
Stacy DeBlasio¹, Anding Luo², Daniel Hill², Evan Abbaszadeh², Tara Zadrozny¹, Jing Wang¹, Alexander Goldschmidt¹, Agnes Chan³, Anne W. Sylvester², Dave Jackson¹.

¹ Cold Spring Harbor Lab., 1 Bungtown Road, Cold Spring Harbor, NY 11724; ² Molecular Biology Dept., 1000 E. University Ave., University of Wyoming, Laramie, WY 82071; ³ J. Craig Venter Institute, 9712 Medical Center Drive, Rockville, MD 20850. email: jacksond@cshl.edu; annesyl@uwyo.edu.

Introduction.

With the completion of the draft maize B73 genome, we have entered into a new scientific era where computational genome annotations must now be validated in order to understand the precise function(s) of all maize proteins as well as to identify the regulatory elements controlling their expression. Although there are several approaches to experimentally validating gene function, none is more commonly used than fluorescent protein (FP) technology. With the support of the NSF Plant Genome Program, we have set out to generate 100 stable, natively expressed, FP fusion lines to provide a useful, molecular resource for the maize community and to ultimately uncover information about crop improvement. The lines thus far have provided unique views of maize cellular architecture and we can now study the localization and real-time dynamics for over 90-tagged proteins. These transgenic lines mark most major cellular compartments and can be used to study developmental and physiological processes, including hormone signaling and vesicle trafficking. Data on the characterization of these lines, including confocal micrographs and movies, are accessible on the website, http://maize.jcvi.org/cellgenomics/index.shtml. Localization and expression data for several genes already available on the website are shown in the Figures to the right.

In addition to our protein fusion lines, we are optimizing the LhG4 2-component transactivation expression system for use in maize. The project will make use of the latest maize optimized color tags to fluorescently-label subcellular compartments as well as to highlight distinct cell and tissue types. The project will deliver to the research community a permanent stock of stably transformed seeds for 50 promoter/driver lines, 20 new FP tagged lines, fully characterized in vivo/imaging methods, gene and reporter constructs, and a robust pipeline for handling large image datasets. Image metadata will be processed using the bioimage database management system Bisque and migrated to the community database MaizeGDB annually for long-term availability. Seeds will also be distributed to the Maize Stock Center. See below to find out how to access our project website to look for the list of tagged genes/proteins, to access the virtual cell to find images and proteins that localize to the major subcellular compartments, and to order seeds and constructs.

Gene tagging and generation of transgenic lines.

Genes are tagged using either triple template overlap PCR (TT-PCR) or Multistep Gateway (Tian et al. 2004 and Invitrogen website, respectively). PCR products corresponding to 2-3 kb of the 5’ upstream region plus gene ORF (for C-terminal tagging) and 1-2 kb of the 3’ downstream region, are cloned into pDONR vectors using the Gateway® BP reaction system. For N-terminal tagging, the gene ORF is combined with the 3’ downstream region. The expression clone is generated by combing entry clones, including a fluorescent tag entry vector, along with the pTF101.1 maize binary vector that has been converted into a Gateway® destination vector. Constructs are sent to the maize transformation facility at ISU for A. tumefaciens-mediated maize transformation. In addition to the standard YFP, CFP and mRFP tags, we have several maize optimized FPs, including TagBFP, Cerulean, mTFP1, TagRFP and mCherry (see images below).

Images of stable transgenic maize FP reporter lines.

Confocal laser scanning images, except J which was visualized using widefield epi-fluorescence microscopy.

A, CYTOKININ REPORTER: NLs-48-TOMATO (root cap, nucleus); B, HISTONE H1-YFP (ear primordia, chromatin, red-FM4-64); C, TANGLED1-YFP (leaf, preprophase band); D, HISTIDINE PHOSPHOTRANSFER PROTEIN1-RFP (ear spikelet meristems, nucleocytoplasmic); E, LIPOXYGENASE10-YFP (tassel spikelet pair meristems, small plastid-like organelles, red-FM4-64); F, MALATE DEHYDROGENASE-YFP (leaf, mesophyll chloroplasts, red-chlorophyll autofluorescence); G, PEROXIN11-YFP (root epidermis, peroxisomes, red-propidium iodide); H, GFP-RIBOSOMAL PROTEIN L18 (ear primordia, punctate, possibly ribosomes); I, PINFORMED1-YFP (ear floral meristems, plasma membrane, red-autofluorescence); J, EXPANSIN A1-RFP (root, vesicles, cell wall/cytoplasm, blue-cell wall autofluorescence); K, PROTEIN DISULFIDE ISOMERASE-YFP (leaf, perinuclear ER, red-FM4-64); L, FLAVONOL SYNTHASE1-RFP (ear bract, perinuclear ER); M, TONOPLAST INTRINSIC PROTEIN1-YFP (ear primordia, vacuole membrane and ER); N, RAB1A1-YFP (leaf, perinuclear ER and vesicles); O, FIMBRIN ACTIN BINDING DOMAIN-YFP (leaf epidermis, actin cytoskeleton); P, G2AUBULIN-YFP (leaf, microtubules, insets, Tubulin-GFP and RFP, leaf cell during mitosis, spindle microtubules)

LhG4-pOp maize trans-activation system

A tissue specific promoter drives expression of the LhG4 transcription factor, which binds to pOp sequences and activates expression of GUS and NLS-TagRFP in the same cells. This line can also be crossed to another pOp reporter line, to activate any gene in trans. We are developing this system using knowledge gained from our subcellular tagging project (below) and community suggestions. Our goal is to generate a community resource for tissue specific gene expression, knockouts or other genomic applications.

Examples of tissue specific promoters discovered by our project.

Images show tissue specific promoters from G2AUBULIN-RFP, (A), note pink color of seeds and specific expression in inner endosperm (en) but not in aleurone or pericarp, Brittle2-RFP (B), expression in leaf cross-section, green, that appears specific to bundle sheath cells (A.S. and Anding Luo, preliminary data); Jacalin1-RFP (C) shows expression enriched in vasculature; DR5-RFP (D), expression enhanced in zones rich in auxin; RTR7-RFP (E), expression strongest in the mesistem central zone; Tasselsh3-1-RFP (F), expressed in glume leaf primordia in the inflorescence; and Zmm16-1-RFP (G), which shows expression in specific floral organ primordia. See Poster 114 for images of our pZmWuschel1::RFP::NLS and pRamosa3::RFP::NLS lines, which will also used to develop the maize LhG4-pOp maize trans-activation system

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