TRUMPing

Targeted Resequencing Using Massively Parallel Methods

ABI SOLID:

Advantages of Sequencing by Oligo Ligation Detection

- Lower cost < $1000/target
- Sequence entire gene
- > 3 GB sequence/run
- 48-fold pooling of individuals
- Flexibility with PCR conditions
- 2 Base encoding, minimizes errors
- Homopolymers not a problem
- Higher throughput (50 targets/run)

Logistical challenges:

1) Shearing 1-6 kb PCR products to ~200 bp fragments
2) Barcoding DNA fragments in each well of a microtiter plate
3) Optimize library creation and amplification for 96 samples
4) Explore ways to multiplex PCR reactions

The Scheme:

1) Build PCR plate: Starting with 36 plates arrayed 6x6 with DNA from individuals, create a 2D arrayed 96 well plate. The 2304 individuals are pooled 48-fold and each is re-purposed twice and with a unique set of co-ordinates.

2) Shear DNA and attach barcoded adapters. With DNA in the 96 well plate, use the Covaris E series sonicator to randomly shear PCR products to ~150-200 bp fragments. DNA is in a 96 well plate and the bottom of each well is subjected to a focused acoustic beam. This is a non-invasive way to randomly generate small DNA fragments from gDNA and PCR products.

We have attached a unique 5 base sequence to the 3' end of the ABI SOLID P1 primers so DNA in a given well will have a specific tag that will be read in the subsequent sequencing reactions.

3) Pooling, amplification and emulsion PCR (ePCR). The PCR products for each target are pooled and enriched for the ~150 bp fragments. This library is amplified then the template for emulsion PCR (ePCR). During ePCR, each DNA molecule is attached to a bead and suspended in a droplet of oil containing all the reagents to further amplify that molecule. After ePCR, beads containing amplified target are separated from the "empty" beads.

4) Bead attachment to slide and sequencing by ligation. The beads are attached to the slide and a 35 bp sequence is generated by 5 distinct cycles of primer binding and ligation. The primers contain fluoroprobes that are read in the subsequent reactions.

Sonication optimization using a 1.4 kb PCR product (FLP). Two protocols were tested each with 6 different time points. The first program had a 10 sec burst at intensity setting 10 followed by 6 different time points. The second program had a 10 sec burst at intensity setting 10 followed by 6 different time points. The yellow line and dot indicate the desired 200 bp size. Variables to be optimized include type of plate (96 or round bottom), beam intensity and time.

EcOTILLING Industrial Germplasm

HAVE CURRENT BREEDING PROGRAMS REALLY BEGIN TO EXHAUST THEIR GENETIC DIVERSITY?

While gains continue to be made, concern is growing about how long those gains can continue. Maize EcOTILLING queries natural variation for specific gene targets in comparison to the B73 reference genome. We also know the intron-exon structure of the target sequence and can compare exon sequence diversity found among older, widely diverse inbreds from the Maize Diversity Lines and that found among ten, recently off-patent, industrial inbreds (the Elite Maize Association Mapping Panel or EMAMP lines).

While diversity is usually assessed using SSR or other markers chosen because they are both polymorphic and occur throughout the genome, the relevant interest is that within coding exons (or regulatory sequences). We compared a broad, representative set of inbreds from the Maize Diversity Lines to the ten EMAMP lines, looking at haplotype variation within exon sequence.

These data suggest that the commercial lines examined retain only slightly less exon diversity than the germplasm overall. The genes tested are not believed to have been overt targets of selection during domestication, though some may have been selected in modern breeding programs. The relatedness of some of the EMAMP lines may also explain the reduction in haplotypes for Targets 11 and 12. Note that, while in several cases all the EMAMP lines have only one haplotype relative to B73, this does not necessarily mean that they are identical to one another.

While the alleles of some genes in commercial germplasm may be fixed, the germplasm remains healthy diverse for many other genes. Genes for which allelic diversity has not been lowered by choice (selection) are prime candidates for directed mutagenesis.