Genome and Agricultural Biotechnology LLC Services at SIUC.

1. Soybean Marker Assisted Selection at GAAB-SIUC ($0.5-$2 per marker)

2. Plant, animal and fungal BAC Library Construction ($0.3- 1 per clone)

3. Rapid (1-6 month) Genome Wide Physical Map Generation (~$15 per clone)

4. Microarray analysis of expression (enquire).

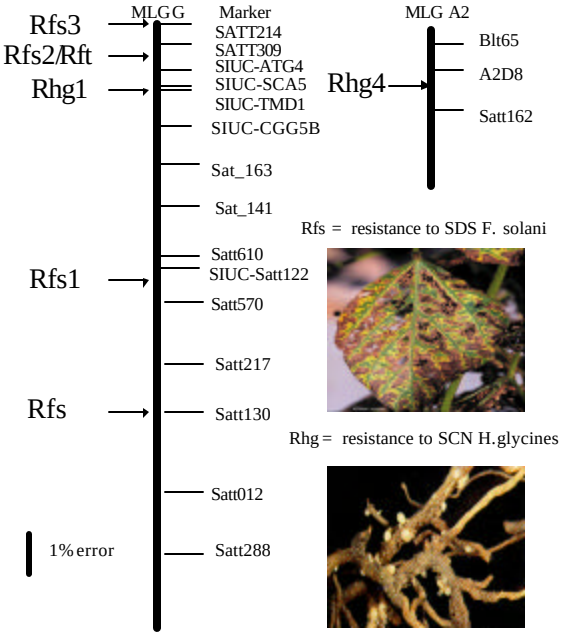
5. Custom DNA sequencing ($5-15 per read).

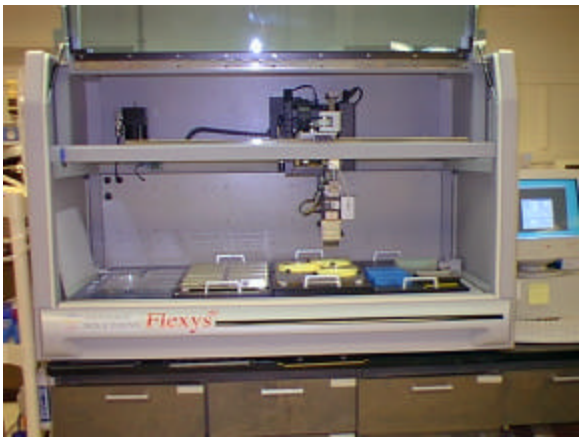
6. GMO content testing for food and feed ($2-5 per sample).

7. Plant Identity Testing ($2-200 per marker depending on type)

Contact:
David A. Lightfoot, Director, PO Box 312, Dunn-Richmond Business Incubator Room 174, Carbondale IL 62903-0312
Tel: 618 453-8296 618 529 8283 or Fax: 618 529 8283 or 618 453 7457 Cell Phone 618 528 5305. e-mail: DALightfoot@hotmail.com **or** ga4082@siu.edu **www.gaabdal.com**

Figure1. The Integrated Physical-Genetic Map of the Soybean Genome for genes underlying SDS resistance(Rfs 1-3) and SCN resistance (Rhg1) showing SIUC proprietary markersavailable





Genome and Agricultural Biotechnology LLC – Marker Assisted Breeding

1. Soybean Marker Assisted Selection at GAAB-SIUC ($0.5-$2 per marker)

We undertake selections for dual resistance to SDS and SCN for public breeders or private companies at cost. We have six SDS resistance genes, three are co-located with SCN resistance *rhg1* (Fig. 1) allowing dual selection. Importantly these include the root resistance gene *rfs1*. There are two major genes for SCN resistance covered by our patents, one is essential for all genotypes (*rhg1*) the second in Hartwig resistance (*Rhg4*). We have sequenced alleles of the major SCN resistance genes *rhg1* and *Rhg4* so we have perfect markers. We also have patented markers for increasing Phytoestrogen content 2 fold and a yield locus worth about 4 bushel/acre from the Hartwig type background .

Capacity: The capacity of our lab has expanded recently, through equipment purchase and Taqman deployment we can run 2,000 samples per day, about 500,000 per year. We are committed to just 40,000 at present.

Costs: <4,000 samples $1.50 first marker, $0.5 the second, third etc.
>4,000 samples $1.25 first marker, $0.4 the second, third, etc
With about 95% of data points returned (1 pass)
For selections involving SCN there may be technology fees (5-10%) to pay to Pioneer on top of these charges depending on volume and target gene. As we adopt our markers to micro-arrays formats we expect prices will drop.

Material: We prefer whole seed (dry) or leaflets (on ice). If you want to send a seed fragment we will charge an extra $2.50 per line for DNA extraction because it is much more labor intensive. With heterogeneous lines we use a composite sample of DNA extracted from 4-8 seed. That gives us better than 99% probability of detecting heterogeneous lines. If possible then each sample should consist of 8-16 seed in a packet with a distinct code or label. The parents should ALWAYS be included if possible and be clearly labeled so we can quickly select the best markers.

Second Markers: Can be for other SDS loci or loci you determine.
Recombinant Identification: We can multiplex three markers per gene to identify resistant recombinants that have no yield drag.

Accuracy: We expect more than 95% correct prediction of phenotype.

Licensing of Markers and Methods: About 10% of gross is paid to SIUC patent office.

Proprietary Issues: So that our patents applies to what we do we will always report dual resistance to SDS with SCN, or only SDS. Checks for payment are made out to the "Dual Selection" or GAAB rather than SIUC so there is no college liability. If you in future market varieties selected from dual selection there would be not be any necessity to license with the SIUC proprietary rights office since GAAB holds license to that patent. Commercialization fees would be nominal or zero. Please contact Pioneer attorney Robert Giaquinta (at 515-270 3600) for details of their licensing terms if you want selection for their genes and markers. GAAB does not use any Pioneer markers or any technology claimed in their patents.

Lightfoot D.A., Meksem K., P.T. Gibson. 2001. Soybean Sudden Death Syndrome resistant soybeans, soybean cyst nematode resistant soybeans and methods of breeding and identifying resistant plants. US Patent # 6,300,541.

Lightfoot D.A., Meksem K., P.T. Gibson. 2001. Soybean Sudden Death Syndrome resistant soybeans, soybean cyst nematode resistant soybeans and methods of breeding and identifying resistant plants. US Patent #pending.

Iqbal J., Njiti, V., Meksem K. Kilo, V., Johnson J., P.T. Gibson and D.A. Lightfoot. 2001. Common Loci underlie Resistance to Soybean Sudden Death Syndrome (SDS) in Pyramid, Forrest, Douglas and Essex. Theor Appl Genet (2002) 104:294–300.

Yuan, Z., Njiti V., Meksem K., Iqbal MJ., Triwitakorn K., Kassem MA, Davis GT, Schmidt ME and D.A. Lightfoot. 2002 Identification of yield loci in soybean populations that segregate for disease resistance. Crop Science 42(1):271-277.

Njiti, V., K. Meksem, D.A. Lightfoot W.J. Banz and T.A. Winters. 2003 (anticipated). A method for breeding and genetically manipulating phytoestrogen content in soybeans. US Patent pending issue. Application # 10/008,789.

Meksem K. and D.A. Lightfoot. 2004 (anticipated). Novel polynucleotides and polypeptides relating to loci underlying Resistance to Soybean Cyst Nematode and methods of use thereof. Patent pending. # 11/009,154.

2. Plant, animal and fungal BAC Libraries, Filters or Clones ($0.3- 1 per clone)

We have constructed BAC libraries from twelve species since 1998. There are two bryophyte species libraries, one animal species library, two fungal species libraries, and seven crop plant species libraries. Crop species include soybean (*G.max* and *G.soja*), pea, chickpea, wheat, potato and pepper.

3. Rapid (1-6 month) Genome Wide Physical Map Generation (~$15 per clone).

Maps are constructed from a *Hind*II *Hae* III fingerprint of BAC clones. Map based genome sequencing was successful for *Ustilago maydis.*

4. Microarray analysis of expression (enquire).

Microarrays for soybean, rat and *Fusarium solani* are available. Arrays for fathead minnows, cow embryo and ginseng root are under development.

5. Custom DNA sequencing ($5-15 per read).

Sequencing uses PE377. Discount rates for bulk orders.

6. GMO content Testing at GAAB

We undertake selections for GMO content public or private companies. There is one sequence common to all GMOs licensed to date, the 35S promoter for which we test by PCR. The test is sensitive to 0.001% of GMO in the product tested (Fig. 2) allowing rapid and quantitative detection..

Capacity: The capacity of our lab has expanded recently, through equipment purchase and Taqman deployment we can run 2,000 samples per day, about 500,000 per year. We are committed to just 40,000 at present.

Costs: <4,000 samples $5.00 first gene, $2.50 the second
>4,000 samples $4.00 first marker, $2.00 the second
With about 95% of data points returned (1 pass).

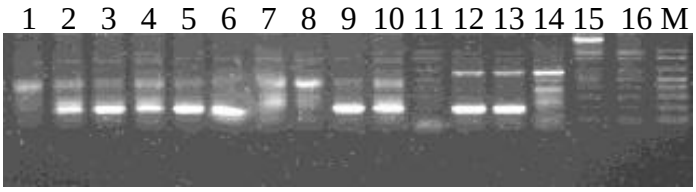
Material: Amount will depend on DNA content range from 1g (feeds) to 1 Kg (oils, soy sauce etc.). Should be shipped in sterile disposable containers.

Accuracy: We expect more than 95% correct prediction of GMO content when present. We will miss its presence in about 2-5% of contaminated samples.

Licensing of Markers and Methods : About 10% of gross

Proprietary Issues: None

Fig. 2: GMO Detection in corn feedstuffs with the viral 35S promoter probes



1: Line 1, 1X, RR corn
2: Line 1, 10X RR corn, +ve for 35S Promoter
3: Line 2, 4X LL Corn, + ve for 35S Promoter
4: Line 2, 7X LL Corn, + ve for 35S Promoter
5: Line 3, 3X BT Corn, +ve for 35S Promoter
6: Line 3, 9X BT Corn, +ve for 35S Promoter
7: Line 4, 5X Pioneer 33G26, -ve for 35S Promoter
8: Line 4, 6X Pioneer 33G26, -ve for 35S Promoter
9: Line 5, 8X GDH Corn, +ve for 35S Promoter
10: Line 6, 2X GDH –ve, GUS +ve, + ve for 35S Promoter
11: -ve Control, 1 KB ladder
12: GDH +ve Tobacco amplified with 35S promoter primers
13: GDH +ve Tobacco amplified with 35S promoter primers
14: GDH –ve Tobacco DNA + 1Kb Ladder
15: GDH +ve amplified with GDH primers
16: GDH –ve with GDH primers
M: 1Kb DNA ladder

7. Plant Identity Testing

We undertake determination of the identity of plants and plant parts for public or private companies. We use hyper-variable sequences detected by AFLP, micro-satellite DNA or a combination of both (SAMPL), for which we test by PCR. The test is sensitive to distinguish identical twins from clones (Fig. 2) allowing rapid and robust detection.

Capacity: The capacity of our lab has expanded recently, through equipment purchase and deployment we can run 100 samples per day, about 30,000 per year.

Costs: $200 per sample

Material: Amount will depend on DNA content range, typically 1g. Should be shipped in sterile disposable containers.

Accuracy: We expect more than 95% correct prediction of identity. The test will fail in about 2-5% of samples.

Licensing of Markers and Methods: About 10% of gross

Proprietary Issues: None