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Development of an assembly-based, recombineering pipeline for overcoming recalcitrance to DNA transfer caused by RM systems in C1 chassis

Current energy and chemical needs are met by the extraction and processing of the fossil fuels. Such resources are finite and their use causes environmental pollution and greenhouse gas (GHG) emissions. The challenge facing humankind is, therefore, to identify new, sustainable and cleaner processes for chemical and energy generation. Biological routes offer a promising alternative, and the newly emerging field of gas fermentation offers the opportunity to capture carbon from waste gases produced by a wide range of industrial processes, including steel mill off-gas, gas generated from landfill sites, and waste gas from the combustion of non-food biomass. This is achieved through the feeding of these single carbon greenhouse gases to microorganisms in a liquid fermentation vessel, which are then able to convert the carbon directly into useful chemical and fuels. This not only supplies us with an additional source of fuel and industrially useful products, but it reduces the emission of harmful GHGs.

The challenge we now face is to use synthetic biology to engineer these organisms so that they are able to more efficiently produce the chemicals and fuels that we desire from them, and even to increase the range of products that they are able to synthesize. This is a newly emerged scientific discipline that has arisen through the merger of several core areas of science, principally biology, engineering, chemistry and Information Communication Technology (ICT). However, there is one crucial barrier preventing us from engineering many exciting microorganisms with specific desirable traits, and that is their own form of genetic self-defense, known as restriction modification (RM) systems.

RM systems exist so that in the wild these organisms are protected against the abundant bacterial viruses, known as bacteriophages, which aim to inject their DNA into the bacteria in order to proliferate, however they also prevent us from introducing any DNA into the organism to implement our plethora of genetic tools. The bacteria’s own DNA is encoded with a unique methylation fingerprint, and any DNA which does not conform to this fingerprint is cut and destroyed. The aim of this project is to replicate the unique fingerprint found on the bacterial hosts DNA within our plasmid DNA, crucial for implementation of our genetic tools, thus overcoming the bacterial defenses, and allowing us to explore a whole new range of microorganisms capable of gas fermentation which were previously genetically inaccessible.