SINGLE COPY INTEGRATION MARKERS TO TRACK BACTERIAL SYNTHETIC COMMUNITIES IN THE RHIZOSPHERE

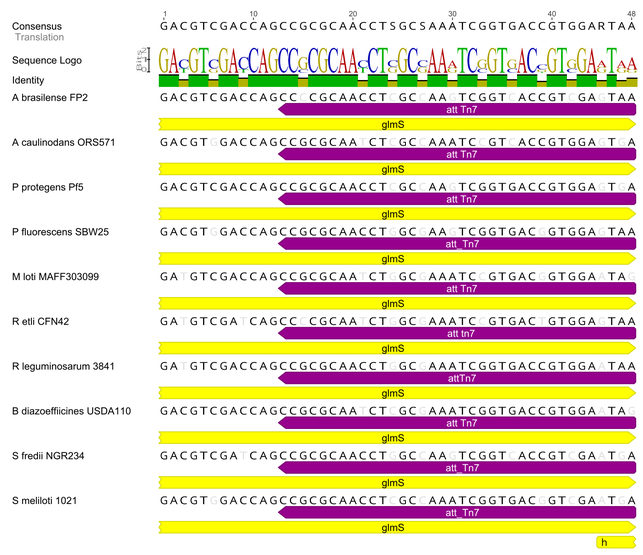
PAS6-P05

Beatriz Jorrín, Andrzej Tkacz and Philip Poole
Department of Plant Sciences, University of Oxford

In order to track rhizobia associated with plants it is needed to tag them, however using plasmids to mark them is ineffective due to the absence of an antibiotic pressure during plant colonisation. In 2005 Choi *et al* developed a broad host cloning-expression tool based on the mini-Tn*7* transposition. It integrates permanently in a single copy in the chromosome, downstream *glmS*, in the presence of the transposase plasmid pTNS3. We have developed a family of these plasmids (pUC18T-miniTn*7*) marked with different fluorescent and chromogenic proteins. First, we assembled the expression cassettes using Golden Gate cloning. All of them were constructed the followed way: a very strong constitutive promoter, a standard ribosome binding site, a marker gene and a terminator. Secondly, these constructions were PCR amplified and cloned into the MCS inside the transposon element in pUC18T plasmid. This family of plasmids will allow us to easily study the interaction of different bacteria at the same time *in planta* in order to elucidate the forces governing in the rhizosphere.

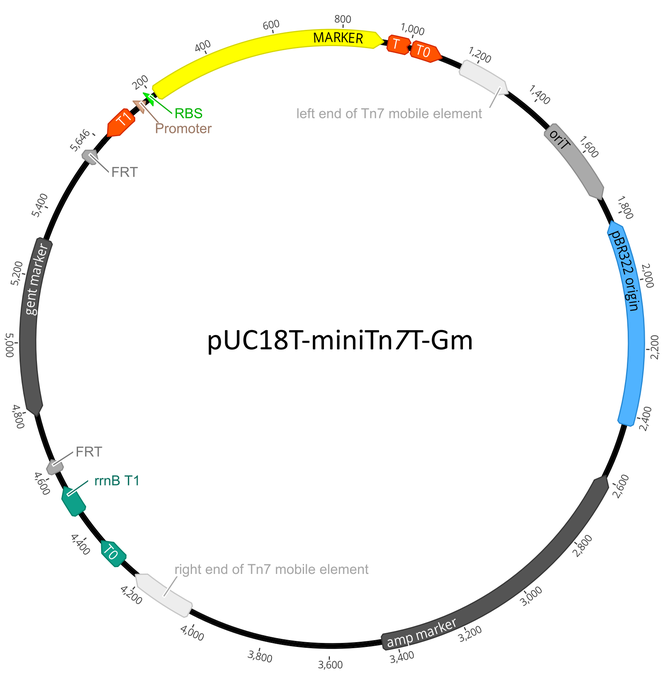
Bioinformatic analysis of the Tn*7* integration site

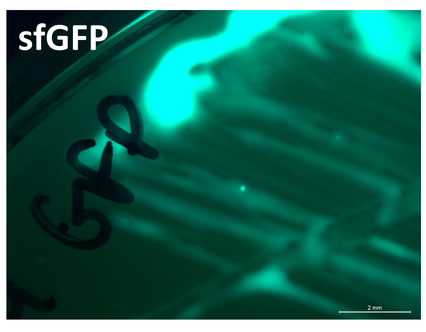
The *att* Tn*7* recognition site is conserved among different rhizobia

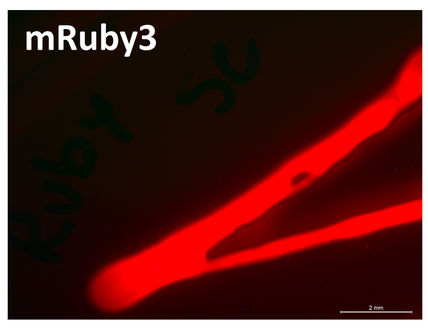


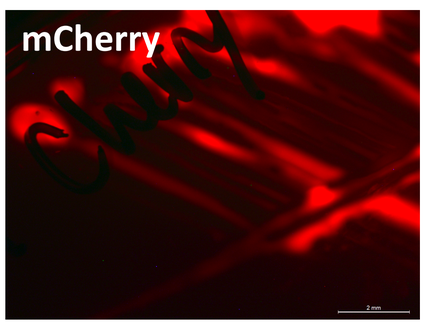
The Tn*7* integration site is not conserved among different rhizobia. Therefore integration efficiencies may vary in different species.

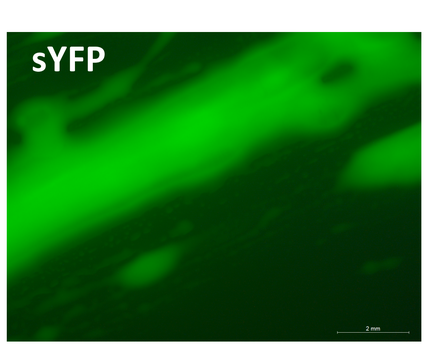
New miniTn*7* plasmids

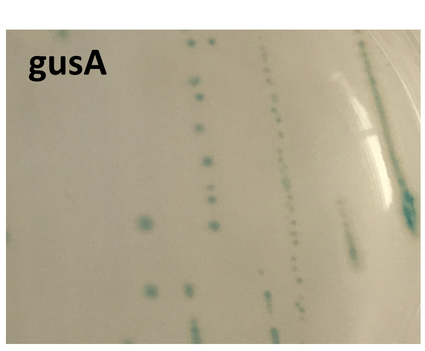










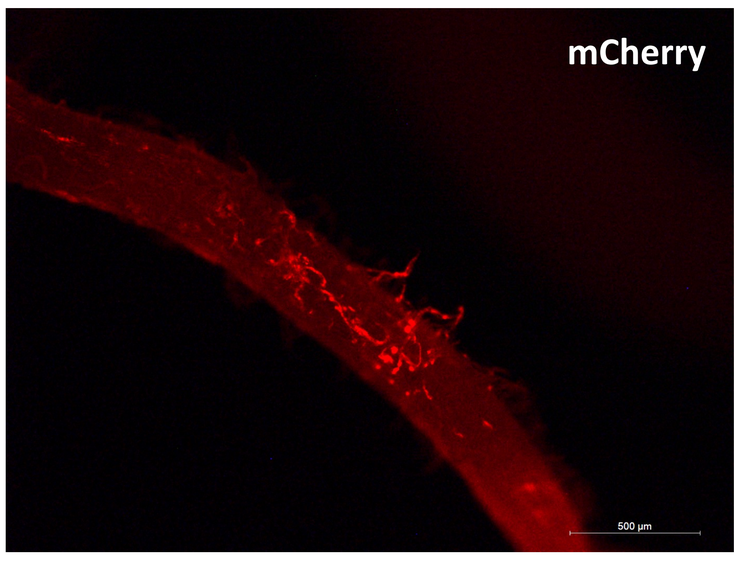


Stereomicroscope images of *Rhizobium leguminosarum* 3841 expressed single copy chromosomic markers on TY plates

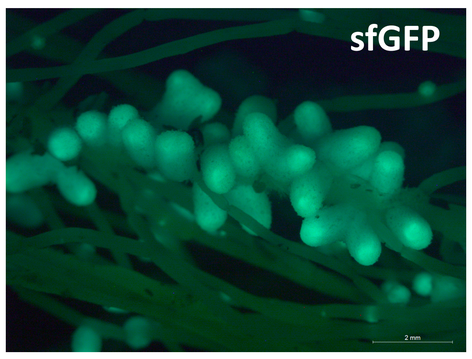
In Planta visualization of Tn*7* tagged *Rhizobium leguminosarum*

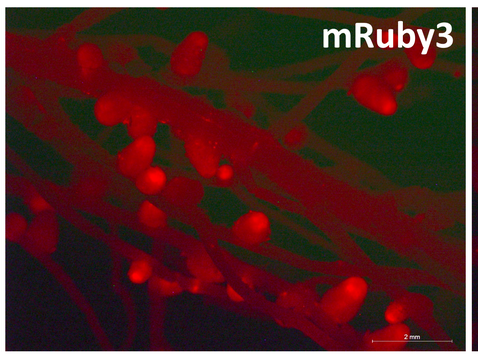
Stereomicroscope images of *R. leguminosarum* 3841 Tn*7* marked strains on pea roots after 1 wpi (A) and after 3 wpi (B)

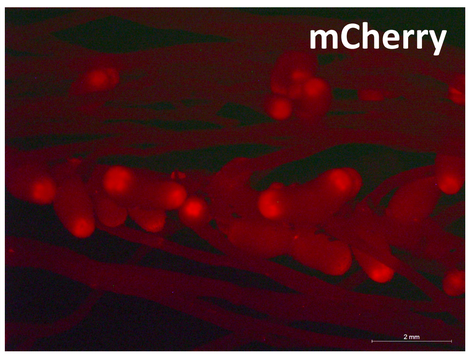
A

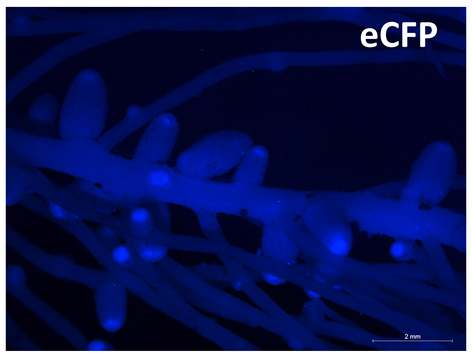


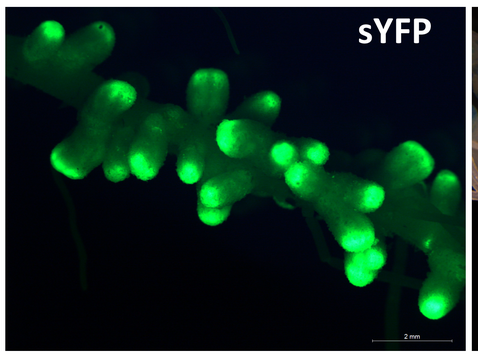
B

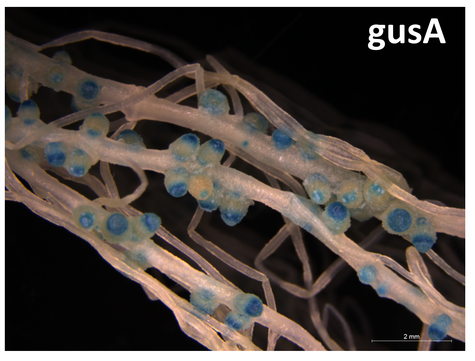












This work was funded by BBSRC (BB/N013387/1)
Contact: beatriz.jorrin@plants.ox.ac.uk