

Transgenic Research

VOLUME 5 NUMBER 3
MAY 1996
ISSN 0962–8819

LIBRARY
JUN 1 0 1996

CHAPMAN & HALL

Electronic in 1996
see inside

Transgenic Research

Aims and scope

Transgenic Research is dedicated to the publication of fundamental research on transgenic animals including gain-of-function, gene targeting and gene ablation modeling. The Journal provides a forum for the cross-fertilization of ideas and techniques in all areas of transgenic technology, from fungi to higher eukaryotic organisms. The areas covered include:

— methods and techniques for their production
— characterization and properties
— development of basic biological research models
— use as experimental tools
— biomedical and disease modeling
— agricultural and industrial exploitation
— environmental impact

Transgenic Research accepts full-length articles and short communications describing original research, reviews and mini-reviews in the areas of transgenic technology (including regulatory and environmental aspects) and short discussion articles aimed to stimulate debate

Copyright
© 1996 Chapman & Hall
Apart from any fair dealing for the purpose of research or private study, or criticism or review, as permitted under the Copyright, Designs and Patents Act, 1988, this publication may only be reproduced, stored or transmitted, in any form or by any means, with the prior permission in writing of the publishers, or in the case of reprographic reproduction in accordance with the terms of licences issued by the Copyright Licensing Agency in the UK

US Copyright Law Applicable to Users in the USA

Subscriptions
The 1996 subscriptions for volume 5 are: institutions EU £205.00; USA/Canada US $335.00; Rest of the World £220.00. Personal subscription rates: EC £70.00; USA/Canada US $105.00; Rest of World £70.00. (Personal subscriptions can only be made through the publisher and can only be paid using a personal cheque or credit card.)

For subscription orders: Subscription Dept, Chapman & Hall, International Thompson Services Ltd, Cheriton House, North Way, Andover, Hampshire SP10 5BE, UK. Tel +44 (0)1264 332424, Fax: +44 (0)1264 359704. E-mail: chsub@itps.co.uk.

All subscription enquiries and prices for single issues should be addressed to: USA/Canada, Journals Promotions Dept, Chapman & Hall Inc., 115 Fifth Ave., New York, NY 10003, USA, Tel: +1 (212) 780 6235, Fax: +1 (212) 260 1363; rest of world, Journals Promotions Dept, Chapman & Hall, 2–6 Boundary Row, London SE1 8HN, UK, Tel: +44 (0)171 865 0066, Fax: +44 (0)171 522 9624.

Journals are sent second-class in the UK and by air speeded post or air freight to most other countries. Journals can be sent at air mail letter rate at an additional cost, details of which are available on request. Second-class postage paid at Rathway, NJ, USA, Postmaster: send address corrections to *Transgenic Research*, c/o Mercury Airfreight International Inc., 2323 Randolph Avenue, Avenel, NJ 07001, USA.

In 1996 *Transgenic Research* consists of 6 issues in January, March, May, July, September and November.

Editorial enquiries to: Publisher's Editor *Transgenic Research*, Chapman & Hall, 2–6 Boundary Row, London SE1 8HN, UK (e-mail: lisa.grant@rapidcom.co.uk).

INSTRUCTIONS FOR AUTHORS

Submission of manuscripts
The original manuscript (plus three copies) should be submitted directly to one of the Editors.

Dr R. Forster, C.I.T., Miserey, P.O. Box 563, 27005 EVREUX Cedex, France. E-mail: 100633.327@compuserve.com

Dr C.A. Pinkert, Department of Comparative Medicine, University of Alabama at Birmingham, 227 Volker Hall, 1670 University Boulevard, Birmingham, AL 35294-0019, USA. E-mail: pinkert@cmed.bhs.uab.edu

In order to facilitate the refereeing process, authors are invited to suggest up to five potential reviewers, giving names, addresses, telephone numbers and E-mail addresses. The Editors reserve the right to appoint referees to their own choice.

Journal policies
Submission of a manuscript to *Transgenic Research* implies that it reports unpublished work, not currently being considered for publication elsewhere. If previously published tables, illustrations or text exceeding 200 words are to be included then the copyright holder's written permission should be obtained, and included in a preliminary form. Further justification for using previously reported information can be given in the covering letter if required.

It is understood that researchers who submit papers to this journal are prepared to make available to qualified researchers materials not available from commercial sources, which are needed to duplicate their research results (probes, clones, sequences, cells, antibodies and such like), for non-commercial research purposes only.

Where manuscripts contain new sequence information it is a requirement of acceptance for publication that these sequences are added to the EMBL database.

It is implicit in the submission of manuscripts to *Transgenic Research* that all experimentation has been conducted with close adherence to the relevant regulations pertaining to the use of animals, genetic manipulation and the transport or release of genetically altered organisms. The Editors and Publishers reserve the right to reject manuscripts if they have reason to believe that such regulations have not been respected.

There are no page charges.

Transgenic Research

Volume 5 Number 3 May 1996 ISSN 0962–8819

STEENBOCK LIBRARY
JUN 10 1996

CONTENTS

REVIEW

IYENGAR, A., MÜLLER, F. and MACLEAN, N. *page* 147
Regulation and expression of transgenes in fish - a review

PAPERS

RUSSELL, A.J., KNIGHT, M.R., COVE, D.J., KNIGHT, C.D., TREWAVAS, A.J. and WANG, T.L. 167
The moss, *Physcomitrella patens*, transformed with apoaequorin cDNA responds to cold shock, mechanical perturbation and pH with transient increases in cytoplasmic calcium

MCFARLANE, M. and WILSON, J.B. 171
A model for the mechanism of precise integration of a microinjected transgene

KHAN, M.R.I., CERIOTTI, A., TABE, L., ARYAN, A., MCNABB, W., MOORE, A., CRAIG, S., SPENCER, D. and HIGGINS, T.J.V. 179
Accumulation of a sulphur-rich seed albumin from sunflower in the leaves of transgenic subterranean clover (*Trifolium subterraneum L.*)

CECIM, M., ALVAREZ-SANZ, M., VAN DE KAR, L., MILTON, S. and BARTKE, A. 187
Increased plasma corticosterone levels in bovine growth hormone (bGH) transgenic mice: effects of ACTH, GH and IGF-I on *in vitro* adrenal corticosterone production

HERMINGHAUS, S., THOLL, D., RÜGENHAGEN, C., FECKER, L.F., LEUSCHNER, C. and BERLIN, J. 193
Improved metabolic action of a bacterial lysine decarboxylase gene in tobacco hairy root cultures by its fusion to a *rbcS* transit peptide coding sequence

SHANMUGAM, V., HAINES, D., LAKE, J.P. and SAHA, B.K. 203
Tracking *H-2* alleles in transgenic mice by RFLP and heteroduplex analysis

TECHNICAL NOTE

NADON, N.L. and DRAEGER, K. 209
Genomic DNA analysis from mouse toe lysates

CHRISTENSEN, A.H. and QUAIL, P.H. 213
Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants

SHORT COMMUNICATION

XIAYI, K., XIUWEN, Z., HEPING, S. and BAOJIAN, L. 219
Electroporation of immature maize zygotic embryos and regeneration of transgenic plants

The papers published in *Transgenic Research* are indexed in *CABS* (*Current Awareness in Biological Sciences*), *Chemical Abstracts*, the *Biomedical Citation Index*, the *Biotechnology Citation Index, Research Alert, Current Contents/Life Sciences, Index Medicus/MEDLINE, Biotechnology Abstracts* and *Biosis*.

Transgenic Research is published by Chapman & Hall, 2–6 Boundary Row, London SE1 8HN, UK.

This material may be protected by Copyright law (Title 17 U.S. Code)

Transgenic Research **5**, 213–218 (1996)

TECHNICAL NOTE

Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants

ALAN H. CHRISTENSEN‡ and PETER H. QUAIL*

Dept. of Plant Biology, University of California, Berkeley, CA 94720, and USDA/ARS Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710, USA (Fax: 1 510-559-5678)

Received 7 July 1995; revised 21 August 1995; accepted 24 August 1995

A set of plasmids has been constructed utilizing the promoter, 5′ untranslated exon, and first intron of the maize ubiquitin (*Ubi-1*) gene to drive expression of protein coding sequences of choice. Plasmids containing chimaeric genes for ubiquitin-luciferase (*Ubi-Luc*), ubiquitin-β-glucuronidase (*Ubi-GUS*), and ubiquitin-phosphinothricin acetyl transferase (*Ubi-bar*) have been generated, as well as a construct containing chimaeric genes for both *Ubi-GUS* and *Ubi-bar* in a single plasmid. Another construct was generated to allow cloning of protein coding sequences of choice on *Bam* HI and *Bam* HI–compatible restriction fragments downstream of the *Ubi-1* gene fragment. Because the *Ubi-1* promotor has been shown to be highly active in monocots, these constructs may be useful for generating high-level gene expression of selectable markers to facilitate efficient transformation of monocots, to drive expression of reference reporter genes in studies of gene expression, and to provide expression of biotechnologically important protein products in transgenic plants.

Keywords: gene expression; transgenic monocots; ubiquitin

Introduction

The general availability of strong promoters active in all or most cell types of monocotyledonous plants would be useful in a variety of applications in gene transfer studies with this plant group (McElroy and Brettell, 1994). Although the widely-used cauliflower mosaic virus (CaMV) 35S promoter is active in monocot cells, its relative strength is substantially less than in dicot cells, and it is inactive in some cell types, e.g. pollen (Bruce *et al*., 1989; Christensen *et al*., 1992; McElroy and Brettel, 1994). The maize *Adh1* promoter has also been used in monocot transformation studies (Fromm *et al*., 1990), but its activity appears to be restricted to root and shoot meristems, endosperm, and pollen (Kyozuka *et al*., 1991). Because of their expected involvement in fundamental processes in all cell types, the genes for rice actin (*Act-1*) (McElroy *et al*., 1990) and maize ubiquitin (*Ubi-1*) (Christensen *et al*., 1992) have been investigated as potentially useful alternatives to the CaMV 35S and *Adh1* sequences. Both of these monocot promoters have been shown to be significantly more active than the CaMV 35S promoter in monocot cells (Bruce *et al*., 1989; McElroy *et al*., 1990; Christensen *et al*., 1992; Cornejo *et al*., 1993; Gallo-Meagher and Irvine, 1993; McElroy and Brettell, 1994) with the *Ubi-1* promoter being somewhat stronger than the *Act-1* promoter where compared directly (Cornejo *et al*., 1993; Gallo-Meagher and Irvine, 1993; Schledzewski and Mendel, 1994; Wilmink *et al*., 1995).

Since our initial reports on the use of maize *Ubi-1* promoter constructs in transient (Christensen *et al*., 1992) and stable (Toki *et al*., 1992; Uchimiya *et al*., 1993) cereal transformation studies, we have distributed to a large number of researchers a variety of constructs with the *Ubi-1* promoter fused to a spectrum of selectable and scorable markers. Certain of these constructs or their derivatives have been used successfully in transforming a number of different monocot species (Wilmink *et al*., 1995), including several cereals (McElroy and Brettell, 1994) and *Lemna* (Rolfe and Tobin, 1991), with reports of transgenic plants having been generated for rice (Cornejo *et al*., 1993), wheat (Weeks *et al*., 1993), and

*To whom correspondence should be addressed.
‡Present address: Dept. of Biology, George Mason University, Fairfax, VA 22030, USA

0962–8819 © 1996 Chapman & Hall

barley (Wan and Lemaux, 1994). This report presents the structural details of the complete set of these constructs.

Materials and methods

The cloning and sequencing of the maize ubiquitin gene and its promoter have been reported previously (Christensen *et al.*, 1992). All DNA cloning and manipulations reported here were performed according to standard protocols (Ausubel *et al.*, 1989). Restriction endonuclease digestions were carried out according to manufacturers' recommendations. GeneClean (BIO 101, La Jolla, CA, USA) was used to isolate specific restriction fragments from agarose gels. Recovery of DNA fragments was quantified by comparison of ethidium bromide fluorescence of an aliquot of the fragment with known masses of DNA on agarose gels. The *Hin* dIII linker (5′ CAAGCTTG 3′) used in the construction of pAHC27 was obtained from New England Biolabs. DNA ligations and subsequent transformations into competent *Escherichia coli* strain XL1-Blue or HB101 cells and plasmid DNA preparations were carried out using standard protocols (Ausubel *et al.*, 1989). Analysis of DNA sequences was performed using the UWGCG package of programs (Fromm *et al.*, 1990) and DNA Inspector II (Textco, W. Lebanon, NH, USA).

Results

All of the constructs described here were generated by fusing the same 1992 bp *Pst* I fragment from the maize *Ubi-1* gene upstream of the relevant polylinker or marker sequence (Fig. 1). This *Ubi-1 Pst* I fragment contains 899 bp of promoter sequence, 83 bp of 5′ untranslated exon, and 1010 bp of first intron sequence, terminating through reconstitution of the *Pst* I site precisely at the G in the AG dinucleotide of the 3′ splice junction of the intron (Christensen *et al.*, 1992). The nucleotide sequences at the fusion junctions at the 3′ end of the *Ubi-1* DNA are shown for each construct in Fig. 2.

pAHC17

This plasmid is a *Ubi-1* promoter expression vector for *Bam* HI (or *Bam* HI-compatible) cloning of protein coding regions. It contains the *Ubi-1* promoter, 5′ untranslated region and intron upstream of an unique *Bam* HI site (Fig. 1). About 250 bp of nopaline synthase (NOS) 3′ untranslated sequence and polyadenylation signals are located downstream of the *Bam* HI site. The 1992 bp *Pst* I fragment of the *Ubi-1* gene had been previously cloned into the *Pst* I site of M13mp19 for sequencing (Christensen *et al.*, 1992). A *Hin* dIII-*Bam* HI fragment from the replicative form of that clone was isolated and ligated to a 3175 bp *Hin* dIII–*Bam* HI fragment of pMF6 (Goff *et al.*, 1991) containing pUC8 sequence and 250 bp of NOS 3′ polyadenylation sequence adjacent to the *Eco* RI site.

The polylinker sequence is located between the end of the *Ubi-1* intron and the *Bam* HI cloning site and between the *Bam* HI site and the NOS sequence (Fig. 2). Thus, a reporter gene cloned into the *Bam* HI site is flanked by polylinker sequence on both the 5′ and 3′ sides. *Sal* I and *Xba* I sites from the M13mp19 polylinker are upstream of the *Bam* HI site and a *Sal* I and a *Pst* I site from the pUC8 polylinker are on the 3′ side.

pAHC15 and pAHC27 (pUbi-GUS)

These plasmids contain the maize *Ubi-1* promoter, 5′ untranslated region and first intron fused to the coding region of the *E. coli uid*A gene (GUS) (Fig. 1). To produce pAHC15, *Hin* dIII–*Eco* RI fragment of pBI101.2 (Jefferson *et al.*, 1987) containing the *Hin* dIII to *Sma* I region of the pUC19 polylinker, the GUS coding sequence, and 260 bp of the nopaline synthase gene polyadenylation signal was cloned into the *Hin* dIII and *Eco* RI sites of pUC19 (pUC19-GUS-NOS). The 1992 bp *Pst* I fragment of the maize *Ubi-1* gene (Christensen *et al.*, 1992) was cloned into the *Pst* I site of the polylinker sequence upstream of the GUS coding sequence in pUC19-GUS-NOS. The construct contains the *Ubi-1* sequence in an orientation such that transcription will proceed through the ubiquitin 5′ exon, intron and the GUS coding sequence, terminating in the NOS 3′ sequence.

pAHC27 contains the same Ubi-GUS-NOS construct as pACH15 but as a *Hin* dIII fragment cloned into the *Hin* dIII site of pUC19 (Fig. 1). This construct was generated to facilitate the production of pAHC25 (see below). The *Eco* RI site at the 3′ end of the chimeric gene in pAHC15 is not unique as there is an additional *Eco* RI site in the *Ubi-1* intron. However, the *Hin* dIII site at the 5′ end of the chimaeric gene is unique. To allow the entire construct to be removed as one fragment for further subcloning, a *Hin* dIII site was introduced at the 3′ end of the chimaeric gene. This was achieved by partially digesting pAHC15 with *Eco* RI, optimizing the digestion for linear fragments. The *Eco* RI sites were filled in with dNTPs and Klenow fragment of DNA Polymerase and a *Hin* dIII linker (5′ CAAGCTTG 3′; New England Biolabs) was added. Addition of the linker also restored the *Eco* RI site. The DNA was digested with *Hin* dIII to remove excess linker and to cut at the 5′ end of the chimaeric gene. The 4.15 kb *Hin* dIII fragment containing the *Ubi-1 gus* chimaeric gene was gel-purified and subcloned into *Hin* dIII–digested pUC19. The chimaeric gene in the resultant pAHC27 is oriented such that the entire pUC19 polylinker is upstream of the *Ubi-1* promoter (Fig. 1).

pAHC18 – pUbi-LUC

This plasmid contains the *Ubi-1* promoter-5′ exon-first

Corteva Exhibit 2070 - Page 5 of 9
Inari v. Corteva - PGR2023-00022

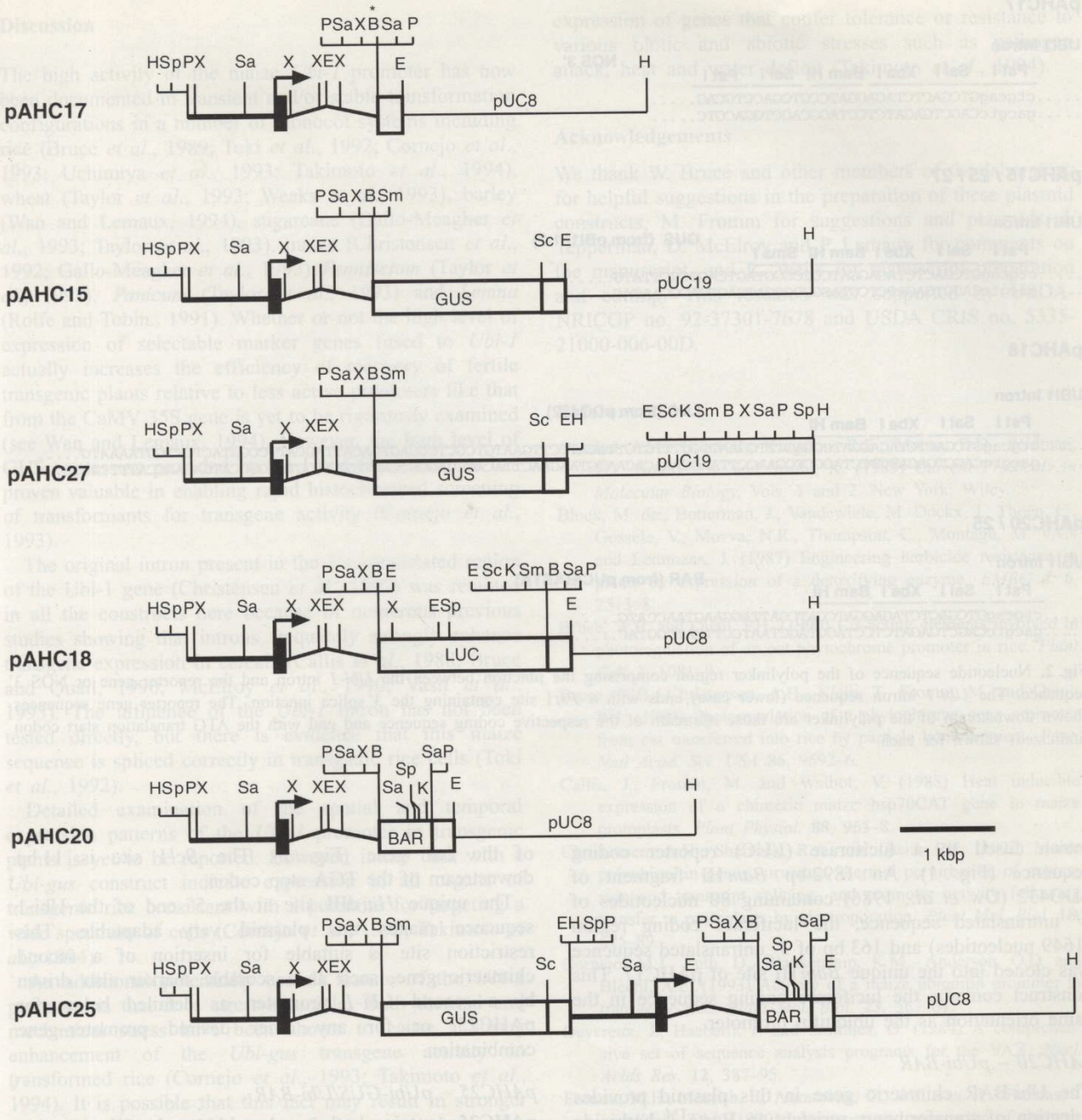


Fig. 1. Schematic diagrams of expression vectors based on maize *Ubi-1* sequences. The relative sizes of the various segments of the linearized plasmids are drawn to scale. Bold straight line, *Ubi-1* promoter sequences; filled box, *Ubi-1* exon; angled line, *Ubi-1* intron; labelled open boxes, reporter gene sequences; blank open box, nopaline synthases 3′ untranslated sequence; thin straight line, pUC8 (pAHC17, 18, 20, and 25) or pUC19 (pAHC15 and 27) sequence. Arrow at the Ubi-1 exon signifies transcription start site and direction. GUS, β-glucuronidase (Jefferson *et al*., 1987); LUC, firefly luciferase (Ow *et al*., 1986); BAR, phosphinothricin acetyltransferase (De Block *et al*., 1987). Restriction sites used in construction of the chimaeric genes and in adjacent polylinker sequences are shown. The *Bam* HI site marked with an asterisk in pAHC17 is an unique site for cloning *Bam* HI or *Bam* HI-compatible fragments. (Note: The *Xba* I sites located in the Ubi-1 intron are subject to methylation interference in *dam* + *E. coli* strains. Also, although the *Eco* RI site in the *Ubi-1* intron upstream of the *gus* sequence in pAHC15 is cleaved efficiently, in both pAHC27 and pAHC25 the corresponding *Eco* RI site is cut very inefficiently). B, *Bam* HI; E, *Eco* RI; H, *Hin* dIII; K, *Kpn* I; P, *Pst* I; Sa, *Sal* I; Sc, *Sac* I; Sm, *Sma* I; Sp, *Sph* I; X, *Xba* I.

Corteva Exhibit 2070 - Page 6 of 9
Inari v. Corteva - PGR2023-00022

pAHC17

UBI1 Intron **NOS 3'**

Pst I Sal I Xba I Bam HI Sal I Pst I

.....ctgcagGTCGACTCTAGAGGATCCGTCGACCTGCAG.....
.....gacgtcCAGCTGAGATCTCCTAGGCAGCTGGACGTC.....

pAHC15 / 25 / 27

UBI1 Intron **GUS (from pBI101.2)**

Pst I Sal I Xba I Bam HI Sma I

.....ctgcagGTCGACTCTAGAGGATCCCCGGGTAGTCAGTCCCTTATG.....
.....gacgtcCAGCTGAGATCTCCTAGGGGCCCATCAGTCAGGGAATAC.....

pAHC18

UBI1 Intron **LUC (from pDO432)**

Pst I Sal I Xba I Bam HI

.....ctgcagGTCGACTCTAGAGGATCCGAGCTTGGAATTCCTTTGTGTTACATTCTTGAATGTCGCTCGCAGTGACATTAGCATTCCGGTACTGTTGGTAAAATG.....
.....gacgtcCAGCTGAGATCTCCTAGGCTCGAACCTTAAGGAAACACAATGTAAGAACTTACAGCGAGCGTCACTGTAATCGTAAGGCCATGACAACCATTTTAC.....

pAHC20 / 25

UBI1 Intron **BAR (from pUC/BASTA)**

Pst I Sal I Xba I Bam HI

.....ctgcagGTCGACTCTAGAGGATCCATCGATTAGGAAGTAACCATG.....
.....gacgtcCAGCTGAGATCTCCTAGGTAGCTAATCCTTCATTGGTAC.....

Fig. 2. Nucleotide sequence of the polylinker region comprising the junction between the *Ubi-1* intron and the reporter gene or NOS 3′ sequence. The *Ubi-1* intron sequence (lower case) ends with a *Pst*I site containing the 3′ splice junction. The reporter gene sequences shown downstream of the polylinker are those upstream of the respective coding sequence and end with the ATG translation start codon (italicized) shown for each.

intron fused to a luciferase (LUC) reporter coding sequence (Fig. 1). An 1892 bp *Bam* HI fragment of pDO432 (Ow *et al.*, 1986) containing 80 nucleotides of 5′ untranslated sequence, the luciferase coding region (1649 nucleotides) and 163 bp of 3′ untranslated sequence was cloned into the unique *Bam* HI site of pAHC17. This construct contains the luciferase coding sequence in the same orientation as the ubiquitin promoter.

pAHC20 – pUbi-BAR

The Ubi-BAR chimaeric gene in this plasmid provides selection of transformants resistant to Basta™ herbicide (phosphinothricin) (De Block *et al.*, 1987). The Ubi-BAR construct was formed by ligating a 570 bp *Bam* HI–*Bcl* I fragment containing the *bar* gene into the *Bam* HI site of pAHC17. The *bar* gene fragment was excised from a plasmid (pUC8/BASTA) obtained from Dr M. Fromm (Fromm *et al.*, 1990). The resultant pAHC20 plasmid has *bar* in the same orientation as the maize Ubi-1 promoter (Fig. 1). The construct contains 18 bp of sequence between the *Bam* HI site and the translation start codon of the *bar* gene (Fig. 2). The *Bcl* I site is 11 bp downstream of the TGA stop codon.

The unique *Hin* dIII site at the 5′ end of the Ubi-1 sequence makes this plasmid very adaptable. This restriction site is suitable for insertion of a second chimaeric gene, such as a scorable marker also driven by a second *Ubi-1* promoter, as detailed below for pAHC25, or for any other desired promoter–gene combination.

pAHC25 – pUbi-GUS/Ubi-BAR

pAHC25 contains both a selectable marker (*bar*) and a scorable marker (GUS), each under the transcriptional control of a separate *Ubi-1* promoter (Fig. 1). The two chimaeric genes were first assembled separately in pAHC20 and pAHC27 and then the double construct was formed. This was achieved by excising the Ubi-GUS-NOS-containing *Hin* dIII fragment from pAHC27 and subcloning it into *Hin* dIII-digested pAHC20. The resultant pAHC25 plasmid has both Ubi-BAR and Ubi-GUS chimaeric genes in the same orientation.

Discussion

The high activity of the maize *Ubi-1* promoter has now been documented in transient and/or stable transformation configurations in a number of monocot systems including rice (Bruce *et al.*, 1989; Toki *et al.*, 1992; Cornejo *et al.*, 1993; Uchimiya *et al.*, 1993; Takimoto *et al.*, 1994), wheat (Taylor *et al.*, 1993; Weeks *et al.*, 1993), barley (Wan and Lemaux, 1994), sugarcane (Gallo-Meagher *et al.*, 1993; Taylor *et al.*, 1993), maize (Christensen *et al.*, 1992; Gallo-Meagher *et al.*, 1993) *Pennisetum* (Taylor *et al.*, 1993), *Panicum* (Taylor *et al.*, 1993) and *Lemna* (Rolfe and Tobin., 1991). Whether or not the high level of expression of selectable marker genes fused to *Ubi-1* actually increases the efficiency of recovery of fertile transgenic plants relative to less active promoters like that from the CaMV 35S gene is yet to be rigorously examined (see Wan and Lemaux, 1994). However, the high level of GUS expression provided by the Ubi-GUS constructs has proven valuable in enabling rapid histochemical screening of transformants for transgene activity (Cornejo *et al.*, 1993).

The original intron present in the 5′-untranslated region of the Ubi-1 gene (Christensen *et al.*, 1992) was retained in all the constructs here because of numerous previous studies showing that introns frequently strongly enhance transgene expression in cereals (Callis *et al.*, 1988; Bruce and Quail, 1990; McElroy *et al.*, 1990; Vasil *et al.*, 1993). The influence of the *Ubi-1* intron has not been tested directly, but there is evidence that this maize sequence is spliced correctly in transgenic rice cells (Toki *et al.*, 1992).

Detailed examination of the spatial and temporal expression patterns of the *Ubi-1* promoter in transgenic plants is yet to be reported. However, initial data with a *Ubi-gus* construct indicate expression in all organs of transgenic rice consistent with a potential for targeting a wide spectrum of cells (Cornejo *et al.*, 1993; Takimoto *et al.*, 1994).

An additional potentially usefull feature of the *Ubi-1* promoter is that it is stress-inducible. Both thermal and mechanical stress have been shown to cause a strong enhancement of the *Ubi-gus* transgene activity in transformed rice (Cornejo *et al.*, 1993; Takimoto *et al.*, 1994). It is possible that this fact may result in stronger expression of selectable marker fusion genes during the early stages of transformation, where recipient cells are exposed to a variety of stresses such as high osmotic pressures, particle bombardment and growth on toxic compounds. A subsequent decrease in expression level is expected upon removal of the selective conditions so that regenerated transgenic plants would presumably not continue to express the marker at high levels when it is no longer needed. The stress-inducibility of the *Ubi-1* promoter might also be useful for driving conditional expression of genes that confer tolerance or resistance to various biotic and abiotic stresses such as pathogen attack, heat and water deficit (Takimoto *et al.*, 1994).

Acknowledgements

We thank W. Bruce and other members of the laboratory for helpful suggestions in the preparation of these plasmid constructs, M. Fromm for suggestions and plasmids, J. Tepperman, D. McElroy and P. Lemaux for comments on the manuscript, and R. Wells for manuscript preparation and editing. This research was supported by USDA-NRICGP no. 92-37301-7678 and USDA CRIS no. 5335-21000-006-00D.

References

Ausubel, F.M., Brent R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1989) *Current Protocols in Molecular Biology*, Vols. 1 and 2. New York: Wiley.

Block, M. de., Botterman, J., Vandewiele, M. Dockx, J., Thoen, C., Gossele, V., Movva, N.R., Thompson, C., Montagu, M. VAN and Lemmans, J. (1987) Engineering herbicide resistance in plants by expression of a detoxifying enzyme. *EMBO J.* **6**, 2513–8.

Bruce, W.B. and Quail, P.H. (1990) *Cis*-acting elements involved in photoregulation of an oat phytochrome promoter in rice. *Plant Cell* **2**, 1081–9.

Bruce, W.B., Christensen, A.H., Klein, T., Fromm, M. and Quail, P.H. (1989) Photoregulation of a phytochrome gene promoter from oat transferred into rice by particle bombardment. *Proc. Natl Acad. Sci. USA* **86**, 9692–6.

Callis, J., Fromm, M. and Walbot, V. (1988) Heat inducible expression of a chimeric maize hsp70CAT gene in maize protoplasts. *Plant Physiol.* **88**, 965–8.

Christensen, A.H., Sharrock, R.A. and Quail, P.H. (1992) Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. *Plant Mol. Biol.* **18**, 675–89.

Cornejo, M.J., Luth, D., Blankenship, K.M., Anderson, O.D. and Blechl, A.E. (1993) Activity of a maize ubiquitin promoter in transgenic rice. *Plant Mol. Biol.* **23**, 567–81.

Devereux, J., Haeberli, P. and Smithies, O. (1984) A comprehensive set of sequence analysis programs for the VAX. *Nucl. Acids Res.* **12**, 387–95.

Fromm, M.E., Morrish, F., Armstrong, C., Williams, R., Thomas, J. and Klein, T.M. (1990) Inheritance and expression of chimeric genes in the progeny of transgenic maize plants. *Bio/Technology* **8**, 833–9.

Gallo-Meagher, M. and Irvine, J.E. (1993) Effects of tissue type and promoter strength on transient GUS expression in sugarcane following particle bombardment. *Plant Cell Rep.* **11**, 567–70.

Goff, S.A., Cone, K.C. and Fromm, M.E. (1991) Identification of functional domains in the maize transcriptional activator C1: comparison of wild-type and dominant inhibitor proteins. *Gen. Dev.* **5**, 298–309.

Corteva Exhibit 2070 - Page 8 of 9
Inari v. Corteva - PGR2023-00022

Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**, 3901–7.

Kyozuka, J., Fujimoto, H., Izawa, T. and Shimamoto, K. (1991) Anaerobic induction and tissue-specific expression of maize *Adh1* promoter in transgenic rice plants and their progeny. *Mol. Gen. Genet.* **228**, 40–8.

McElroy, D. and Brettell, R.I.S. (1994) Foreign gene expression in transgenic cereals. *Trends Biotech.* **12**, 62–8.

McElroy, D., Zhang, W. and Wu, R. (1990) Isolation of an efficient actin promoter for use in rice transformation. *Plant Cell* **2**, 163–71.

Ow, D., Wood, K.V., DeLuca, M., Wet, J.R. de, Helinski, D.R. and Howell, S.P. (1986) Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* **234**, 856–9.

Rolfe, S.A. and Tobin, E.M. (1991) Deletion analysis of a phytochrome-regulated monocot *rbcS* promoter in a transient assay system. *Proc. Natl Acad. Sci. USA* **88**, 2683–6.

Schledzewski, K. and Mendel, R. (1994) Quantitative transient gene expression: comparison of the promoters for maize polyubiquitin 1, rice actin 1, maize derived *Emu* and CaMV 35S in cells of barley, maize and tobacco. *Transgenic Res.* **3**, 249–55.

Takimoto, I., Christensen, A.H., Quail, P.H., Uchimiya, H. and Toki, S. (1994) Non-systemic expression of a stress-responsive maize polyubiquitin gene (*Ubi-1*) in transgenic rice plants. *Plant Mol. Biol.* **26**, 1007–12.

Taylor, M.G., Vasil, V. and Vasil, I.K. (1993) Enhanced GUS gene expression in cereal/grass cell suspensions and immature embryos using the maize ubiquitin-based plasmid pAHC25. *Plant Cell Rep.* **12**, 491–5.

Toki, S., Takamatsu, S., Nojiri, C., Ooba, S., Anzai, H., Iwata, M., Christensen, A.H., Quail, P.H. and Uchimiya, H. (1992) Expression of maize ubiquitin gene promoter-*bar* chimeric gene in transgenic rice plants. *Plant Physiol.* **100**, 1503–7.

Uchimiya, H., Iwata, M., Nojiri, C., Smarajeewa, P.K., Takamatsu, S., Ooba, S., Anzai, H., Christensen, A.H., Quail, P.H. and Toki, S. (1993) Bialaphos treatment of transgenic rice plants expressing a bar gene prevents infection by the sheath blight pathogen (*Rhizoctonia solani*). *Bio/Technology* **11**, 835–36.

Vasil, V., Srivastava, V., Castillo, A.M., Fromm, M.E. and Vasil, I.K. (1993) Rapid production of transgenic wheat plants by direct bombardment of cultured immature embryos. *Bio/Technology* **11**, 1553–8.

Wan, Y. and Lemaux, P.G. (1994) Generation of large numbers of independently transformed fertile barley plants. *Plant Physiol.* **104**, 37–48.

Weeks, T.J., Anderson, O.D. and Blechl, A.E. (1993) Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum*). *Plant Physiol.* **102**, 1077–84.

Wilmink, A., van de Ven, B.C.E. and Dons, J.J.M. (1995) Activity of constitutive promoters in various species from the Lilaceae. *Plant Mol. Biol.* **28**, 949–55.

Corteva Exhibit 2070 - Page 9 of 9
Inari v. Corteva - PGR2023-00022