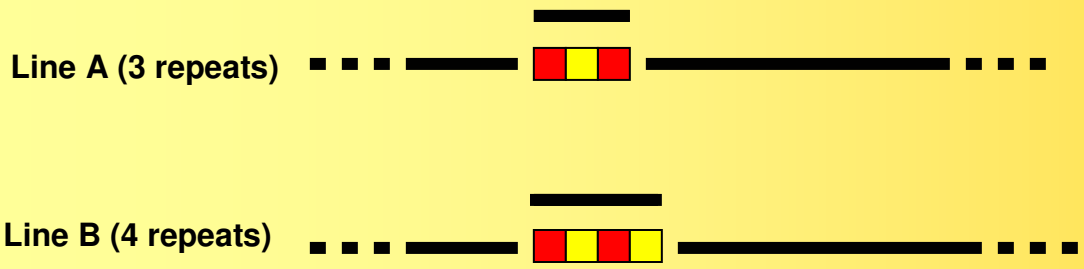
Marking Success

Simple sequence repeats (SSR)

- Usually consist of di or tri nucleotide repeats
- Variation in the number of repeats occurs between cultivars



The difference in length of the SSRs can be detected on a gel or by a microcapillary platform

Electric current causes fragments to move – smaller fragments move more quickly

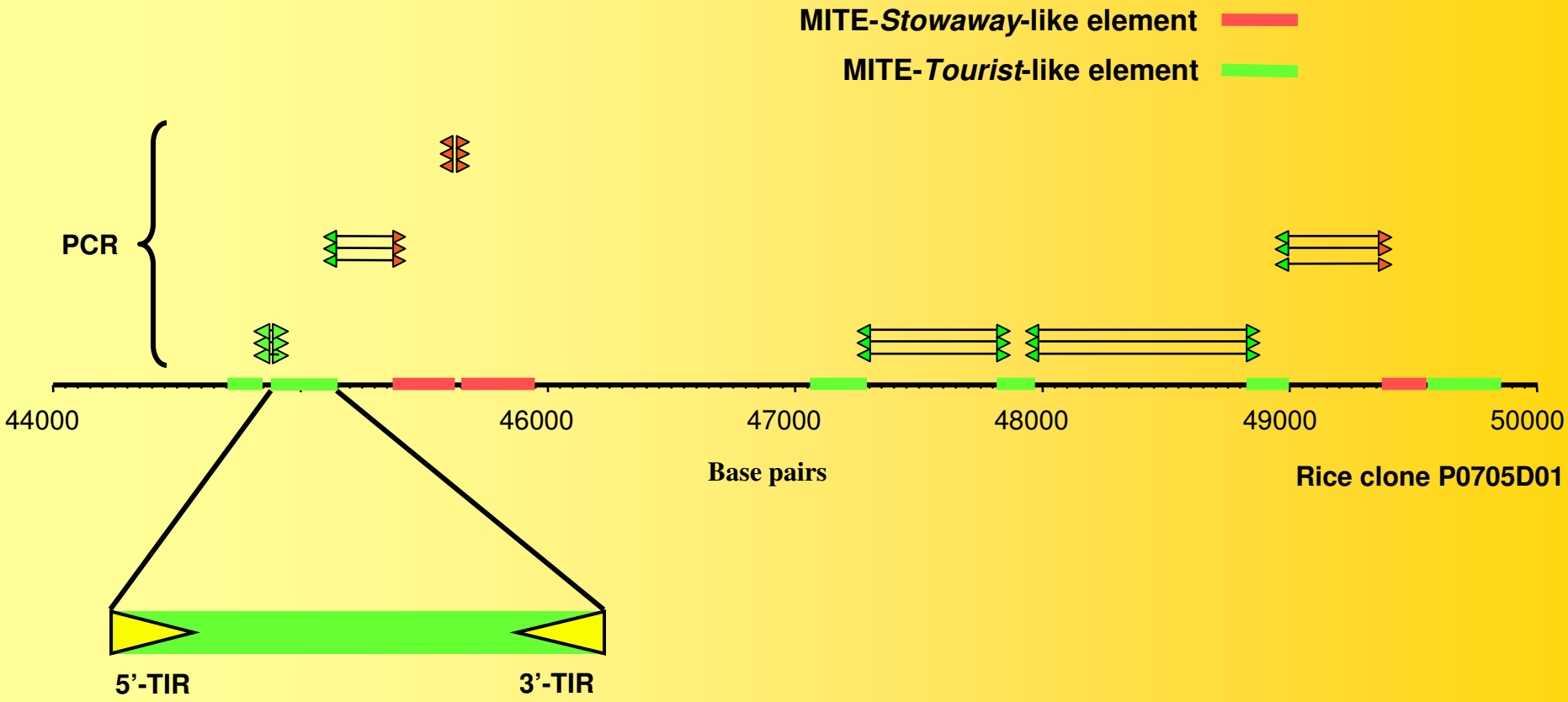
Scoring the position of the markers allows tracking of allelic variation

- SSRs are the most common marker system in use today
- One reaction yields one data point
- Greater efficiencies possible if more than one primer is combined (multiplexing)
- Co-dominant marker system (more informative when dealing with heterozygotes)

What are IMP markers?

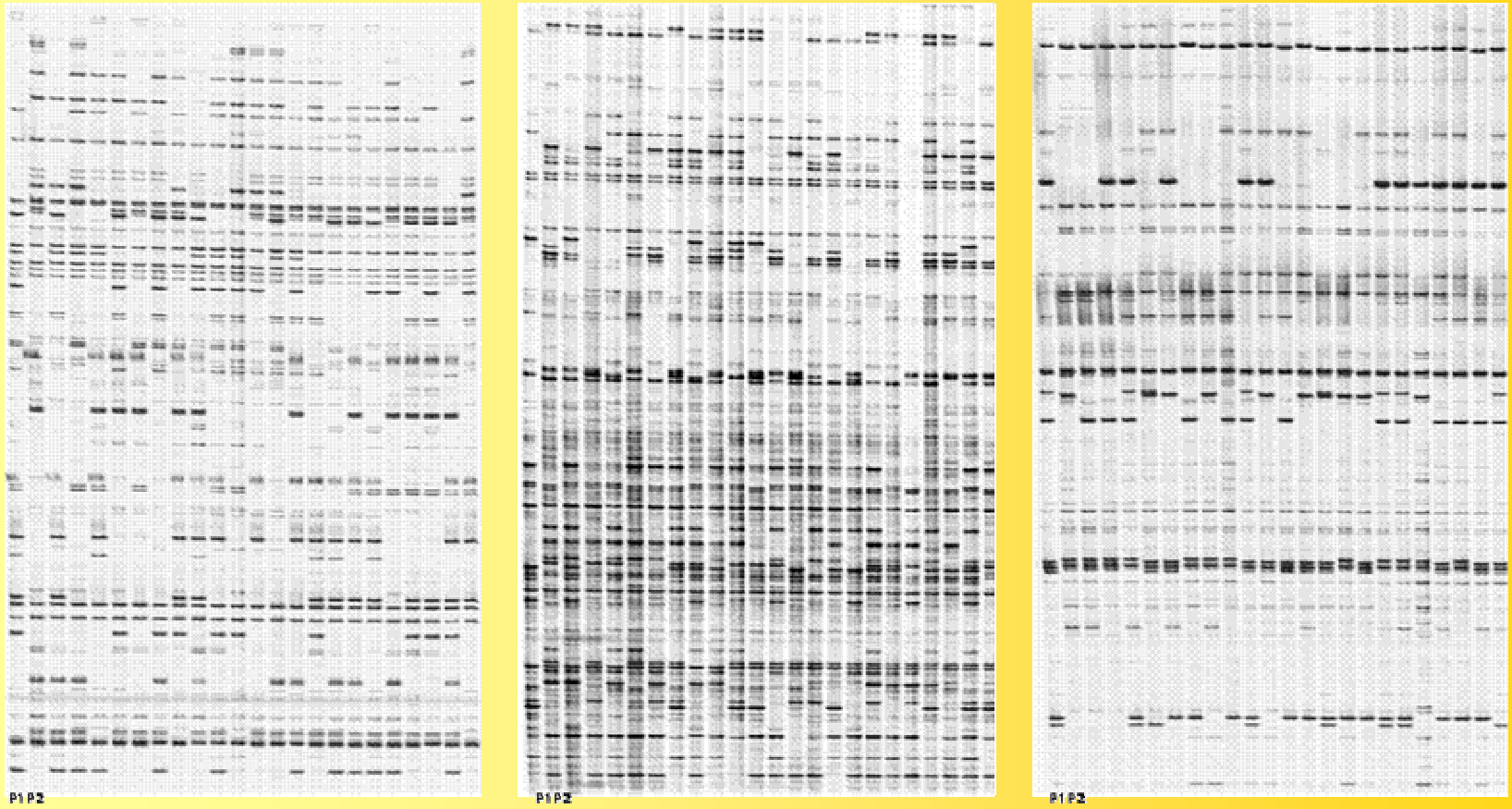
- Developed by and exclusive to DNA LandMarks
- Inter-MITE Polymorphism (IMP) markers are based on Miniature Inverted-repeat Transposable Elements (MITEs)
- MITEs are short interspersed DNA transposons with terminal inverted repeats (TIRs),
- Small size (< 500 bp), conserved TIRs, high A+T content
- Several distinct families: Tourist-like, Stowaway-like
- In plants highly associated with genes (flanking regions, introns)
- Present in plants, fungi, vertebrates, fishes, insects
- Abundant in plants (several thousand copies per genome)

Basis of the IMP Technology



Naturally multiplexed – Low cost/data point

IMP-26 IMP-41 IMP-31



IMPs in maize: Fragments run from 500 bp - 1.1 kb in length

Cross-species applicability

- DLM has mined various genomes for consensus sequences used to develop IMP primers
- Current IMP library includes 64 markers
- MITE families show up in abundance in all genomes we have tested to date
- Between 50-60% of IMP primers will amplify in a new species
- Polymorphism levels vary by crop
- On average an IMP primer will yield 10 markers from a single PCR
- With a quick, inexpensive pilot DLM can usually generate a few hundred markers for a new crop
- Even greater range possible if sequences of new families were mined – e.g. Solanaceae, Cucurbits, Asteraceae

Recent examples:

Lettuce

Potato

Petunia

Tomato

Lobolly Pine

IMP applications in marker-assisted breeding

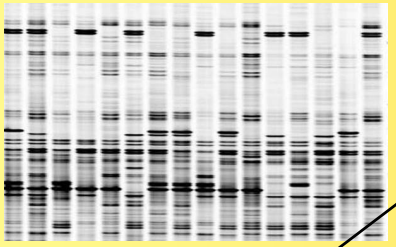
- Fingerprinting
  - Variety protection
  - Germplasm characterization
  - Identity preservation – quality control
- Trait conversion – recurrent parent genome selection
- QTL mapping & trait-linked marker development
  - Map IMP bands to key traits of interest
  - Potentially convert linked bands to locus-specific markers (future development of technology)

Trait-linked marker development

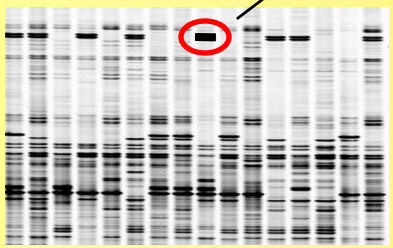
- IMPs are very efficient for mapping traits of interest

Phenotype

Genotype



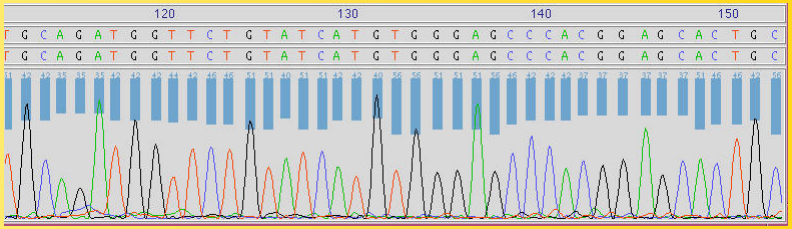
Mapping



Isolate band and flanking sequences linked to trait of interest

Clone

Sequence



Bioinformatics

Locus specific, co-dominant markers (e.g. SSR, SNP)

IMP applications in marker-assisted breeding

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Single Nucleotide Polymorphism (SNP)

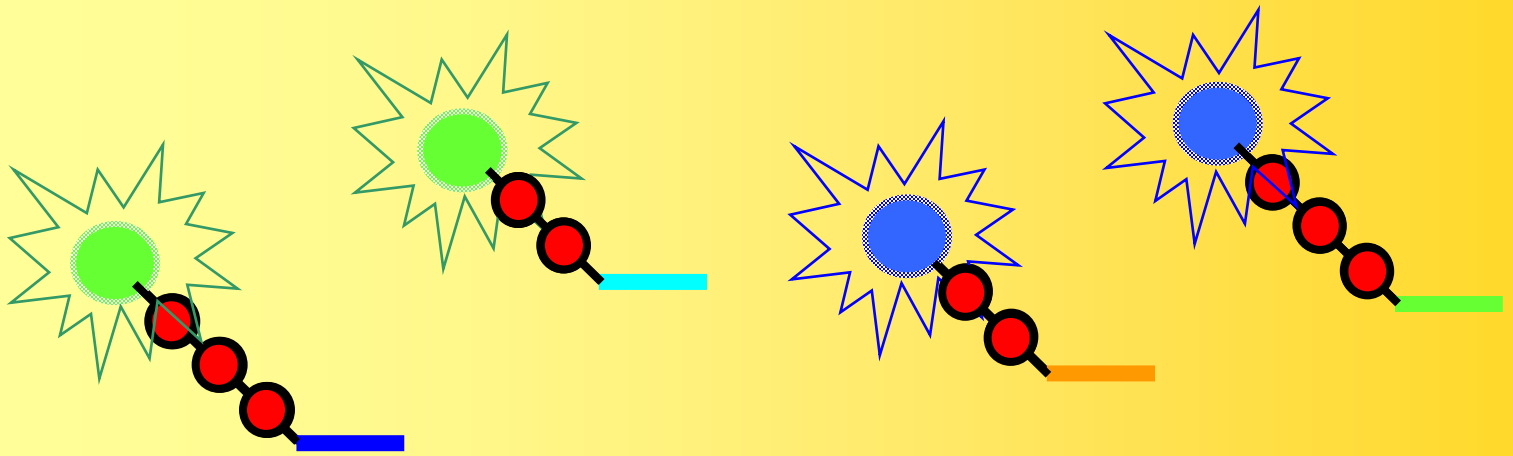
CCTGTTAA**T**GGTACATT

CCTGTTAA**C**GGTACATT

- Co-dominant marker system
- Most prolific of all marker types
- Very efficient and inexpensive to use once developed
- Holds enormous potential for multiplexing
- ABI SNPlex technology multiplexes 48 SNPs in a single reaction
- 96-plex is in development and will soon be released
- Cost is below a dollar per datapoint
- DLM is at the forefront if this technology

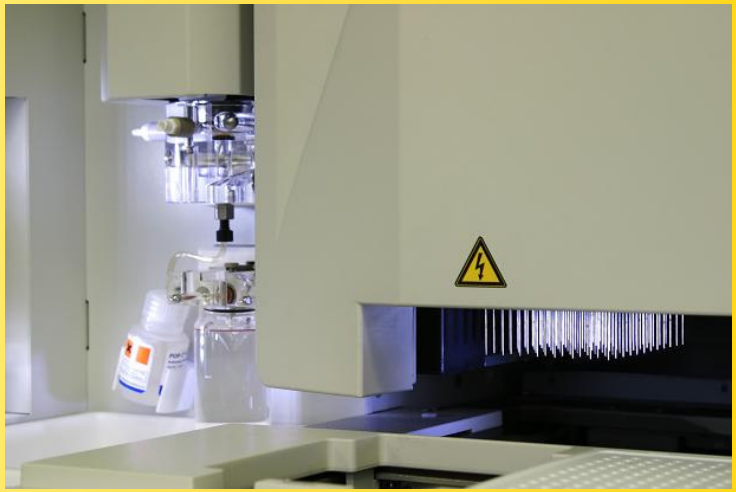
SNPlex Technology

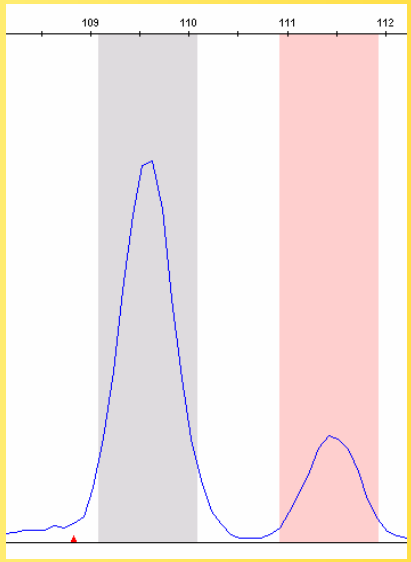
- Based on Oligonucleotide Ligation Assay (OLA)
- Hybridize amplified products to ZipChutes (universal reporter probes)
- ZipChutes are eluted then loaded onto the 3730XL
- ZipChutes are tagged with mobility modifiers which allow separation along a microcapillary even in a dense multiplex
- Also tagged with fluorescent dyes
- Combination of colour and size separations allows very high-throughput when run on a 3730 XL – over 400,000 dp/day

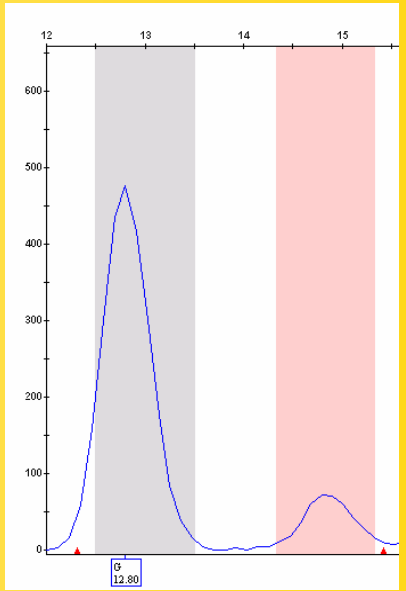


SNPlex Technology

- ZipChutes are loaded onto ABI3730XL
- Output of raw data demonstrates the density of the multiplex
- Can zoom in on individual data points for closer study



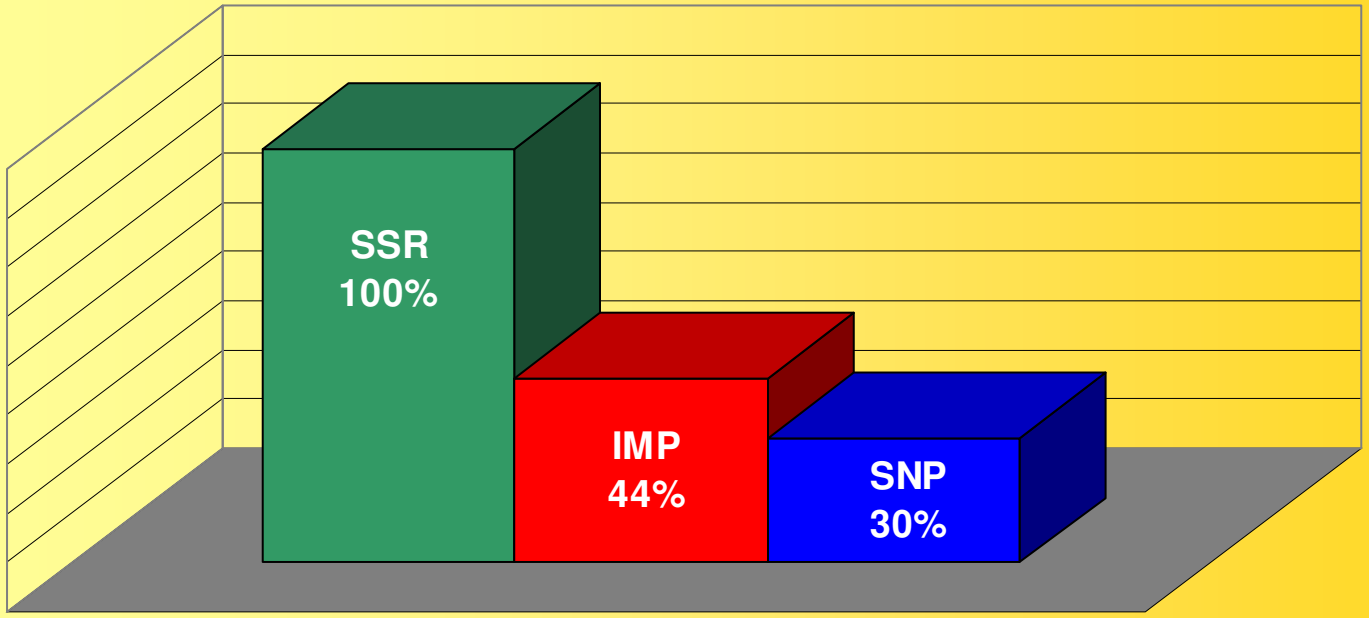




Marker technology development

- Each generation of technology becomes increasingly more powerful but also less costly

Trait Conversion Cost Comparison
(Corn)



Note: Costs quoted are estimates only. Actual cost will vary on a project by project basis.