

**Supplementary Table S1. List of strains used to search for genes specific to symbiotic *Frankia* genomes.**

**Symbiotic strains**

Cluster 1 strains. Able to form effective nodules on *Alnus* and *Myricaceae*. Most strains can grow in pure culture on TCA intermediates except a few lineages called Sp+ not considered here. They have *nif* genes and can fix N<sub>2</sub> in pure culture.

ACN14a (*Frankia alni*, isolated from *Alnus*, infective on *Alnus*)

QA3 (undescribed species, isolated from *Alnus*, infective on *Alnus*)

Cc13 (*Frankia casuarinae*, isolated from *Casuarina*, infective on *Casuarina*)

Cluster 2 strains. Able to form effective nodules on *Datisca*, *Coriaria* and *Ceanothus*. Most strains cannot be grown in pure culture except one BMG5.1 that can fix N<sub>2</sub> in pure culture. They have *nif* genes and fix N<sub>2</sub> in nodules.

BMG5.1 (*Frankia coriariae*, isolated from *Coriaria*, infective on *Coriaria*)

Dg1 (*Candidatus Frankia datiscae*, not isolated, infective on *Datisca*)

Cluster 3 strains. Able to form effective nodules on *Elaeagnaceae* and *Gymnostoma*. Most strains can grow in pure culture on TCA intermediates and on hexoses. They have *nif* genes and can fix N<sub>2</sub> in pure culture.

EAN1pec (*Frankia soli*, isolated from *Elaeagnus*, infective on *Elaeagnus*)

EUN1f (undescribed species, isolated from *Elaeagnus*, infective on *Elaeagnus*)

**Non-Symbiotic strains**

Cluster 4 strains. Can be grown in pure culture but are not symbiotic. Some (CN3 and DC12) although isolated from nodules cannot reinfect their host and cannot fix N<sub>2</sub> in pure culture. Others (AgB1.8 and Eu11c) can reinfect their host but cannot fix N<sub>2</sub> either in nodules nor in pure culture. They do not have *nif* genes.

CN3 (*Frankia saprophytica*, isolated from *Coriaria*, non-infective). Used to subtract.

DC12 (undescribed species, isolated from *Datisca*, non-infective). Used to subtract.

AgB1.8 (isolated from *Alnus*, infective on *Alnus*, non-effective). Not used to subtract.

Eu11c (isolated from *Elaeagnus*, infective on *Elaeagnus*, non-effective). Not used to subtract.

**Supplementary Figure S1. A flow chart of research and methods used in this work from comparative genomics to experimental studies.**

