with simulated mercury containing polluted water was recorded by digital image at 10h, 15h, 20h and 30h. 3 replicates of 1 biosensor strain behaved very similarly with respect of indicating the mercury concentration range in a wide time window**

After the heavy metal concentration determined, it's time for our bioabsorbent to decontaminate the heavy metal ions from water. Firstly, bacteria switch on MBP generating device and facilitation module to get ready-to-use (Fig 4A). Then bioabsorbent will be applied to the contaminated water, to absorb heavy metal ions, for instance, mercury (II) with high performance (Fig 4B). At the same time, a genetic cascade will amplify the input (presence of mercury) and Ag43 will be expressed finally with a time delay and high intensity. When the bacteria sedimentate, post treatment will be ready to be conducted (Fig 4C).

A





C

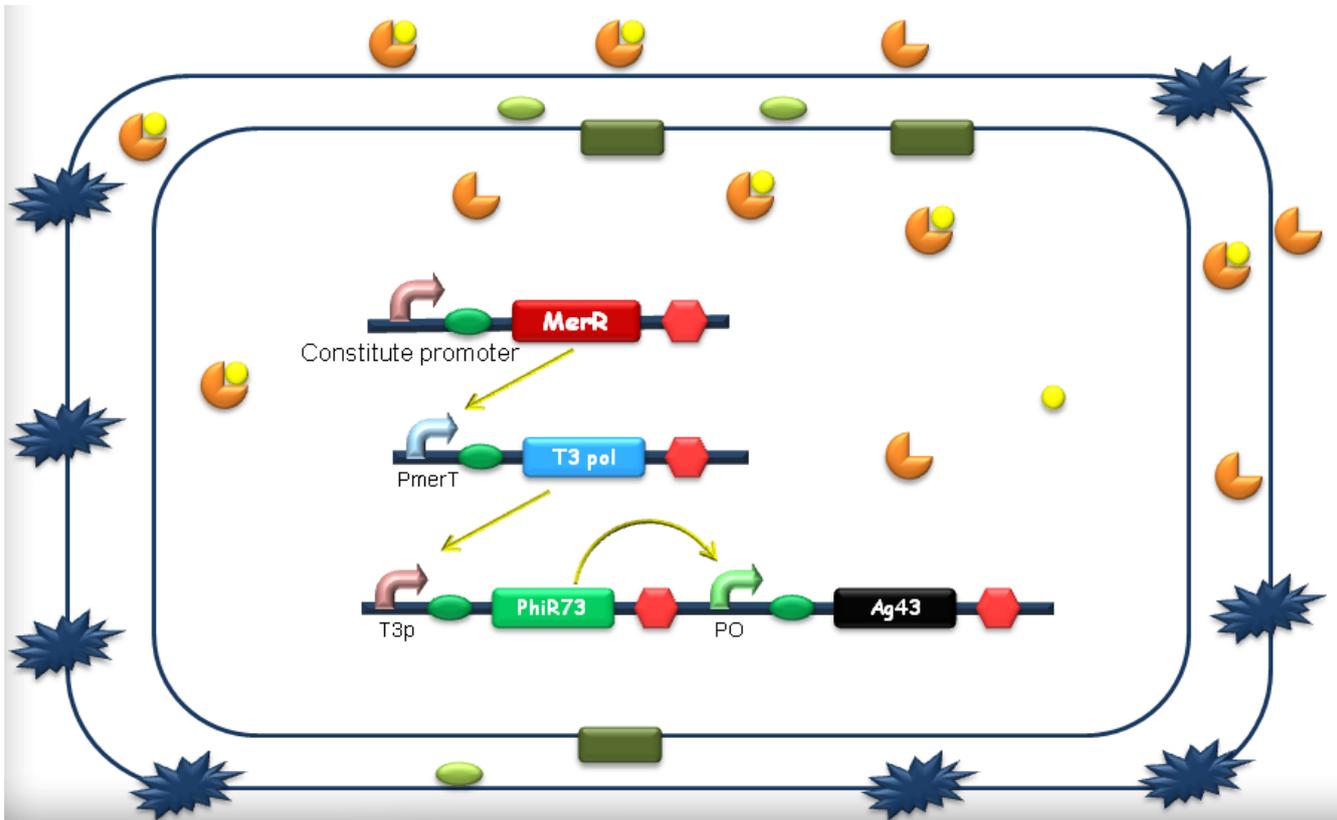


Fig 4. The schematic of how our bioabsorbent functions. A: bioabsorbent gets ready-to-use; B: bioabsorbent absorbs heavy metal ions from water; C: bioabsorbent autoagregate at a population level.

To conclude the above work, we have developed a streamlined method to construct heavy metal decontamination kits consisting of efficient bioreporters and bioabsorbents both for in field application and in lab use. Compared with the technologies we have now, this biological engineering approach gives advantages to its bioavailability assessment, relatively low cost and less resource consumption. More importantly, it clearly points the way of future development. As a revolutionary progress, we believe, the conceptual advancement is of great value when the potential of bio-decontamination is unveiled. At present, it is highly probable that bioremediation is confined in the laboratory study, taking possible defects of mass application and public panic over transgenic products into consideration. However, as long as this promising field is constantly being probed into, we are firmly convinced that, there will be more experts devoted into relevant research, thus optimizing the bio-decontamination method. This is not remote, but imminent; for the biological science and technology is marked by tremendously rapid and ever-accelerating change. Hopefully, we are involved in the exciting improvement and contribute to the rescuing of the environment and human beings ourselves.