Molecular markers

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What are they?

How are they detected?

Molecular markers

- Are sites where differences in DNA sequences occur among members of the same species
- Reveal polymorphisms at the DNA level
- Can be in either coding or non-coding regions

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Variations at the DNA level

- Single nucleotide polymorphisms (SNPs)
- Insertions or deletions (Indels)
- Variable number of tandem repeats (VNTRs)

Markers detect one or more of these variations

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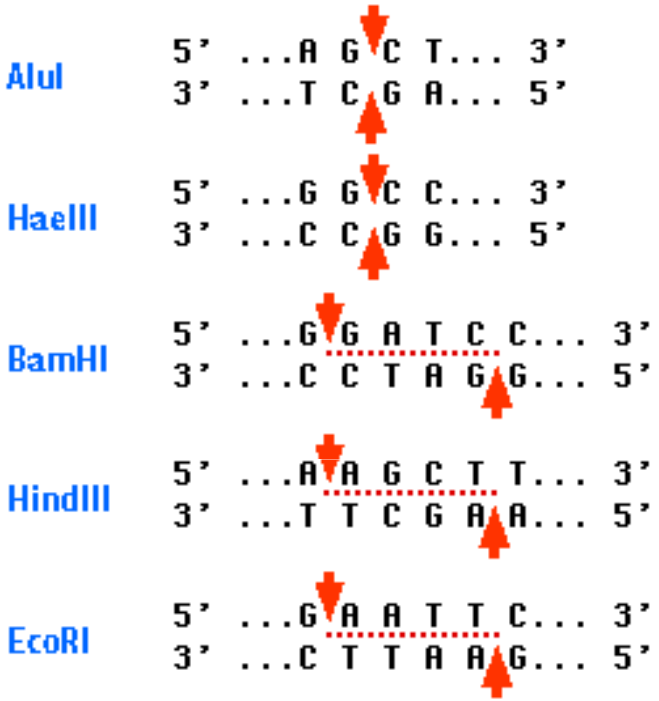
RFLP markers

- Restriction fragment length polymorphisms
- Restriction enzymes recognise and cut DNA at specific sites
- Different sized fragments are produced depending on whether the restriction site exists or not



AluI and HaeIII produce blunt ends

BamHI HindIII and EcoRI produce "sticky" ends

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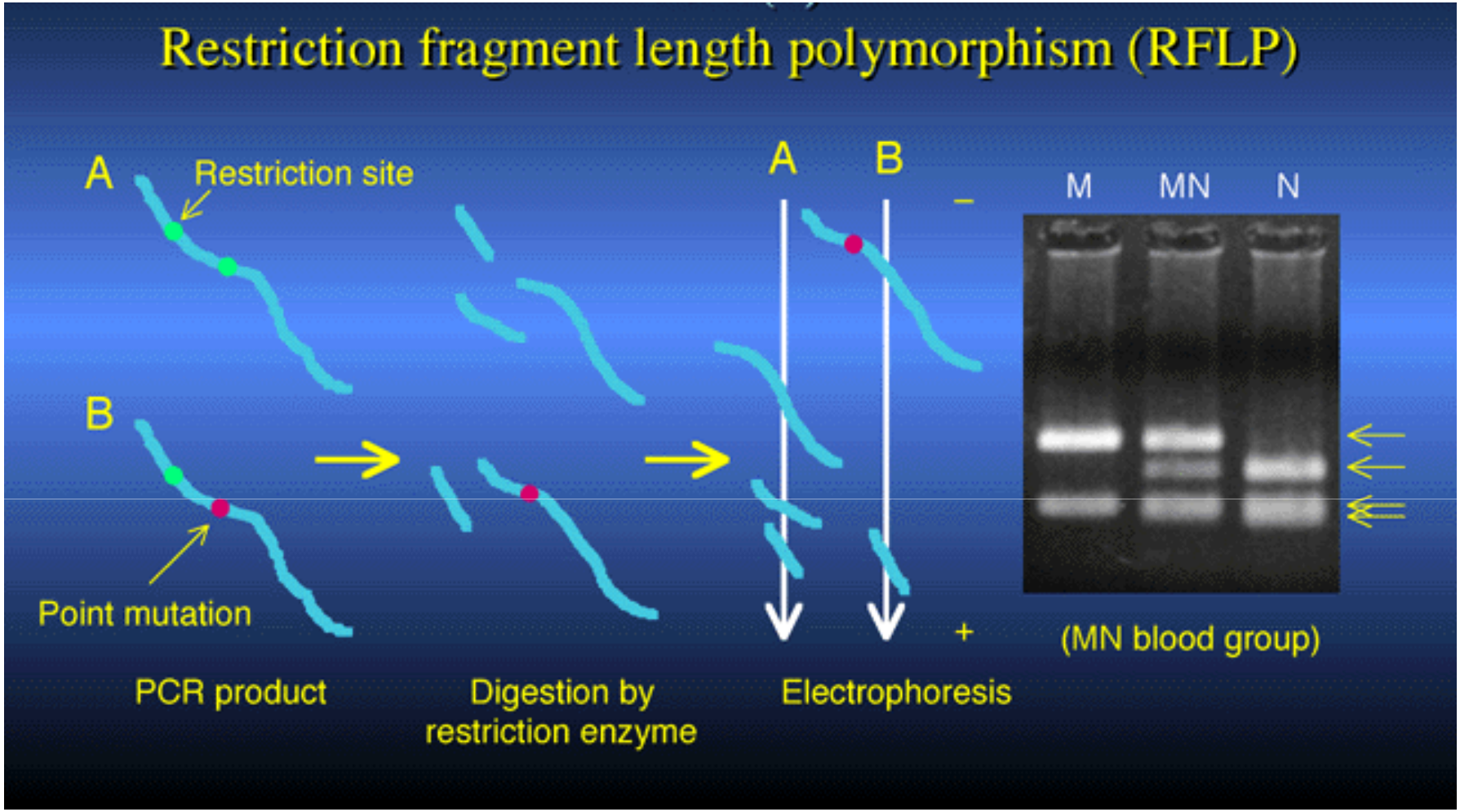
Restriction fragment length polymorphism (RFLP)
A
Restriction site
B
Point mutation
PCR product
Digestion by
restriction enzyme
A
B
–
+
Electrophoresis
M
MN
N
(MN blood group)

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Microsatellite markers

- Type of VNTR, which are multiple copies of a sequence of base pairs arranged end to end

- Length of repeating unit varies
  - if <4 base pairs: microsatellite

    5′ CACACACACACA 3′

    3′ GTGTGTGTGTGT 5′

  - if >4 base pairs: minisatellite

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Microsatellite markers

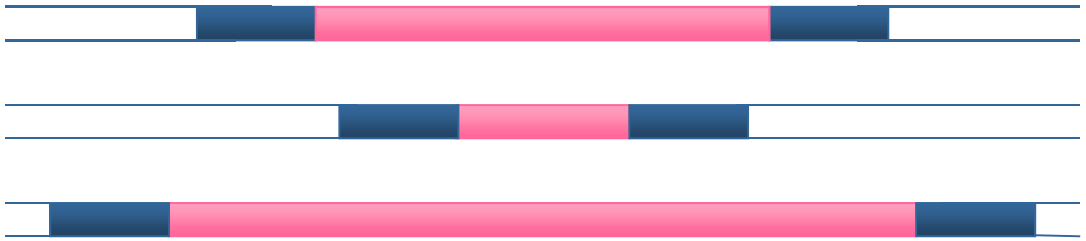
BL25

5’ 3’

GGCAATGGAAGTGG CACACA...CACACA CACTCACCCACTAGATC

CCGTTACCTTCACC GTGTGT...GTGTGT GTGAGTGGGTGATCTAG

3’ 5’



Alleles differ in length

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Typing microsatellites

- Most commonly use PCR based methods

- Steps are
  - amplify region by PCR
    - *primers labelled via radioactivity or fluorescence*
  - separate PCR products according to size
    - *polyacrylamide gel, capillary based systems*
  - determine size of amplified product
    - *autoradiography, fluorescent traces*
  - score alleles

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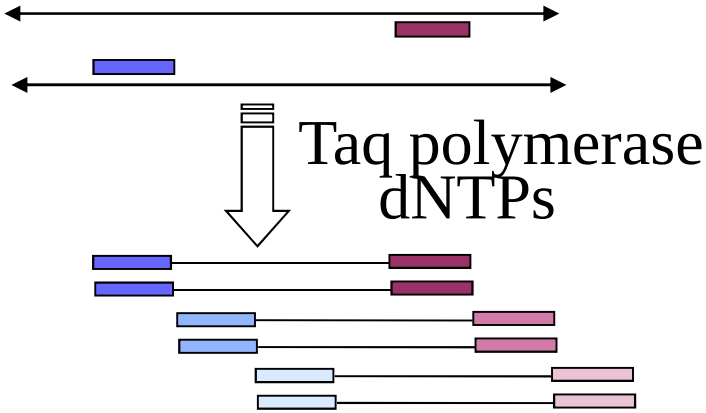
PCR

Denaturation

Annealing

Extension

~25 cycles



PCR machines

http://www.uni-koeln.de/math-natfak/botanik/bot2/agflue/HOME/equipment/pcr.htm

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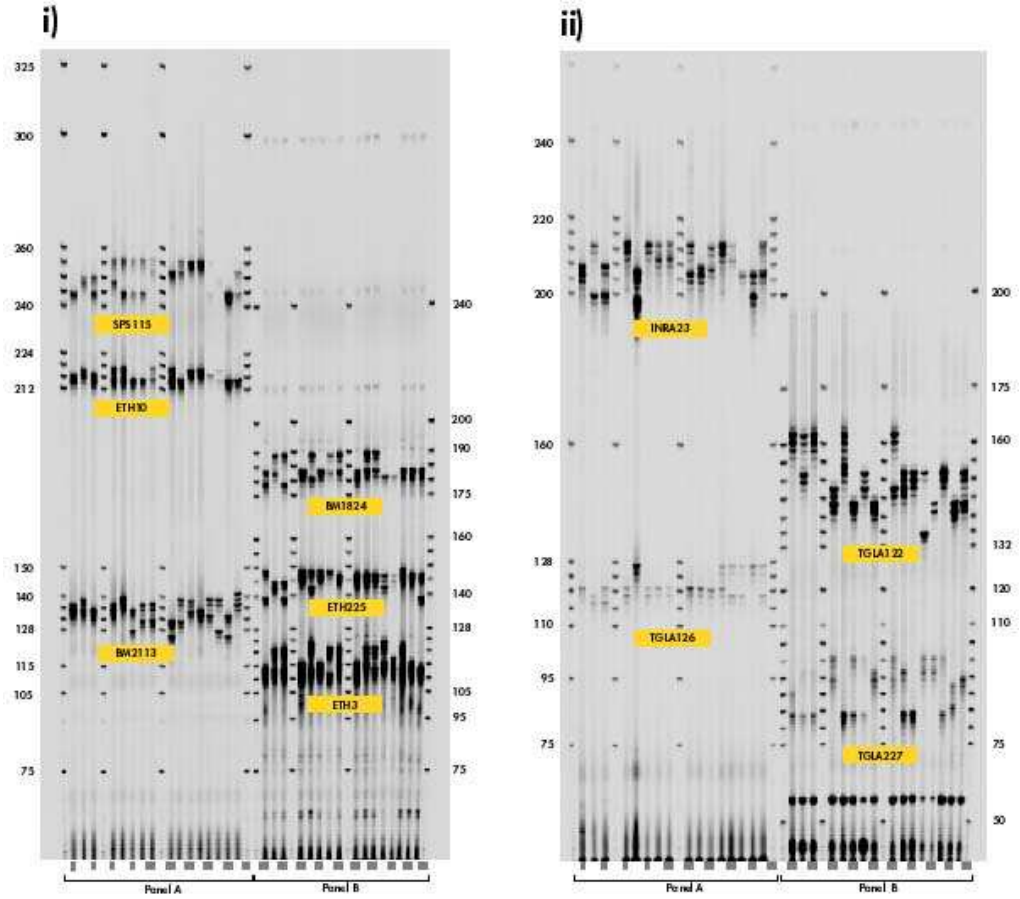

http://www.licor.com/bio/Posters/PAG_520/F1.jpg

Microsatellite genotyping via autoradiography:

bands at different positions represent different alleles

Bovine Microsatellite Multiplexing for Herd Evaluation and Parentage

J. Kovar, J. Walker, D. Steffens, J. Harford, and J. Qiu
LI-COR Inc., 4308 Progressive Avenue, Lincoln, NE 68504, USA

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Loading an ABI377 for genotyping

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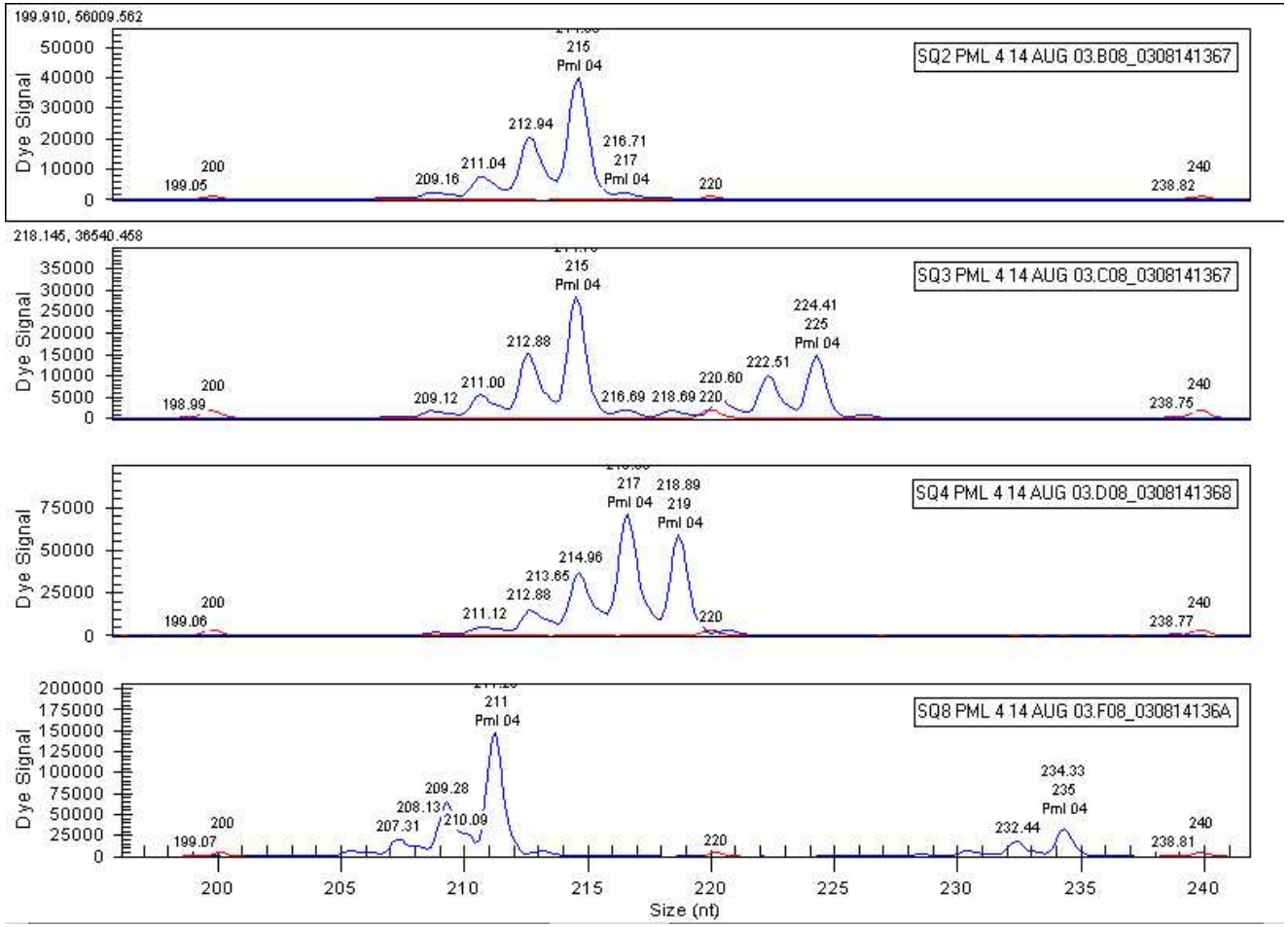

Microsatellite genotyping where a trace is produced. Peaks at different positions represent different alleles.

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SNP markers

- Single base change in DNA sequence
- Usually two alternative nucleotides at a single position
- Least frequent allele present at 1% or greater

- *Why not 4 alternative nucleotides?*
  - low probability of 2 independent base changes occurring at any single position
    - (1-5 x 10^{-9} / nucleotide / generation at neutral position)
  - bias for transitional mutations (A ⇔ G, C⇔ T) over transversions

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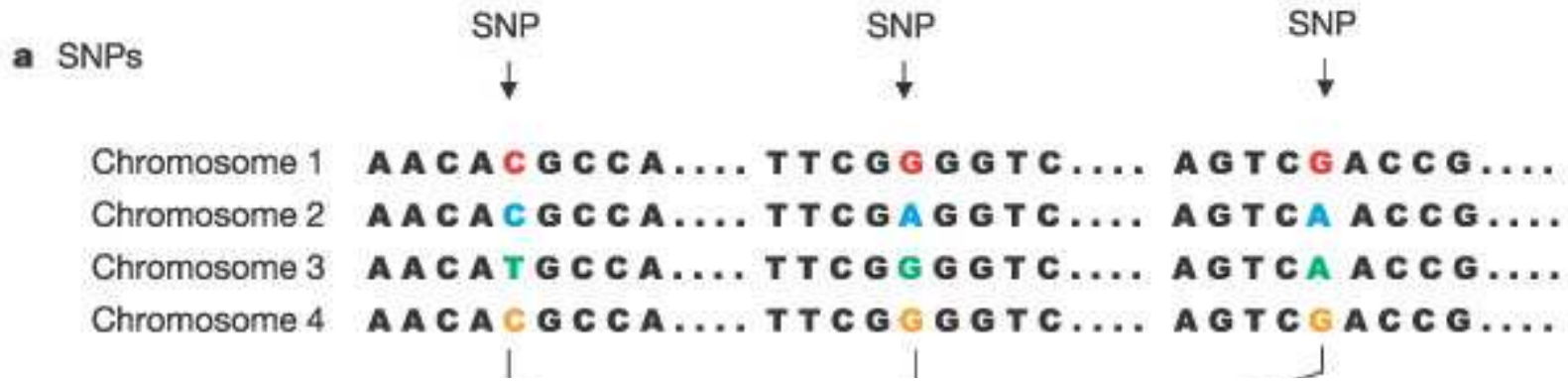

Examples of SNPs as found from sequence alignment

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Typing SNPs

- Numerous methods
  - Direct sequencing
  - DNA chips (potential for very high throughput)
  - Other e.g. SSCP (single stranded confirmation polymorphism), primer extension, pyrosequencing etc. (see Vigal et al. GSE 2002)

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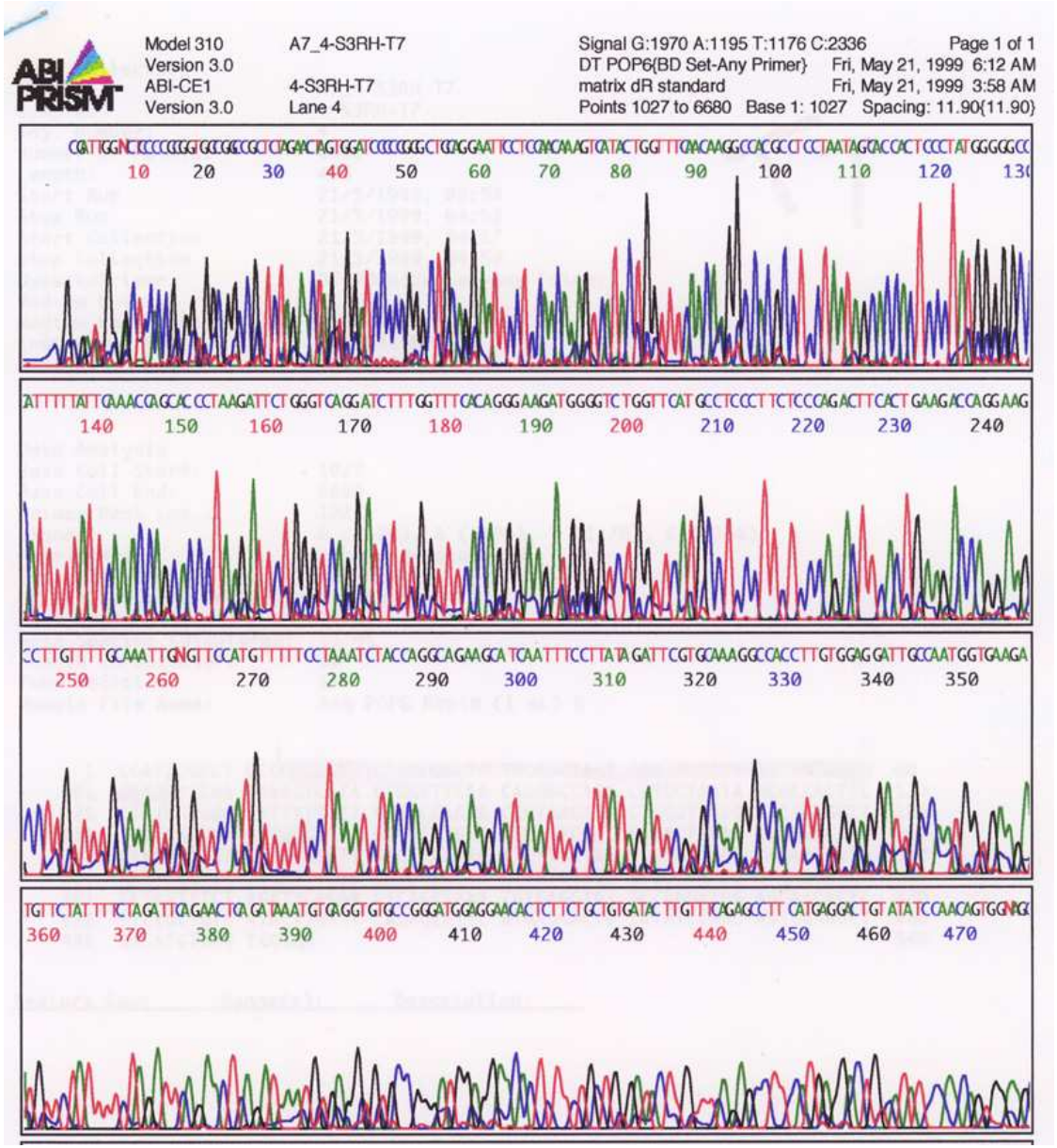

http://www.genelink.com/images/Seqfull.jpg

Example of DNA sequence output

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Variations detected by markers

Marker	Variation type		
	SNP	Indel	VNTR
RFLP	+	(+)	(+)
Microsatellite	-	(+)	+
SNP	+	(+)	-
RAPD (random amplification of polymorphic DNA)	+	(+)	(+)
AFLP (amplified fragment length polymorphism)	+	(+)	(+)
SSCP (single stranded confirmation polymorphism)	+	(+)	(+)

From: Vignal et al. GSE 2002.

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Properties of markers: statistical considerations

- Heterozygosity
  - SNPs: two co-dominant alleles
  - microsatellites: numerous co-dominant alleles
  - thus, lower heterozygosity of single locus SNPS compared to microsatellites
  - note, however, that marker heterozygosity is always population dependent

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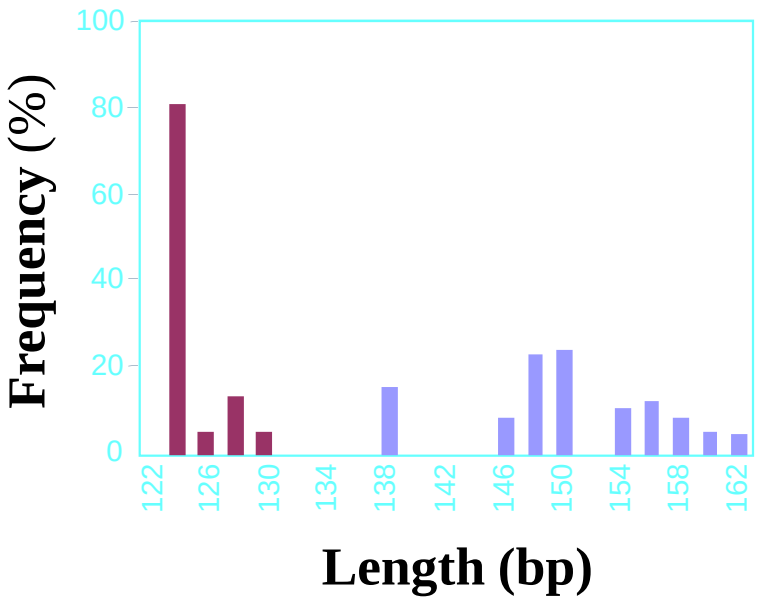
Microsatellite Allele Frequency

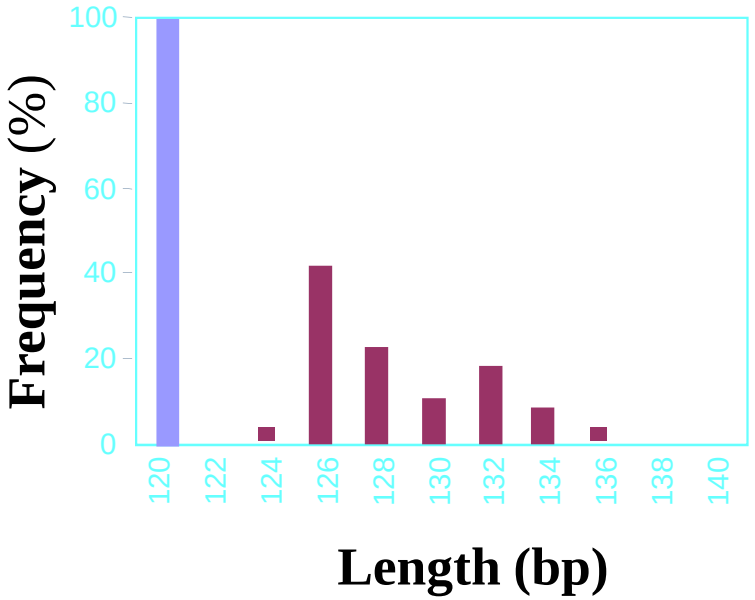
RME23



Merino

Bos taurus x Bos indicus, N'Dama, Boran, Brangus

CSDR240



Brahman, Hereford, Afrikaner

Merino, Suffolk, Border Lester, Romney, Poll Dorset

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Properties of markers: statistical considerations

- Density
  - SNPs (~1 every 1000 bp)>> microsatellites
- Neutrality
  - imp. assumption of pop'n genetics
  - microsatellites usually in non-coding regions, whereas neutrality of SNPs is case dependent
- Mutation rate
  - microsatellites ($1x10^{-5}$) > SNPs ($1x10^{-9}$)
- Rate and type of genotyping errors

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History of markers

- 1980's : 1st major effort to produce a human genetic map, mainly used RFLPs
- 1990's : Shift to microsatellites
  - More informative and easier to type
- 2000's : Movement to SNPs
  - need for very high density of markers
- Technology is very rapidly changing

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