

Genetic fine-mapping technology

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Genetic maps provide a powerful tool for gene identification, study, utilization and isolation. Fine-mapping involves the identification of markers that are very tightly linked to a targeted gene. In plants, traditional genetic markers (morphological traits, isozymes, etc.) usually did not permit fine mapping because they were too rare, too widely spaced and often too difficult to use. The advent of DNA markers has made fine mapping possible in any plants that can be crossed. The advantages of DNA markers include their very large number, a relatively high rate of polymorphism in many populations, usually robust/routine technologies for scoring, frequent comparability across species, clear dominance or codominance in most situations, and no problems with penetrance or epistasis.

A genetic fine map of a specific locus will usually have as its goal the identification and location of markers that flank the targeted gene and are within one or fewer centiMorgans (cM). With a fine map, marker-assisted selection can be very precise. Also, comparisons of map positions can be accurately made with other species to see if they share similar traits at that chromosomal location. Finally, fine-mapping a gene is usually an essential step in map-based gene isolation, whereby the researcher can acquire the gene for further precise study and for use in crop improvement by transgenic technology.

Several problems are associated with the generation of a fine map around any plant gene. First, relative to bacteria and many other simple organisms, plants have relatively large genomes. Hence, most DNA markers are unlinked to any given locus (e.g., on a different chromosome) and most linked markers are many cM away from the targeted gene. Second, recombination is relatively rare in plants. On the average, a particular chromosome may have one meiotic recombination event per chromosome arm per sexual generation. Mapping markers to a resolution of one cM requires the investigation of a few hundred sexual progeny. Third, marker polymorphism can be low in a mapping population. Whether a particular genetic marker is near the targeted gene can only be determined if there is polymorphism,

In: B.I.G. Haussmann, H.H. Geiger, D.E. Hess, C.T. Hash, and P. Bramel-Cox (eds.). 2000. Application of molecular markers in plant breeding. Training manual for a seminar held at IITA, Ibadan, Nigeria, from 16-17 August 1999. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

so that populations with low levels of polymorphism will not allow location of most tightly-linked markers. This problem can be partly alleviated by using mapping populations derived from crosses between distantly related parents, often individuals classified as different species. However, these interspecific crosses are prone to segregation distortion and/or recombinational suppression/enhancement phenomena that can severely limit the quantitative accuracy of mapping studies.

The importance of fine-mapping studies is indicated by the large number of techniques that have been developed to facilitate this process. Many different DNA marker types have been developed, varying tremendously in their cost, ease of generation, dependability, and long-term usefulness. Different mapping populations have been developed, partly to take optimal advantage of different DNA marker approaches and partly to optimize the subsequent power of the maps generated. For instance, recombinant inbred populations are particularly useful for fine mapping because they provide at least two-fold higher frequencies of recombination in any small chromosomal region compared to an F₂ or an F₁ backcross, and provide a permanent source of mapped individuals. Bulk segregant analysis can be a useful approach to the first identification and crude location of markers near a targeted gene. Recombination between easily-scored flanking markers can simplify and reduce costs for the screening of rare crossovers within a particular small region. The use of markers already mapped in the targeted species, and in colinear/syntenic relatives, can provide a large collection of DNA markers that are already known to be fairly close to the targeted gene. Similarly, a genetic map that is anchored to a physical map generated from cloned segments can provide access to tightly linked markers with minimal additional experimental effort, and can provide a simple route to cloning the targeted gene. Intragenic recombination screens can be used to provide very precise linkage data, and can confirm a map-based gene isolation approach. Finally, the cloned gene is (by definition) its own most-tightly-linked marker.

With the tremendous number of tools available, the generation of genetic fine maps can become a routine approach in almost any plant species. It remains a laborious activity, however, primarily because of the large populations that must be screened, low levels of marker polymorphism, and a lack of correlated physical and genetic maps for multiple plant species. However, genomic technologies are beginning to generate better tools for high throughput mapping, including automation of the process. With this new generation of tools, developed over the next few years, genetic fine maps and their many uses will become inexpensive and routine for the entire plant science community.

TRANSPARENCIES

Genetic Fine Mapping Technology

Value

Problems

Approaches

The Value of a Genetic Fine Map

More effective and precise marker-assisted selection

Syntenic/colinear comparisons across species

Map-based gene isolation

Problems with Genetic Fine-Mapping

Large genomes

Rare recombination events
(per kb of DNA)

Large populations required

Possible problems with low polymorphism levels,
segregational distortion, recombinational distortion

Approaches to Genetic Fine Mapping

Multiple DNA marker types
(AFLPs, RAPDs, RFLPs, SNPs, SSRs, STSs, etc.)

Different population types
(Bulked segregants, recombinant inbreds, etc.)

Flanking morphological marker recombination
(minimizes number of uninformative progeny that need to be screened)

Comparative maps and markers
(minimizes the number of probes that need to be tested, accenting only those that are most likely to be useful)

Genetic/physical map comparison
(can provide the gene and markers guaranteed to be tightly linked)

Intragenic recombination
(very fine maps and cloned gene confirmation)

Gene isolation
(the most-tightly linked marker is the cloned gene itself)
