Hello everyone!

I am Prachi Singh from Dr. Anil Kumar Saxena lab (ICAR-NBAIM) Mau, Uttar Pradesh, India. My lab is one of the participating labs in India-UK Nitrogen Fixation Centre, working on optimization of symbiosis between *Rhizobium* and Pigeon pea for nodulation, nitrogen fixation and grain yield in the area of Indo-Gangetic plain soils of India.

I visited the Dr. Philip Poole Lab in April 2019 and spent a short but fruitful time from 15 April to 8 June 2019. The Poole lab is one of the most prestigious labs in the field of rhizobial genetics and it was great experience to work there.

My projects involved :

1. Modular assembly of Golden Gate-Compatible vectors

We have grown all bacterial strains containing plasmids on Luria agar plate amended with respective antibiotics. Then transfer the single bacterial colony into LB broth to isolate plasmid. Then golden gate vector assembly was performed in a thermocycler in 15µl total volume, containing 40 fmols of each cloned module, 1.5 µl Bovine Serum Albumin, 1 µl of BsaI, 1.5 µl T4 DNA ligase buffer,1 µl of concentrated T4 DNA ligase buffer with water to 15 µl. We have transformed into *E. coli* competent cell. Colonies showing the positive color were screened by plasmid restriction digestion and sequenced to verify the correct constructs. Correct plasmid assembly was observed at high frequencies and compatible those described for Golden Gate cloning reactions. We have designed the bacterial vector assembly system with a series of discrete key modules.

2. Labelling of the endosymbiotic and endophytic *Bradyrhizobium* by mini-Tn7 based pUC18 based suicide delivery plasmids

We selected two strains of NBAIM for labelling based on antibiotic profiling. For this, transposase PTNS3 (ST18) and pUCR6KT based transposon was used for conjugation. Transconjugants supplemented with respective antibiotics and observed under confocal microscopy.

My visit to Oxford was for a short duration, but , as it was a time of festive mood all around due to Easter and bank spring holidays, it doubled my joy to explore the city of learning spires but more holidays led to some scarce of time for conducting experiments. Dr. Beatriz Jorrin helped me throughout from searching strains/plasmids from database to conducting

experiments, to gain the maximum from this short span. She was always there to help around, while conducting experiments I learnt many small but significant things from her. I am grateful to Dr. Philip Poole for giving me this opportunity to work in his lab. Moreover, I should say that Poole lab is very organized and every member work towards that. Every lab member is very cooperative and it is a group of frolicsome people. Thank you everyone for this remarkable experience.