REQUIREMENTS FOR TRANSFORMATION/WHAT WE WILL NEED FROM YOU

* **Plasmid map**

A map of the construct(s) to be transformed is required for central records including its overall size so that equimolar ratios can be calculated should co-bombardment be used. If available, sequence data of the inserted gene cassette should also be provided.

* **Plasmid DNA**

Plasmids should be prepared using a Qiagen kit or similar to give pure DNA. For bombardment, 50µg plasmid at 1mg/ml is requested to give plenty to work with although 25µg should be sufficient if stocks are limited. A nanodrop reading to show the concentration of the plasmid plus a scan to show purity is required and if possible a gel photo of the uncut plasmid and/or test digests is useful to show it is cut as expected and is not degraded. For bombardment of isolated gene cassettes, fragments should be cut out from vectors with some base pair overhang to allow for possible degradation of ends. For constructs to be transformed via *Agrobacterium*, only small amounts are necessary to carry out the initial transfer into the *Agrobacterium* strain, so ~5-10µl at 10-20ng/µl is sufficient.

* **PCR primers and conditions**

Although not required for a few months after transformation has commenced, primer sequences, PCR conditions and the expected size of the amplified product will be needed ready to test putative transgenic plants as they are generated. Primers can be ordered from sequence data but if some are already available which have been tested then some of these can be supplied, preferably at 100µM (pmol/µl).

* **Risk assessment**

A risk assessment will need to be completed before transformation commences; this should cover the gene being introduced and any effects it may have on increased toxicity, allergenicity etc. and its possible effect on the environment. A short form is available for genes which pose no particular hazard (see Risk assessment form tab) which can be appended to the main Transformation Group Project risk assessment. However, any gene which is considered to pose a more considerable risk will require a separate, full risk assessment for which forms are available on the Intranet (see Genetically Modified Organisms Procedures/Section 4. Registration of new projects).

* **Grant number for costings**

A valid grant number will be required including the approval of the grant holder who will be charged for the transformation work.