Root-microbe interactions in *Rhizobium*-legume symbioses

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Background

Nitrogen fixing *Rhizobium*-legume symbioses depend on attachment of *Rhizobium* to legume roots. The mechanisms used for root attachment remain unclear, but glucomannan and rhicadhesin seem to be important at acid and alkaline pH, respectively. This project is using novel imaging techniques and genetic approaches to better characterise root attachment.

Insertion Sequencing (INSeq) is being adapted to query the importance of genes in the *Rhizobium leguminosarum* biovar *viciae* (Rlv)3841 genome for attachment to pea roots. A luminescence-based imaging assay for characterising root attachment phenotypes has been developed, and is being used to investigate the pH dependency in attachment mechanisms. Tracking Root Interaction Systems (TRIS) is being established as a real-time imaging tool for visualising root-microbe interactions, as well as the spatio-temporal localisation of root attachment factor expression.
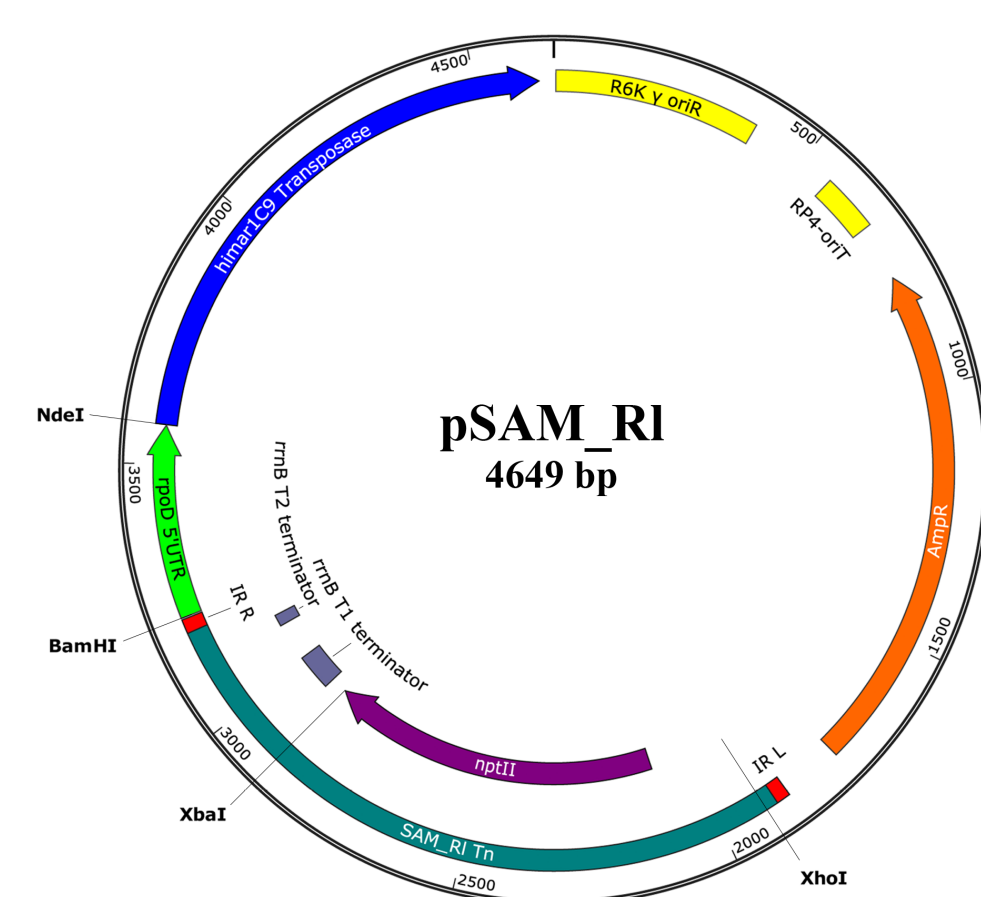
Lux assays

Lux labelled *Rhizobium* incubated with pea roots for one hour, before washing and imaging. Luminescence allows semi-quantitative assessment of bacterial attachment levels.

Root attachment of Rlv3841 strains at pH7 after one hour
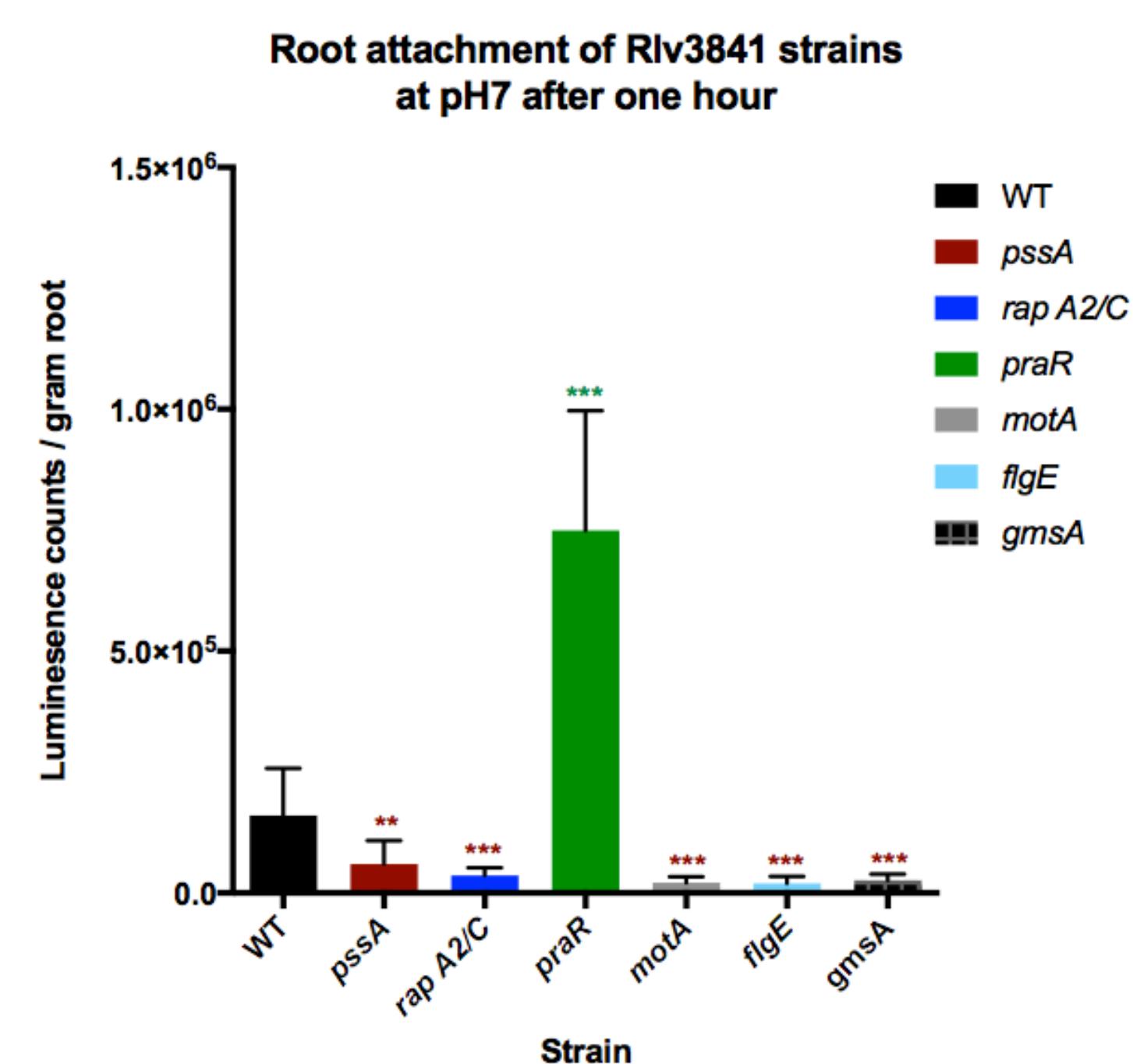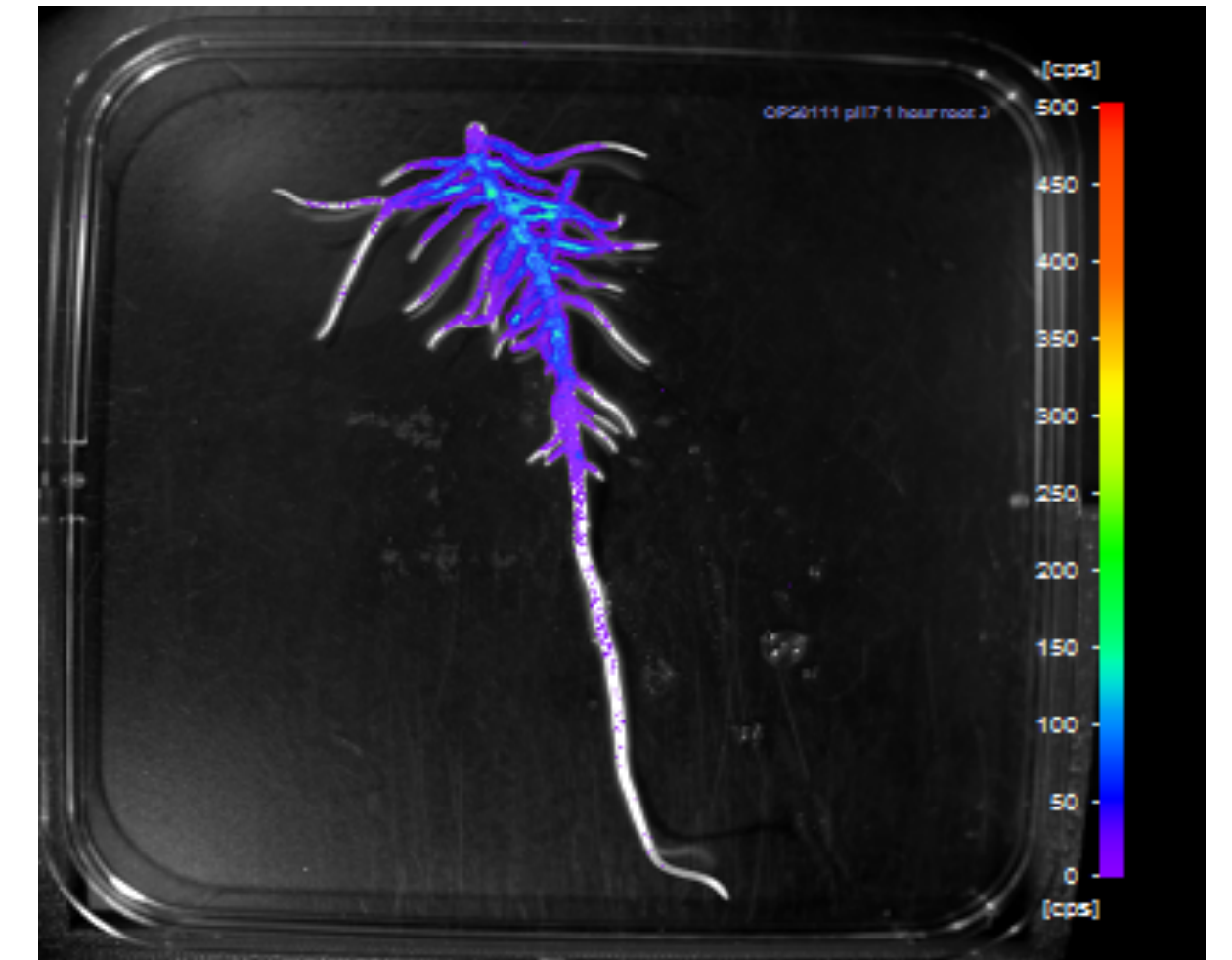
At neutral pH, *pssA* (acidic EPS), *rapA2/C* (adhering proteins), *motA* (motor protein), *flgE* (flagellar subunit) and *gmsA* (glucomannan) mutants are impaired in attachment. A *praR* regulator mutant demonstrates a hyper attachment phenotype.

INSeq

- Large libraries of single insertion mutants
- Root attachment assay (1 hour attachment)
- Mutant recovery and sequencing
- Quantify frequencies of insertion mutants
- Assess how insertion mutation affects growth
- Quantify fitness values of genes

pSAM_Rl
4649 bp

Above – the mariner transposon mutagenesis vector

INSeq (1,2) is a technique for the study of gene fitness.
Libraries of single mutants are analyzed to assess the effect of gene mutation on root attachment at acid, neutral and alkaline pH. INSeq is used with a hidden Markov model (HMM) for analysis. This assigns genes to classification states (growth essential, defective, neutral or advantaged) based on the fitness of mutants. This is inferred from their population frequency among root attached microbes.

TRIS
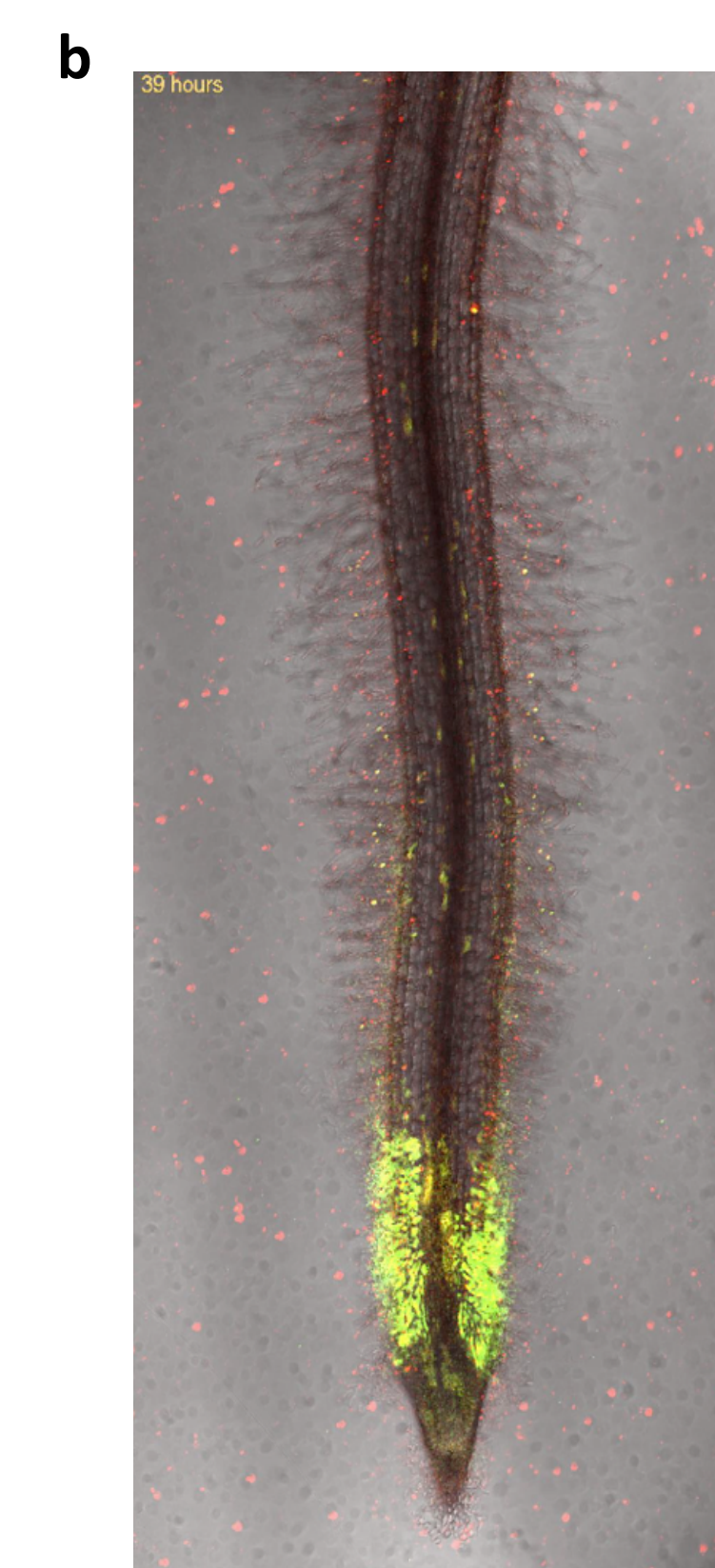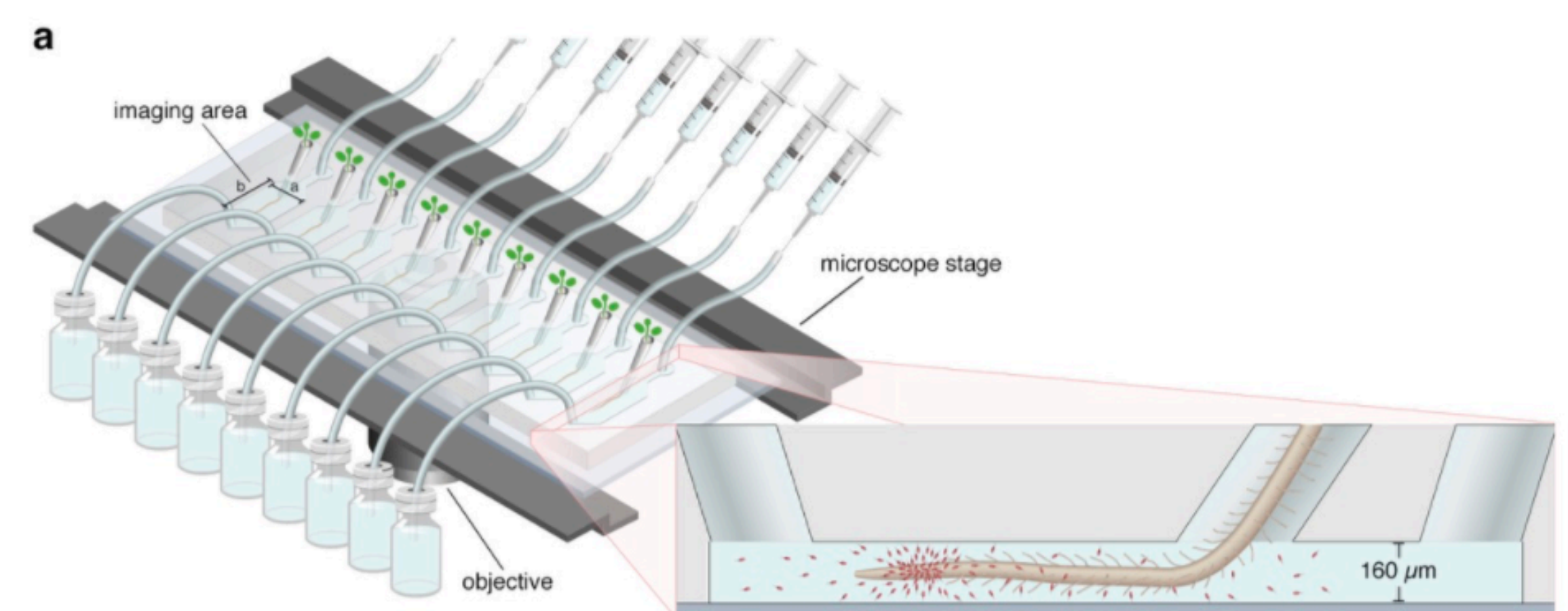
a) The TRIS (3) setup. Plants are germinated into a PDMS chip with inlet and outlet channels for fluorescently labelled bacteria. Real time confocal imaging can track the root-microbe interactions.

b) TRIS imaging of a *Trifolium repens* (white clover) root inoculated with Rlv3841 carrying a constitutive mCherry / *lppE* promoter GFP fusion. *lppE* operon expression (involved in early root attachment) is seen from ~2 hours onwards, localized to the root elongation zone.

Summary / References

- INSeq represents a high-throughput technique for the classification of gene's importance in root attachment
- *Lux* assays can validate INSeq data, and provide a facile tool for attachment phenotyping of *Rhizobium* strains across pH ranges
- TRIS is a powerful imaging tool for characterizing root-microbe interactions and mapping root attachment factor expression

(1) Perry BJ, Yost CK. (2014) *BMC Microbiol* **14**:298 (2) Wheatley RM *et al.* (2017) *J Bacteriol* **199**:e00572 (3) Massalha *et al.* (2017) *PNAS* **114:17**, 4549-4554