# A FLEXIBLE BINARY TRANSACTIVATION SYSTEM FOR THE EXPRESSION OF 'QUALITY' GENES IN CROP PLANTS

MAY, S. T, RAMSAY, N. A, AND BENNETT, M. J.

Plant Biochemistry, Gibbet Hill, Warwick University, Coventry, W. Midlands, CV4 7AL, UK.

## ABSTRACT

Traditional methods for testing multiple promotor elements, in terms of how they may control single or multiple genes of interest, have relied on recombinational cloning for each promotor with each coding region followed by a time-consuming transformation step for each combination. We have developed a transactivation system utilising a modified yeast GAL4 protein and binding site pair in order to address this and several other problems associated with both combinational cloning and multigene pathways. A transactivation system allows for a minimum of molecular recombination steps followed by genetic crossing of lines to provide a 'mix and match' environment for combinational testing. Another powerful feature is the opportunity for co-ordinate expression of multiple genes uder the control of a single promotor or promotors, with the same potential for combinational testing as above. Our GAL4 system has successfully produced transactivation *in planta* of reporter genes in all tissues tested for several plant species in transient assays. We are currently generating stably transformed lines to futher test this system.

## INTRODUCTION

GAL4 is a transcriptional activator protein from yeast which binds to a defined single or multiple 17-mer Upstream Activating Sequence (UAS) and stimulates transcription of downstream genes (Giniger *et al.* 1985). GAL4 derivatives have been shown to activate transcription from artificially introduced UAS sites in mammalian cells (Lin *et al.* 1988), insects (Fischer *et al.* 1988), and plant protoplasts (Ma *et al.* 1988).

The wild-type GAL4 protein consists of a DNA binding fragment (Db : amino acids 1-147) and two activating regions (I : 148-238, II : 768-881) separated by a glucose response domain (Kang *et al.* 1993). Wild-type GAL4 does not transactivate UAS-bearing reporters in plant protoplasts, this has been previously achieved using a derivative comprising of the DNA binding domain linked directly to activating region II, thereby eliminating region I and the glucose response domain (Ma *et al.* 1988).

We have used the GAL4 Db+II derivative described above to develop a GAL4 // UAS transactivation system for use *in planta* as a flexible tool for overcoming several of the problems associated with combinational cloning and the expression of multigene pathways. Conventional methodology for testing a range of defined promotors for their productivity in driving the expression of open reading frames (ORFs) for desirable traits, requires a separate insertional cloning of each promotor upstream of each ORF followed by a labour intensive transformation step for every combination tested. If a novel promotor is to be tested on a

range of ORFs, or a novel ORF is to be compared for expression from a range of established promotors, every combination of promotor with ORF must be generated from scratch and subsequently transformed.

Using a GAL4 // UAS transactivation system, each promotor of interest needs only to be cloned and transformed in combination with the GAL4 transactivator coding region once. Similarly, each desired ORF needs to be cloned and transformed only once with a 5' UAS attachment. In order to test novel combinations of each promotor with quality ORF, one needs only to cross the required lines and assay the trait in the F1 generation. Each novel promotor or ORF subsequently acquired will then need only one insertional cloning step, and transformation, in order to produce a line which can then be assayed in combination with any existing GAL4 // UAS line or lines available. Novel promotor lines may also be assayed by crossing to an existing UAS-reporter line (e.g. UAS-GUS, UAS-luciferase) in order to observe tissue and/or temporal specificity of the promotor before crossing to the required UAS-linked quality gene. It would also be possible to introduce combinations of defined promotor-linked GAL4 constructs in order to increase the flexibility and potential complexity of the ORF expression patterns (e.g. seed specific *and* leaf specific).

For the expression of multigene pathways, where it may be desirable to produce co-ordinate expression of various ORFs from a defined promotor or promotors, a GAL4 // UAS transactivation system has the potential to provide several useful features which may be difficult to achieve by other means. Several ORFs, each presenting a 5' UAS may be transactivated from a single promotor-GAL4 construct, this not only has the advantage of multiple combinational analysis as described above, but it also addresses the potential problem posed by the recurring phenomenon of the inactivation of multiple copies of transgenes (Finnegan & McElroy, 1994). In a transactivation system, there need only be one inserted copy of the trial promotor driving all of the pathway ORFs rather than one copy of the promotor for each ORF, thereby reducing if not eliminating the probability of copy number driven inactivation.

Transactivation leads to trait expression in the F1 hybrids. This provides its own advantages and lends itself ideally to environmentally aware transgenic containment methods based on male sterility or even on simple segregation (and thereby loss of function) of promotor and ORF.

## EXPERIMENTAL

Our initial experiments were designed to analyse the efficacy of promotor-GAL4 'regulator' constructs in transactivating UAS-reporter 'target' constructs in transient expression experiments in living plant cells and tissues.

A biolistic approach was used, whereby tungsten beads carrying various combinations of constructs were fired into plant tissues resting upon a bed of tobacco suspension cells. Tissues tested including seedlings were from oil seed rape and *Arabidopsis*. Tomato fruits were also bombarded.

The positive control construct was a GUS reporter incorporating an artificially inserted plant intron (GUS-int : Vancanneyt *et al.* 1990) under the ubiquitous CaMV 35s promotor control.

Negative control constructs included:
i) GAL4 Db+II under CaMV 35s promotor control, to confirm that there was no endogenous reporter activity triggered by GAL4 protein or the process of transformation;
ii) UAS-linked GUS-int, which showed both that there were no endogenous transactivation effects that could activate expression from a UAS-linked ORF, and that the UAS-linked ORF was itself 'silent'.
No expression could be detected in experiments using these constructs.

When plant cells and tissues were bombarded with beads carrying a combination of i) CaMV 35s-GAL4 Db+II and ii) UAS-GUS-int and subsequently assayed for GUS activity. Reporter expression was then observed at a level comparable to that shown by the positive control described above. Reporter activity was observed in all tissues examined including cotyledons, leaves, flowers, roots, stem and in tomato fruits and tobacco suspension cells.

We therefore have evidence that efficient transactivation of a UAS-bearing GUS reporter gene by a modified yeast GAL4 protein can successfully take place in a substantial range of plant cells either *in planta* or in cell suspension following transient biolistic transformation. We are currently generating stably transformed lines from related constructs in order to further characterise the GAL4 // UAS system in plants.

ACKNOWLEDGEMENTS

The authors thank Robert Shields (PBI - Cambridge) and the members of his lab for technical help and the use of their facilities.

REFERENCES

Finnegan and McElroy (1994) Bio/Technology, 12, 883-888.

Fischer *et al.* (1988) Nature, 332, 853-856.

Giniger *et al.* (1985) Cell, 40, 767-774.

Kang *et al.* (1993) Journal of Biological Chemistry, 268, 9629-9635.

Lin *et al.* (1988) Cell, 54, 659-664.

Ma *et al.* (1988) Nature, 334, 631-633.

Vancanneyt *et al.*(1990) Molecular and General Genetics, 220, 245-250.