TRANSFORMATION OPTIONS/PLANNING TRANSFORMATION PROJECTS

* **Method of transformation**

Transformation is carried out by either particle bombardment or *Agrobacterium*-mediated transformation. Each method has pros and cons so individual requirements should to be considered to decide the best approach for a particular project.

* **Constructs**

An appropriate type of plasmid needs to be designed according to the method of transformation; cloning vectors for bombardment or *Agrobacterium* are available and these should be discussed with Alison Huttly or Caroline Sparks. There is also the option to bombard a DNA fragment containing only the gene cassette (promoter-gene-terminator) which eliminates contamination with plasmid backbone DNA and so avoids the antibiotic gene used for bacterial selection of plasmids.

* **Number of plants required**

Generally 15-20 independent transgenic lines are generated per construct but more/fewer can be provided as appropriate.

* **Young plantlets or seed**

Young plantlets in soil can be provided if required; this is a cheaper option taking about 4 months from initial transformation and is useful if the original transgenic events (T0 plants) can be studied. Alternatively, threshed, cleaned seed from the T0 plants can be provided within about 9-11 months.

* **Wheat variety**

Cadenza (hard, red, pseudo-winter (non-vernalising) wheat) is the variety of choice for biolistic transformation and Fielder (soft, white, spring wheat) is generally used for *Agrobacterium* transformation. However, over 40 different wheat and wheat-related varieties have been transformed at Rothamsted so if there is a requirement for a particular variety to be transformed this is usually possible; efficiencies may vary and therefore some adjustment in costs may be necessary but there is no restriction to using only model genotypes.

* **Selection of transgenic plants**

The construct supplied is usually co-bombarded with a second construct containing a selectable marker gene for selection of transformed plants in culture. Alternatively, a selectable marker cassette can be present in the same vector as is the case for *Agrobacterium* constructs. The *bar* gene which confers resistance to glufosinate ammonium-containing herbicides (e.g. commercial herbicides such as Basta, Challenge, Liberty etc.) is the recommended selection gene although *nptII* can also be used which provides resistance to the aminoglycoside antibiotic, kanamycin.

* **Project costing**

An online project costing is available on the Intranet (see Finance pages/Useful financial links/Online project costing) which will calculate the costs of transformation by the various methods and whether plantlets or seeds are provided. However, all projects should be discussed with Caroline Sparks prior to submission of grant proposals.