**Biological Justification of Project**

In this OpenPlant Project, we aim to answer the question of when, where and how receptor-like kinases regulate the symbiosis signalling at the early and late stages of the plant-fungi interaction.

To do this, we proposed the use of DNA synthesis for interchangeable kinase modules and parts for Golden Gate cloning. Upon generating these resources (e.g. promoter units), we aimed to create constructs for transformation into stable transgenic lines for the following:

1. transcriptional reporters to understand the tissues where promoter activity is present, and how that changes with stimuli
2. translational protein-fluorescent tag fusion to visualise protein localisation, and how they change with symbiosis
3. creation of kinase ‘parts’ as variants of original Level 0 SC modules so as to allow the generation of chimeric kinases with domain swaps

Overall, the goal was to create resources to advance our understanding of receptor-like kinases in signalling and generate resources for the plant research community.

To this end, **objectives 1 and 2** have been met for the late-stage symbiosis receptor kinase, ARK1. However, because of the obstacles encountered with Golden Gate cloning with promoters of CERK1 and NFR5, we therefore decided to narrow our focus and efforts to generate good resources of a narrower list of receptor-like kinases; rather than to synthesize more Golden Gate modules that may encounter similar problems during cloning. Thus, **objective 2** has been met for CERK1, NFR5 and in the process, CEBiP.

**Detailed Overview of current expenditure:**

|  |  |  |
| --- | --- | --- |
| **Category** | **Items** | **Cost** |
| DNA synthesis | L0 OsCERK1 promoter (2.5kb)L0 OsNFR5 promoter (2kb)L0 OsCERK1 CDS (1.9kb)L0 OsNFR5 CDS (1.9kb) | £250£200£200£200 |
| L0 OsARK1 CDS (3406bp)L0 OsARK1 PU (1951bp) | £300£200 |
| pOsCERK1-OsCERK1-GFP-tHSP (5598bp)pOsNFR5-OsNFR5-RFP-tHSP (5143bp) | £600£520 |
| Subtotal | £2,470 |
| Sequencing &Consumables | Genewiz Account Top-up | £250 |
| Gel Purification and Plasmid Cleanup during cloning | £240 |
| Type IIS Restriction Enzymes for Golden Gate cloning | £250 |
| Subtotal | £740 |
| DNA synthesis – in progress  | OsCEBiP units | £790 |
| Total | £4,000 |

**Follow-on plans objectives:**

To follow up on the lines that we have since created, we intend to use the additional £1,000 towards the thorough characterisation of the stable transformants line that we have since received/or will be receiving. We expect about 10-15 independent transformants per line.

**Below is the itemised breakdown of how we expect to use the £1,000**

|  |  |  |
| --- | --- | --- |
| **Category** | **Items** | **Cost** |
| PCR/qPCR and Sequencing to evaluate transgene expression | Genewiz Account and Top-up  | £300 (good for c. 60 reactions) |
| Primer synthesis | £50 |
| RNeasy Plant Kit (2x 50) | £400 |
| Superscript II  | £250 |
| Total | £1,000 |

The remaining expenditure (Horticultural, transformation costs, gDNA genotyping, GoTaq G2 flexi polymerase costs) will be borne by us.