# Progress report for the characterization of functions of LysM-RLKs in Medicago

*OpenPlant Fund Project: The use of synthetic biology tools to define the roles of LysM receptor-like kinases in legumes and cereals*

**Summary**
Our project made some progresses in the past six months. Firstly, we have synthesized a number of golden gate modules including gene promoters, coding sequences and terminators , and got the final constructs required for this project using gold gate cloning technology. Secondly, we have already expressed these constructs in Medicago truncatula to check the protein expression, now we are focusing on transforming these constructs in Medicago to detect defence and symbiosis phenotype. There are always very good communication between Norwich and Cambridge, JIC has hosted the training of Cambridge collaborators for Golden gate cloning.

**Report and Outcomes**
In this project, we would like to use synthetic biology tools to characterize the roles of LysM receptor-like kinases in *Medicago truncatula* and rice. We firstly submitted 21 level 0 modules with individual EC number to Invitrogen company for synthesis used in our golden gate cloning, but unfortunately we just got 19 modules back, there were 2 modules failed for the synthesis and they were actually required for the objective of this project in rice part (Tab 1), so in that case we had to only focus on *Medicago* for our research. Using 19 level 0 modules, we have finally got 10 level 1 and 6 level 2 constructs by gold gate cloning (Tab 1). The each LysM-RLK contains three extracellular LysM domains (EC), transmembrane domain(TM) and intracellular kinase domain(IC), LysM domain was thought to bind ligand CO8 or Nod factor, the kinase domain was involved in signalling transduction (Fig 1), we have made two chimeric constructs EC21286 and EC21287 to exchange the LysM domains between Nod factor receptors MtNFP and MtLYK3 and CO8 receptors MtLYK5 and MtCERK, EC21283 and EC21285 will be used as a complementation positive control. These constructs will be expressed in *Medicago nfplyk3* and *lyk5cerk1* double mutants to test symbiosis and defense phenotype after treatment of CO8 and Nod factor, respectively. EC20921 and EC20922 were designed to express *MtCERK1* and *MtLYK5* derived by their native promoters to try complementation in *Medicago mtcerk1* and *mtlyk5* mutant. All these level 2 constructs contained a transgenic marker with nuclear expressed 3xYFP for the hair root transformation selection. We have already expressed these constructs in *Medicago* wild type R108 by hair root transformation, and we could detect the protein expression for all constructs using anti-HA and anti-FLAG antibody (Fig 2), from our western blot result, we knew that these constructs worked very well in roots, so we could use these constructs for the following complementation assay in different *Medicago* mutants. As

mentioned in our proposal, JIC has made a good collaboration with Uta Pazskowski group in Cambridge from this project. The two groups worked very closely for constructs design, data updating and material sharing. We communicated the progress every month by email or phone. JIC has hosted the two days' golden gate cloning training for Pazskowski group, now they have set up this system in their lab. The golden gate modules we produced from this project could also share with other people who might be interested in future.