MAIZE COMMUNITY RESOURCES FOR FORWARD AND REVERSE GENETICS

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ABSTRACT - Maize has been intensively studied for agricultural improvement as well as to increase our understanding of biology. Maize is highly amenable to genetic studies, and the current plant genome initiative to obtain an anchored sequence of the maize genome is likely to make maize an important species for functional genome studies in grasses. One aspect of functional genomics is to understand the phenotypic consequence of the loss of any gene within the genome, which is essentially conventional genetic analysis of mutations. Genome scale studies of mutations can be driven either by phenotypes (forward genetics) or by gene sequences (reverse genetics). These approaches are essentially asking converse questions. For forward genetics, what are the genes that cause similar phenotypes? For reverse genetic studies, what are the phenotypes of mutations in related genes? This review discusses the mutagenized populations that have been developed by the maize research community for both forward and reverse genetics resources. In addition, these resources are compared to some of the genetics resources available in Arabidopsis thaliana.

KEY WORDS: Reverse genetics; Forward genetics; Genomics; Transposon.

INTRODUCTION

Arabidopsis thaliana provides a good model for the types of resources that are necessary for rapid forward and reverse genetic analysis in plants. The high-quality, complete genome sequence is essential for both (Arabidopsis Genome Initiative, 2000). The complete genome sequence greatly simplifies map-based cloning for forward genetics by providing physically ordered sequences to develop markers and by identifying candidate gene sequences for the mutation within the mapped genetic interval. For reverse genetics, the genome allows researchers to quickly identify a full complement of closely related genes or genes predicted to be involved in a particular metabolic or developmental pathway (e.g. Lurin et al., 2004). The genome sequence also serves to anchor sequence-indexed insertional mutations caused by T-DNA or transposon insertions. This dramatically simplifies a reverse genetics project by allowing the researcher to identify genes of interest and corresponding knockouts in silico. Further, the knockout resources are, for the most part, integrated in a single web-site that allows researchers to order seed stocks from multiple insertional mutagenesis populations in a few easy steps (SIGNAL TDNA-EXPRESS, 2005).

INGREDIENTS FOR GOOD GENETIC RESOURCES

An important technological advance for the generation of the Arabidopsis knockout resources was the development of a simple and fast method to generate single or low-copy insertional mutations via floral dip T-DNA transformation (Clough and Bent, 1998). This has allowed a relatively small number of laboratories to independently generate hundreds of thousands of independent transformation events. By maintaining each transformation event as a single genetic stock, the location of all events can be determined by amplifying and sequencing genomic DNA flanking each insertion (Sessions et al., 2002; Alonso et al., 2003; Rosso et al., 2003; Samson et al., 2004). The single or low-copy insertions allow relatively simple tests for linkage between the tagged mutation and any mutant phenotype observed. Furthermore, phenotypic mutants from T-DNA populations can be rapidly asso-
associated with specific gene sequences (e.g. Tzafirir et al., 2004).

Developing similar resources for maize will be challenging. Map-based cloning depends on highly integrated genetic and physical maps. For maize, there are multiple versions of both physical and genetic maps, but in recent years, several collaborative research projects have focused on developing physical maps from three bacterial artificial chromosome (BAC) libraries of the B73 inbred and genetic maps based on the intermated B73 x Mo17 (IBM) population (Lee et al., 2002; Tomkins et al., 2002; Yim et al., 2002; reviewed in Coe et al., 2002). The physical map is being developed with two restriction enzyme fingerprinting technologies and by hybridization or amplification of molecular markers on the BACs (reviewed in Meyers et al., 2004). The current Agarose and High Information Content Fingerprint (HICF) maps are available through the Arizona Genomics Institute website (see Table 1). Similarly, the IBM genetic map has multiple classes of molecular markers that were originally developed through the Maize Mapping Project and additional markers are being added by the Maize Genetic Mapping Project (see Table 1 for websites).

The current maize maps and markers have allowed several recent positional cloning successes. Vgt1, Ramosa2, and Tga1 were cloned using map-based strategies (personal communications from S. Salvi, E. Bortiri, and J. Deebly), and it is estimated that positional cloning in maize will require a smaller segregating population than in Arabidopsis to narrow the physical interval to a few or even a single candidate gene(s) (B. Li, personal communication). However, in each case, a significant amount of effort was required to develop additional molecular markers for fine mapping the loci to small physical intervals. Ultimately, a complete maize genome sequence would represent the most complete physical map and would place genetic markers based on sequence. A full genome sequence could also be used to develop additional molecular markers to dramatically facilitate positional cloning.

The maize genome has been partially sequenced with gene enrichment strategies that include: expressed sequence tags (ESTs), high Cot, and methyl-filtered clones (Lunde et al., 2003; Palmer et al., 2003; Whitelaw et al., 2003). These sequencing projects are estimated to identify a fragment from 95% of the genes within the genome with an average coverage of 75% of the nucleotides within the genes identified (Springer et al., 2004). In addition, completely sequenced BACs, BAC end sequences (BES), and some unfiltered genome shotgun sequence add to the coverage of the maize genome (Whitelaw et al., 2003; Messing et al., 2004; Arizona Genomics Institute, 2005). The extensive coverage of the maize gene space allows researchers to readily identify a gene of interest (GOI) for reverse genetic studies. Also, these sequence resources provide the basis for collections of sequence-indexed insertion mutants. However, only a small fraction of the current maize genome sequence is anchored to the physical or genetic maps. The National Plant Genome Initiative has called for proposals to anchor the existing maize sequence to the physical and genetic maps as well as to complete large-scale sequencing to assemble the remaining gene sequences in maize (NSF 04-614, www.nsf.gov/bio/dbi/dbi_pgr.htm). With a full genome sequence, collections of sequence indexed insertion mutations will become valuable for studying complete gene families or biochemical pathways.

The majority of the sequenced knockouts in Arabidopsis derive from transgenic mutagenesis strategies in which most lines have one or two genes tagged with a T-DNA. Maize transgenics are far more difficult to generate and to work with than in Arabidopsis. Maize can be transformed either by particle bombardment or with T-DNA via Agrobacterium tumefaciens (Frame et al., 2002). However, both methods require tissue culture for plant regeneration as well as special permits to grow transgenic plants in the field. The Wang lab directs a public service to allow researchers who do not specialize in maize transformation to work with transgenics. The facility is based at Iowa State University and charges user fees to develop transgenic callus, plantlets, or seed (see Table 1 for website). The current transformation technology and the price of the public facility makes only small collections of transgenics practical. Thus, maize transformation has not yet been used to generate the large number of independent transformation events necessary for genome-wide knockout collections.

As an alternative to transgene insertions, transposable elements can be used as mutagens to develop both forward and reverse genetics resources. Maize has a rich collection of native transposon families, and current projects are focused on developing either the Activator/Dissociator (Ac/Db) or Robertson’s Mutator (Mu) systems for genome-wide genetics resources (summarized in Table 1). The bi-
TABLE 1 - Contact information for maize community genetics resources.

<table>
<thead>
<tr>
<th>Resource</th>
<th>Project Website(s)</th>
<th>Primary Contact (e-mail)</th>
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<tr>
<td>Agarose Physical Map</td>
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<td>Ed Coe (<a href="mailto:coee@missouri.edu">coee@missouri.edu</a>)</td>
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<td>HICF Physical Map</td>
<td><a href="http://www.genome.arizona.edu/fpc_hicf/maize/">http://www.genome.arizona.edu/fpc_hicf/maize/</a></td>
<td>Jo Messing (<a href="mailto:messing@mbcl.rutgers.edu">messing@mbcl.rutgers.edu</a>)</td>
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<td>Maize Mapping Project</td>
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<td>Ed Coe (<a href="mailto:coee@missouri.edu">coee@missouri.edu</a>)</td>
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<td>Patrick Schnable (<a href="mailto:schnable@iastate.edu">schnable@iastate.edu</a>)</td>
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<td><a href="http://www.agron.iastate.edu/ptf/web/mainframe.htm">http://www.agron.iastate.edu/ptf/web/mainframe.htm</a></td>
<td>Kan Wang (<a href="mailto:kanwang@iastate.edu">kanwang@iastate.edu</a>)</td>
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<tr>
<td>Mapped Ac elements</td>
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<td>Tom Brutnell (<a href="mailto:tpb8@cornell.edu">tpb8@cornell.edu</a>)</td>
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<tr>
<td>Transposed Ac elements</td>
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<td>Hugo Dooner (<a href="mailto:dooner@waksman.rutgers.edu">dooner@waksman.rutgers.edu</a>)</td>
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<td>TUSC</td>
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<td>Robert Meeley (<a href="mailto:bob.meeley@pioneer.com">bob.meeley@pioneer.com</a>)</td>
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<td>Virginia Walbot (<a href="mailto:waltbot@leland.stanford.edu">waltbot@leland.stanford.edu</a>)</td>
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<td>Alice Barkan (<a href="mailto:abarkan@molbio.uoregon.edu">abarkan@molbio.uoregon.edu</a>)</td>
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<td>UniformMu</td>
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<td>Donald McCarty (<a href="mailto:drm@ifas.ufl.edu">drm@ifas.ufl.edu</a>)</td>
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<td>Rita Monde (<a href="mailto:maizetilling@purdue.edu">maizetilling@purdue.edu</a>)</td>
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The physiological properties of these transposon classes are different, giving advantages and disadvantages to each system for forward and reverse genetics applications.

**Ac MUTAGENESIS**

*Ac/Ds* are part of the *hAT* family of DNA transposons, which transpose predominantly to closely linked sites within the genome (reviewed in Kunze and Wei, 2002). *Ac/Ds* elements yield germinal revertants and insertion site footprints that can include small insertions or deletions. These properties allow *Ac/Ds* elements to target local segments of the genome for saturation mutagenesis and to generate an allelic series within a single gene. The *Ac* element encodes the transposase required for excision/transposition of itself as well as *Ds* elements. This allows *Ac* elements to be tracked by the generation of somatic revertant sectors in maize genetic stocks that carry transposon-induced mutations. These somatic activity markers are typically in anthocyanin or other non-essential but easily scored mutations. The *Ac* element shows negative dosage that affects the size of revertant sectors allowing...
multiple copies of Ac to be distinguished from a single copy (Fig. 1).

By taking advantage of these properties, the Dooner and Brutnell labs have developed genetic stocks in the W22 inbred to select Ac transposition events. Cowpertzwaite et al. (2002) used the wx-m7 allele tagged with Ac along with the bz1-m2 allele tagged with Ds to select for excision from the wx locus while retaining Ac transposase. This selection identified >1200 transposition events that were predominantly linked to the wx locus. In contrast to this local mutagenesis approach, Kolkmann et al. (2004) used Ac dosage effects to select for Ac transposition events that retained Ac in the donor locus. This allowed selection in a subsequent generation for 158 transposition events to unlinked loci. The unlinked Ac’s identified in both studies are available through the maize stock center and can be used as new sources for local mutagenesis.

Ac is native to maize, and the W22 inbred harbors a background of Ds and inactive Ac elements. Thus, local mutagenesis with Ac produces transposed Ds elements in other genomic locations. When used for local mutagenesis, the wx bz1 stocks showed 61 visible mutant phenotypes (~5% of the transposed lines), but only 6 of the mutants were linked to the transposed Ac indicating that 90% of the visible phenotypes were most likely caused by background Ds elements (Cowpertzwaite et al., 2002). Thus, the Ac transposition lines can be used for forward genetic screens, but they induce visible phenotypes at a lower frequency than Mu elements, and most of the visible mutations will not be tagged by the active Ac. However, the dosage effect of Ac on somatic reversion allows a simple visual screen to identify phenotypes that are linked to the transposed Ac and most likely tagged. The Ac dosage screen has proven effective as both Ac populations were used to clone seed phenotypes tagged by transposed Ac’s (Singh et al., 2003, Ma and Dooner, 2004). To amplify the Ac transposition sites, both the Brutnell and Dooner labs used DNA gel blots and a gel-purification step prior to inverse-PCR in order to remove the inactive Ac elements from the PCR products (Cowpertzwaite et al., 2002; Kolkmann et al., 2004). Interestingly, Ac did not show an insertion bias within genes in contrast to Mu elements, which appear to have a stronger bias for the 5’ end of genes (Cowpertzwaite et al., 2002; Dietrich et al., 2002). This suggests that C-terminal truncations of genes will be easier to identify from Ac mutagenesis populations.

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**FIGURE 1 - Examples of transposon somatic activity markers in maize.**

(A) Ac dosage can be monitored based on the size and timing of reversion of the r-m3 allele. From left to right is one, two, and three doses of Ac (photo courtesy of Tom Brutnell and Liza Conrard). (B) Mu somatic reversion of the bz1-mum9 allele produces densely spotted kernels in which the density of spots is not directly related to the number of MuDR elements. (C) Mu-active kernels with lower density of bz1-mum9 reversion including a Mu-inactive kernel on the right.

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**Mu MUTAGENESIS**

Mu elements are DNA transposons that are characterized by a higher rate of forward mutations than other transposable elements in maize (reviewed in Walbot and Rudenko, 2002). In addition, Mu elements show frequent somatic insertion and excision events, but a low rate of germinal reversion indicating a different mechanism or timing of transposition from Ac. Like other DNA transposons, the maize family of Mu elements comprises of autonomous elements (MuDR) and non-autonomous elements (Mu1, Mu2, etc.) that share terminal inverted repeat sequences. Finally, Mu transposons are subject to genome-wide epigenetic regulation that suppresses transposon activity. In some cases, Mu insertions can cause epigenetically regulated mutant phenotypes that are suppressed when the transposons are inactive (Barkan and Martienssen, 1991; Greene et al., 1994; Grirad and Freeling, 2000; Settles et al., 2001). Due to the transposition properties of Mu, the el-
lements accumulate to high-copy numbers within maize lines harboring active \textit{Mu}, which allows a relatively small population of \( \sim 40,000 \) plants to have a high chance of mutating most genes within the genome. Consequently, multiple groups have developed \textit{Mu} transposon-tagging populations that can be used for both forward and reverse genetics. For reverse genetics, the typical strategy is to grow a large number of \textit{Mu}-active individuals that contain a collection of novel and ancestral transposition events. The plants are self-pollinated to recover the novel transposon gene disruptions in each plant, and DNA is sampled from the plants in pools that can be screened by PCR with specific primers for \textit{Mu} elements and a \textit{GOI}. Usually, the pools are formatted into two dimensional grids of \( 48 \times 48 \) plants, which allows individual plants carrying specific insertions to be identified in a single screening step (Fig. 2). For forward genetics, many of the populations have been scored for common classes of phenotypes that are accessible through each project’s website (Table 1).

Actively transposing \textit{Mu} populations also cause somatic insertion events resulting in false positives that are not recovered in the self-pollinations. One approach to avoid somatic insertions is to sample DNA from two different leaves or from either side of the midvein of the same leaf. Due to the timing of somatic \textit{Mu} insertions, the cells in the separate samples are unlikely to share the same somatic insertions. Insertions that are detected in both DNA samples are thus most likely to be germinal. A second approach to limit the impact of somatic transposon activity has been to use somatic activity markers and/or genetic suppression of \textit{Mu} activity to select plants that have no or greatly reduced levels of somatic transposition. Selecting against transposon activity has the added benefit of stabilizing the number and location of transposon insertion sites within the plant.

\textbf{\textit{Mu} Knockout Service Facilities}

Multiple groups have developed reverse genetics populations in order to identify knockouts in any gene within the maize genome. These include: the Pioneer Hi-bred corporation, the Maize Targeted Mutagenesis project, the Maize Gene Discovery Project, and the BBSRC Gene Function Initiative. The Pioneer Hi-Bred corporation developed the initial reverse genetics resource known as the Trait Utility System for Corn (TUSC) (Benson et al., 1995). The TUSC population includes plants from diverse collections of active \textit{Mu} populations. Consequently, many of the TUSC families do not carry genetic markers for \textit{Mu} activity. Public researchers can screen the population for a \textit{GOI} by entering into a formal collaboration with Pioneer Hi-Bred, which includes shared intellectual property rights relating to the mutant phenotype. However, the PCR screening is completed without any user fees, and there are multiple examples of successful TUSC reverse genetics projects (e.g. Papa et al., 2001; Arthur et al., 2003; Kocsis et al., 2003; Gallavotti et al., 2004).

The Maize-targeted mutagenesis (MTM) project provides an alternative reverse genetics screening service (May et al., 2003). The MTM population contains \( \sim 44,000 \) plants from \textit{Mu} lines. However, MTM plants derive from crosses between \textit{Mu} active individuals carrying either the \textit{bz1-mum9} or \textit{a1-mum2}
anthocyanin mutations as somatic activity markers and a dominant *Mu* activity inhibitor to suppress somatic *Mu* insertions. The dominant inhibitor line also carries a *Les28* leaf lesion mimic mutation that is epigenetically regulated by *Mu* activity (MARTINSEN and BARON, 1994). The *Les28*, inhibitor line clearly reduced *Mu* activity in the MTM ears; however, a significant fraction of the MTM F2 families retain somatic activity (May et al., 2003). To screen the MTM population, individuals provide the project with genomic sequence from the GOI, and the MTM project designs primers and completes the screen for a user fee. Based on the initial 72 genes screened by MTM, there is a 42% chance of recovering one or more insertion alleles from the population (May et al., 2003). As part of the user agreement, both the genes that are screened as well as the phenotypes observed need to be entered into the MTM database, mtmDB. This database also includes phenotypes that have been observed directly by the MTM project.

The Maize Gene Discovery Project (MGDP) has developed a *Mu* transposon population based on a transgenic *Mu* element, Rescue*Mu* (RAIZADA et al., 2001; FERNANDES et al., 2004). The Rescue*Mu* element was engineered to contain pBlueScript between the *MuI* terminal inverted repeats. Flanking sequences for the Rescue*Mu* transposon insertion sites can then be recovered via plasmid rescue. The Rescue*Mu* population was grown in a series of 25 grids, and plasmid libraries have been developed from the rows and columns for each grid. To identify an insertion in a GOI, a user orders the plasmid libraries and completes a PCR screen to determine the specific grid and location of the individual Rescue*Mu* plant. The progeny from individual plants can be ordered through the Maize Stock Center. It is important to note that the Rescue*Mu* element is a low copy element with <1 novel germinal Rescue*Mu* transposition per plant (FERNANDES et al., 2004). Thus, the ~33,000 Rescue*Mu* plants are likely to contain a much smaller fraction of tagged insertions than can be identified by PCR screening of either the MTM or TUSC populations.

The MGDP also sequenced from a subset of the plasmid grids. These sequences can be used to identify potential insertions in a GOI. A positive match in a Genbank GSS database search identifies a specific grid for subsequent screening. Currently, there are 178,000 Rescue*Mu* sequences in Genbank, and they are estimated to represent ~15,000 different insertion sites (FERNANDES et al., 2004). Both the incomplete maize genome sequence as well as the mixed genetic background of the Rescue*Mu* population leads to some uncertainty about whether highly similar insertion site sequences represent different alleles of the same locus or insertions in different paralogous sequences. An additional factor to consider prior to genetic analysis of Rescue*Mu* insertions is that the population was developed in a highly active native *Mu* population, and many of the plasmids sequenced represent somatic insertions. FERNANDES et al. (2004) suggest that Rescue*Mu* sequences that are identified multiple times in a single row are more likely to represent germinal insertions, which could be used to determine if the researcher would like to invest time in screening the grid. By this criteria, ~5,000 of the sequenced Rescue*Mu* insertions are strong candidates for germinal insertions.

The BBSRC Gene Function Initiative is also developing gene knockout resources based on MuAFLP (HANLEY et al., 2000). *Mu* insertion sites are amplified from individual *Mu*-active plants using MuAFLP, and the PCR products are spotted in arrays. These MuArrays are then probed with a cDNA for the GOI and positive signals identify plants with insertions in related genes (http://www.cerealsdb.uk.net/muarray.htm). This approach will identify knockouts in genes with related families, and it is essential to suppress somatic transposon activity in order to identify germinal insertion events. More details on screening the MuArray are available through the project website (Table 1).

**PHENOTYPE DIRECTED *Mu* POPULATIONS**

Two additional *Mu* populations have been developed that are focused on common classes of mutant phenotypes. The Uniform*Mu* population and the Photosynthesis Mutant Library (PML) target seed and non-photosynthetic mutants, respectively (MCARTY et al., 2005; reviewed in STERN et al., 2004). The PML is a collection of 2,200 mutants that have reduced chlorophyll accumulation or high chlorophyll fluorescence phenotypes. By selecting non-photosynthetic mutants, the PML enriches for mutations in genes required for chloroplast biogenesis. Many of the PML mutants have been characterized for chloroplast protein and RNA defects to classify their likely roles in chloroplast biogenesis. These phenotypes can be searched through the project website (Table 1). In addition, the PML is arranged into a grid for PCR screening of knockouts, which
has been used to identify several mutants affecting chloroplast development (TILL et al., 2001; OSTMIEIER et al., 2003; ASAKURA et al., 2004). Currently, the PML project will screen for Mu disruptions without any user fees. However, users should target genes that are likely to be required for chloroplast biogenesis, because the PML population is unlikely to contain insertions in randomly selected maize genes due to the small population size.

The UniformMu population was developed with a focus on seed mutants (McCARTY et al., 2005). For the TUSC, MTM, and RescueMu tagging populations, the Mu active parents carried progenitor seed mutant genes in the mutagenized (M1) individuals used for the reverse genetics pools. Thus, roughly 25% of the M2 families segregate for seed mutants in these populations with a large fraction of the mutations likely to be non-independent (May et al., 2003; SHEN et al., 2003; http://www.maizegenetics.purdue.edu/). Currently, four 48x48 grids have been developed from kernels that are active, based on the somatic activity marker into the W22 inbred parent (McCARTY et al., 2005). The self-pollinations were screened to remove the progenitor seed mutants, and the remaining W22 X Mu-active crosses were used to introgress Mu activity into W22. After 5-8 backcross generations, the W22 x Mu-active crosses were used as M1 plants and self-pollinated to generate M2 families. In addition, each M0 progenitor was used to develop a small number (30-50) of M2 families in order to reduce the number of pre-miotic Mu insertions recovered. This crossing scheme also greatly reduces the impact of progenitor mutations without seed phenotypes, because a large number of different Mu-active plants were used to develop the ~32,000 UniformMu M2 families. McCARTY et al. (2005) identified ~4,200 UniformMu seed mutants and tested ~1,000 mutants for heritability. Currently, ~1,750 different insertion sites based on BLAST searches of known maize sequences (McCARTY et al., 2005).

**CHEMICAL MUTAGENESIS**

Chemical mutagens can be used as an alternative to insertional mutants to develop genetics resources. Targeted Induced Local Lesions IN Genomes (TILLING) is a reverse genetics approach that was originally developed in Arabidopsis (reviewed in HENIKOFF et al., 2004). TILLING uses ethylmethanesulfonate (EMS)-mutagenized populations to generate point mutations throughout the genome. Mutant alleles are identified similarly to PCR screening for insertion lines. DNA is extracted from pools of EMS mutagenized plants that segregate for the EMS induced mutations, and the GOI is amplified from the pools. If a mutation is present within the pool, the PCR products will form a heteroduplex between the normal and mutant allele in the reaction. The CEL I endonuclease is used to specifically cleave heteroduplex DNA, and the cleaved products are visualized using denaturing gel electrophoresis. It is important to note that only a fraction of the point mutations identified by TILLING will have deleterious effects on the gene and that at least 10 EMS induced alleles are generally necessary to assure that one hypomorphic allele will be identified. However, the point mutations that are identified through TILLING do generate an allelic series that can include weak or dominant alleles.

A maize TILLING project has developed EMS mutagenized populations for the B73 and W22 inbreds. The project charges user fees to screen individual
lochi, and the users are responsible for designing primers for screening a GOI. Tiil. et al. (2004) conducted a TILLING screen of a pilot B73 population for mutations in 11 genes involved in chromatin structure. Although the population was small, 17 mutations were identified in the genes with 5 mutations estimated to disrupt protein function. The maize TILLING project has since developed larger EMS populations that are available for public screening. Details for conducting a TILLING screen can be found at the project website (Table 1).

**CONCLUDING REMARKS**

The large variety of maize community genetics resources allows individuals flexibility in their approach towards obtaining a mutant. Most of these resources require an up-front investment to identify the mutant of interest. For example, the Ac transposition lines allow researchers to develop new local mutagenesis experiments, but the Ac strategy requires several field seasons to develop and screen the mutagenesis population. Alternatively, the Mu and TILLING populations provide a large array of reverse genetics capabilities, but PCR screening for mutations in a GOI may require searching multiple populations to ensure a knockout is identified. Each of these populations comes with a different cost ranging from direct user fees of $750 or more to developing specific collaborations in order to conduct the PCR screen. An additional factor to consider in choosing between these resources is the genetic background of the population. The Ac, UniformMu, and TILLING populations were developed in either the W22 or B73 inbreds, while the TUSC, MTM, and RescueMu populations have mixed or heterotic pedigrees. Maize inbreds have significant differences in the W22 or B73 inbreds, while the TUSC, MTM, and TILLING populations were developed in either EMS populations that are available for public screening. Mutations in the W22 or B73 inbreds, while the TUSC, MTM, and TILLING populations were developed in either EMS populations to ensure a knockout is identified. Each of these populations comes with a different cost ranging from direct user fees of $750 or more to developing specific collaborations in order to conduct the PCR screen. An additional factor to consider in choosing between these resources is the genetic background of the population. The Ac, UniformMu, and TILLING populations were developed in either the W22 or B73 inbreds, while the TUSC, MTM, and RescueMu populations have mixed or heterotic pedigrees. Maize inbreds have significant differences in both repetitive sequences and gene content suggesting that the genetic background will mask some phenotypes (Fu and Dooner, 2002; Brunner et al., 2005). In any case, all of the populations contain multiple mutations segregating within the mutagenized families, and significant genetic analysis is required to determine if any phenotype observed is linked to the GOI.

The Arabidopsis research community initially developed reverse genetics grids as primary gene-knockout facilities (reviewed in Parinov and Sundaresan, 2000). These PCR screening resources have essentially been replaced by sequence-indexed mutation databases, because there was a strong interest in having a knockout in every gene within the genome. In maize, it is still an open question whether the genetics community wants a systematic collection of gene knockouts throughout the entire genome. However, several groups have started developing sequence-indexed insertion site databases. Sequencing maize transposon insertion sites is not as efficient as sequencing T-DNA lines from Arabidopsis, because the transposon populations are native and contain multiple insertions in each plant including progenitor elements. Individual insertions have been recovered by excising specific flanking sequences from DNA gels (Hanley et al., 2000; Cowperthwaite et al., 2002; Kolkman et al., 2004). Alternatively, libraries of insertions have been sequenced that include redundant progenitor insertions (Fernandes et al., 2004; Settles et al., 2004; McCarthy et al., 2005).

Although the maize sequencing strategies are less efficient than directly sequencing PCR products from Arabidopsis T-DNA insertions, it is still more cost effective to develop a systematic collection of knockouts than to complete PCR screens for all genes within the genome. In addition, sequence-indexed mutants can be identified much more rapidly using BLAST searches, which increases the speed of reverse genetic studies. The current collection of sequenced insertion sites represent at least 5,000 distinct germinal insertions throughout the genome, but they have not yet been integrated into a single BLAST searchable database. The Gramene database has anchored the RescueMu sequences to the rice genome, but these are not directly searchable by BLAST (Ware et al., 2002). The remaining sequences can be found at individual project websites (Table 1), or in the GSS Genbank database. It is difficult to identify specific matches to transposon flanking sequences in GSS due to the large number of other maize genomic sequences included in this database. Ideally, the MaizeGDB and Gramene databases will integrate these sequenced insertion sites similarly to the SIGnAL TDNA-Express database in the near future (Ware et al., 2002; Lawrence et al., 2004; SIGNAL TDNA-Express, 2004).

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