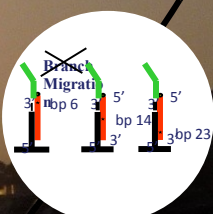
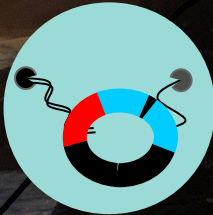
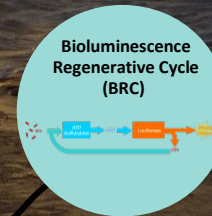
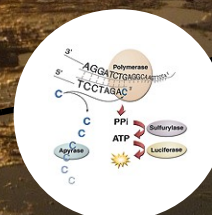
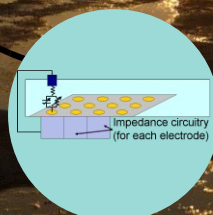
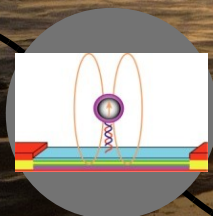
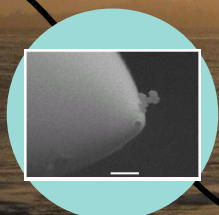
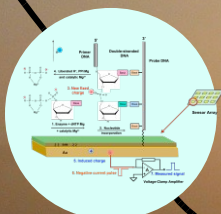


Advances and New Technologies in Forensic DNA

Nader Pourmand, PhD

Department of Biomolecular Engineering,
University of California, Santa Cruz



Assay Development for Human Identification

Methods development for detecting short tandem repeats using microarray and sequencing based platforms

- **Bio Assay:**

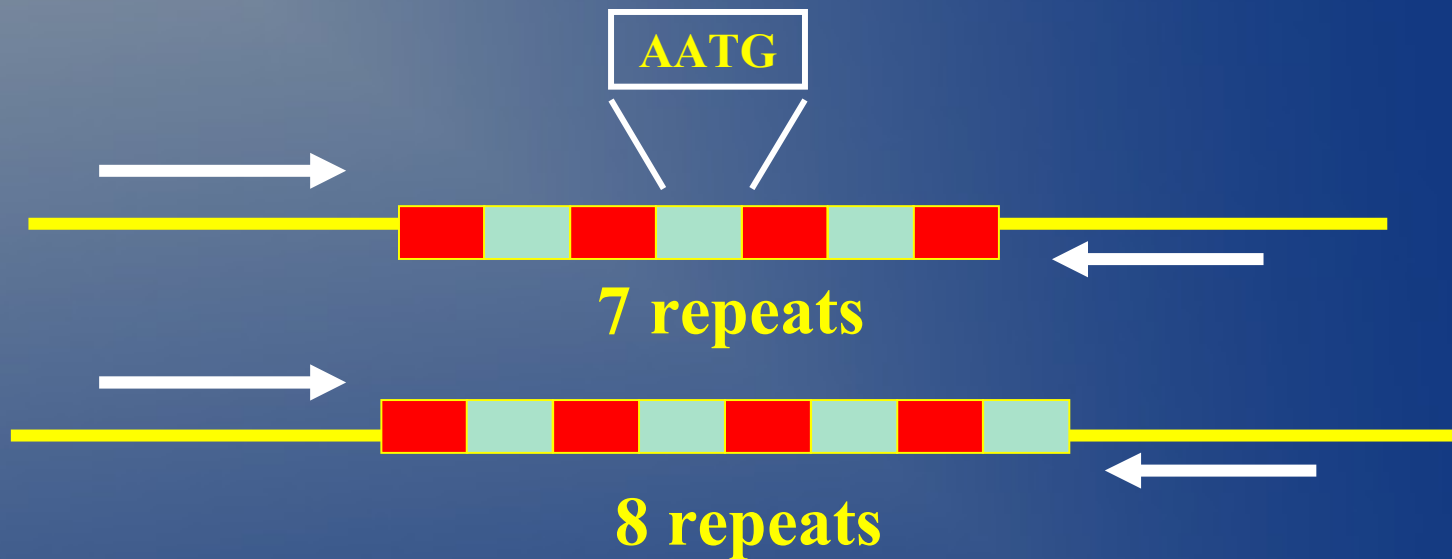
- Branch migration assay (DNA finger printing)
- Connector inversion probe and Trinucleotide Threading (TnT)

- **Platforms:**

- Microarray (printed array)
- Luminex suspension beads array
- Pyrosequencing (DNA template tailoring)

DNA Fingerprinting Using Short Tandem Repeats (STRs)

Located in the non-expressing region of DNA



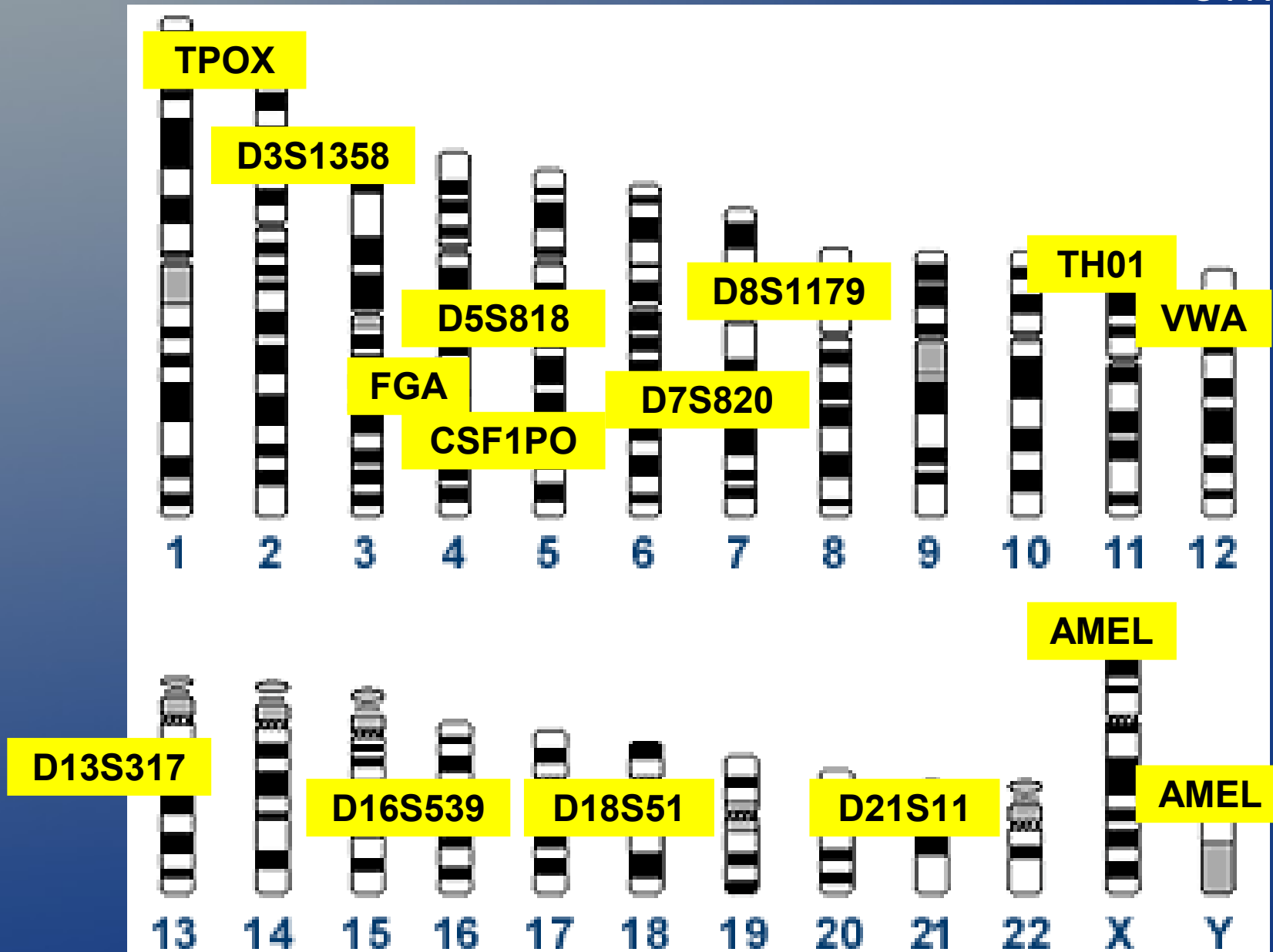
The repeat region is variable between samples while the flanking regions where PCR primers bind are constant

Homozygote = both alleles are the same length

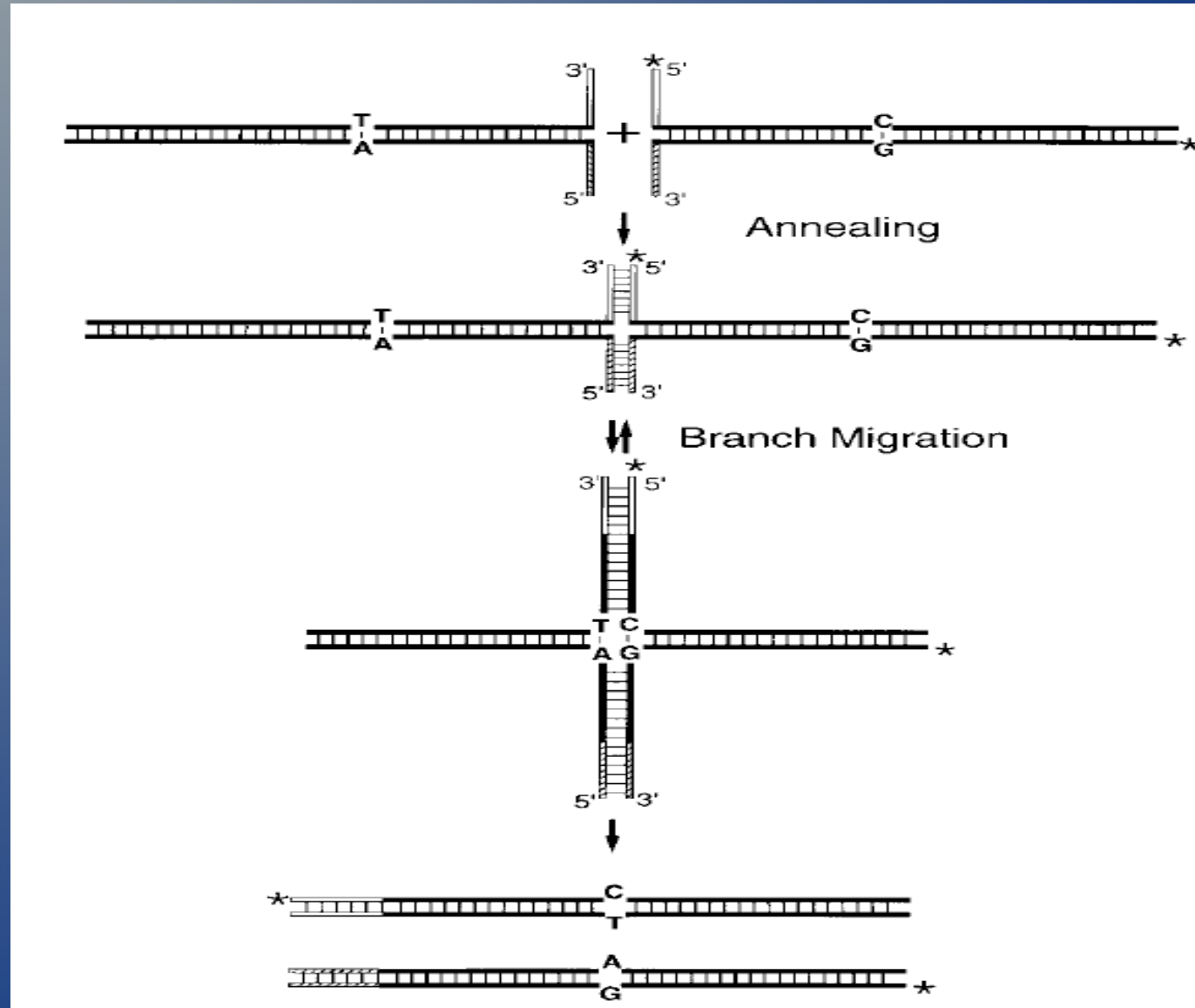
Heterozygote = alleles differ and can be resolved from one another

CODIS Core STR Loci with Chromosomal Positions

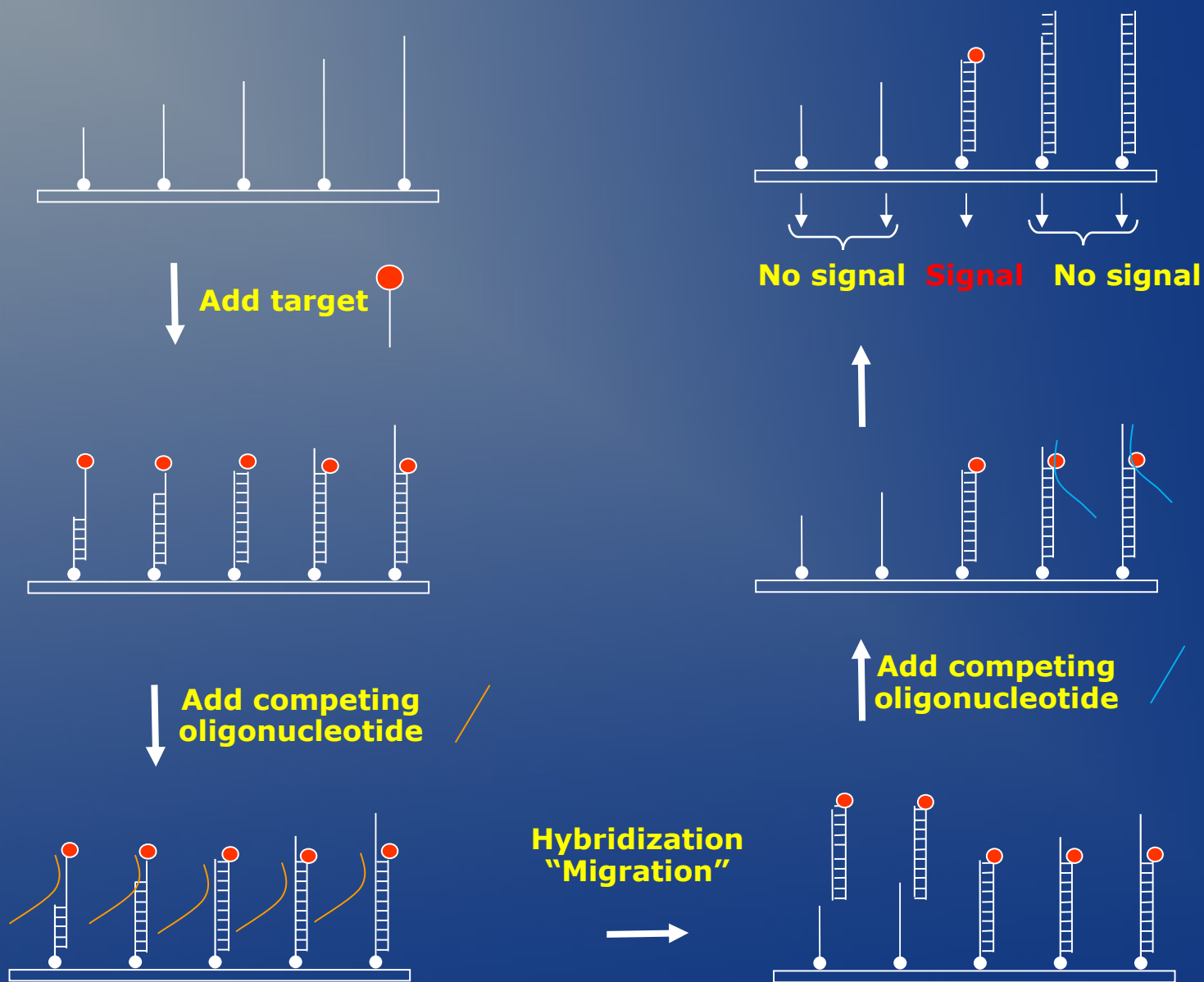
STR website



Spontaneous Branch Migration

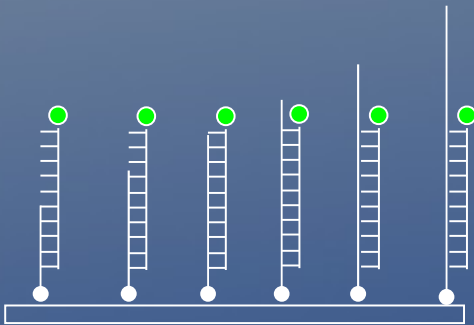


Branch Migration Displacement Assay (For DNA length measurement)

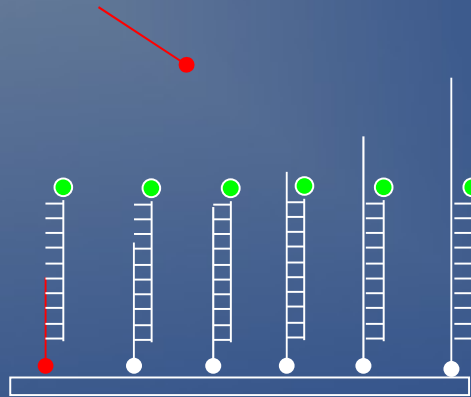


Reliability and Specificity of Biochemistry: Branch Migration Assay

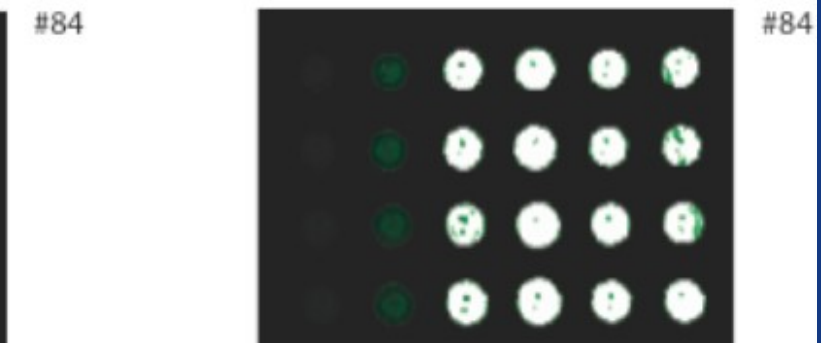
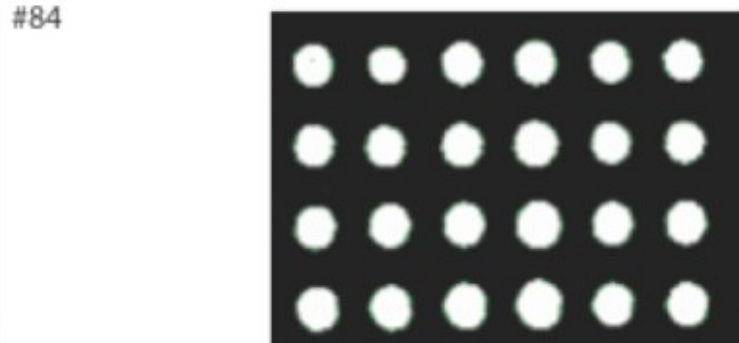
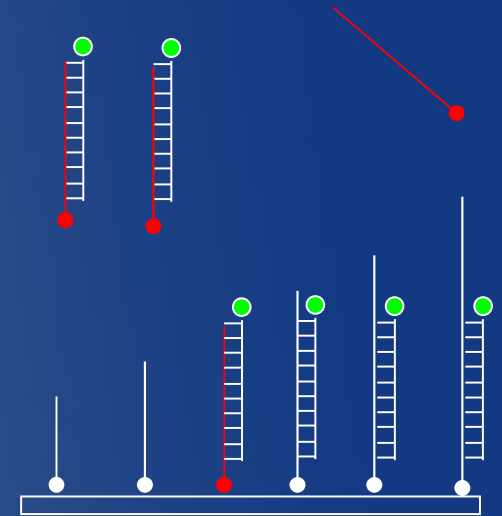
Before assay



Wrong target does not cause branch migration



Correct target causes branch migration

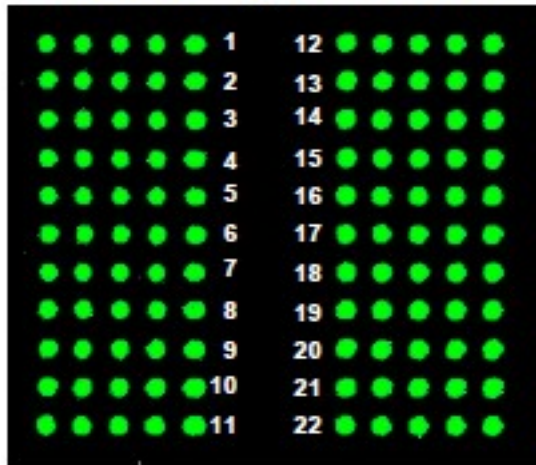


Numerous fluorescent experiments show reliability and specificity of biochemistry!

Microarray (Printed array)

STR D16S539 (Allele 9, 10)

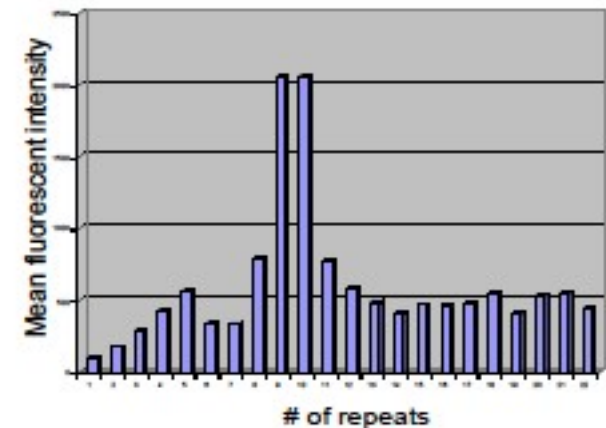
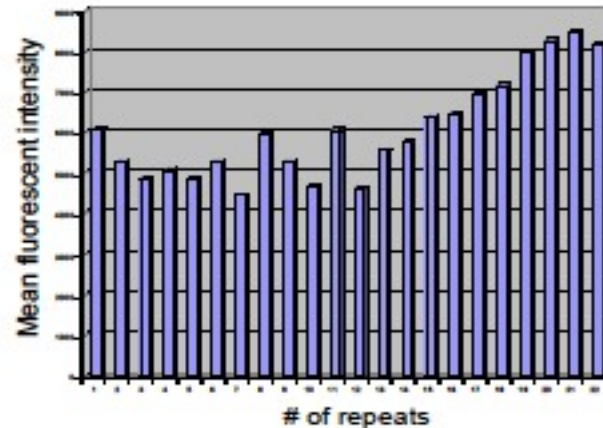
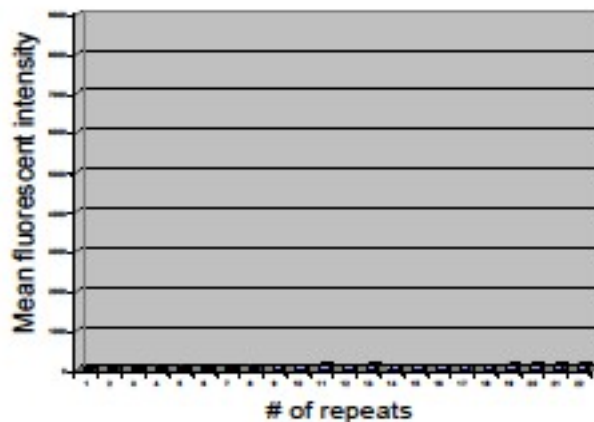
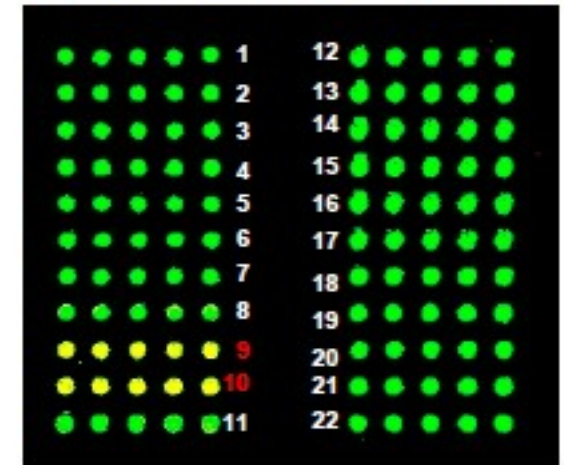
a) Before Hybridization



b) After Hybridization



c) After 1st and 2nd branch

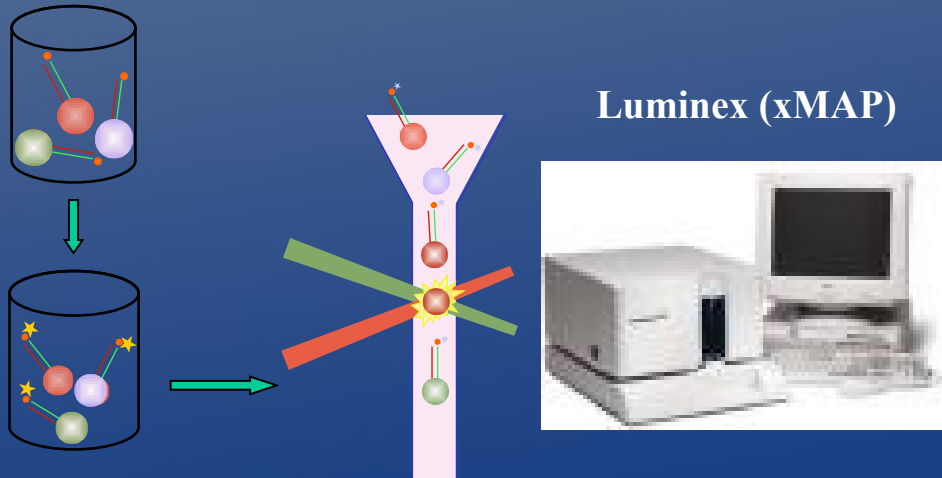


STR measurement by Luminex Bead Array

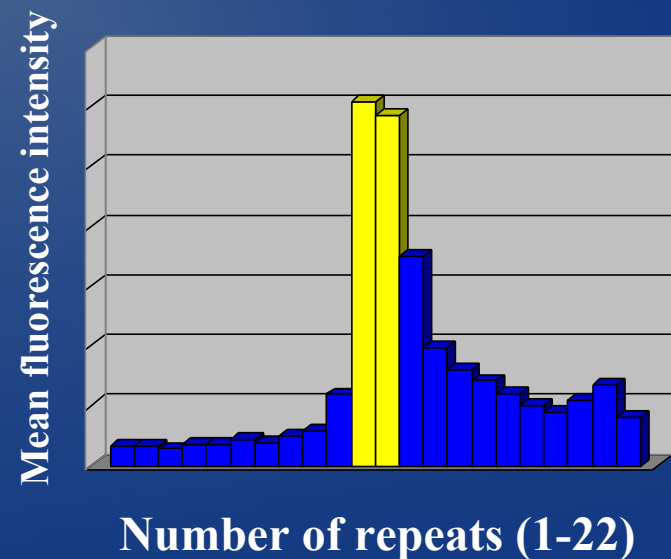
Pourmand et al. Proc Natl Acad Sci U S A. 2007 Apr 10;104(15):6146-51
Villablanca et al. Electrophoresis, 2008

- STR (short tandem repeat) analysis
 - multiplex STR detection
 - 7 different loci
 - 20 DNA samples
 - microarray technology
 - suspension array technology

Villablanca et al. Electrophoresis. 2008 Oct;29(19):4109-14.



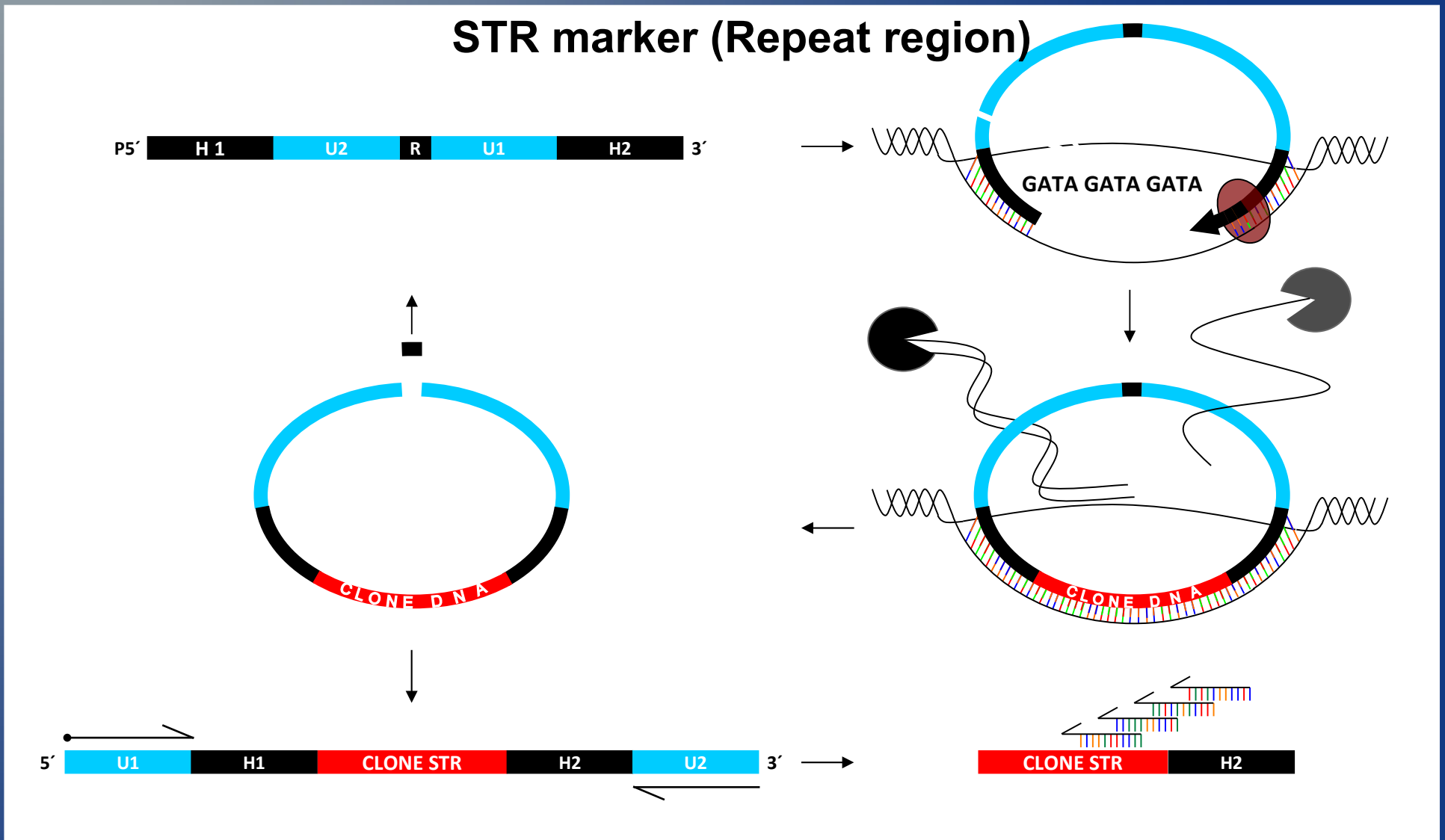
Sample D29623 STR D16 (11/12 repeats)



Summary of Branch migration assay

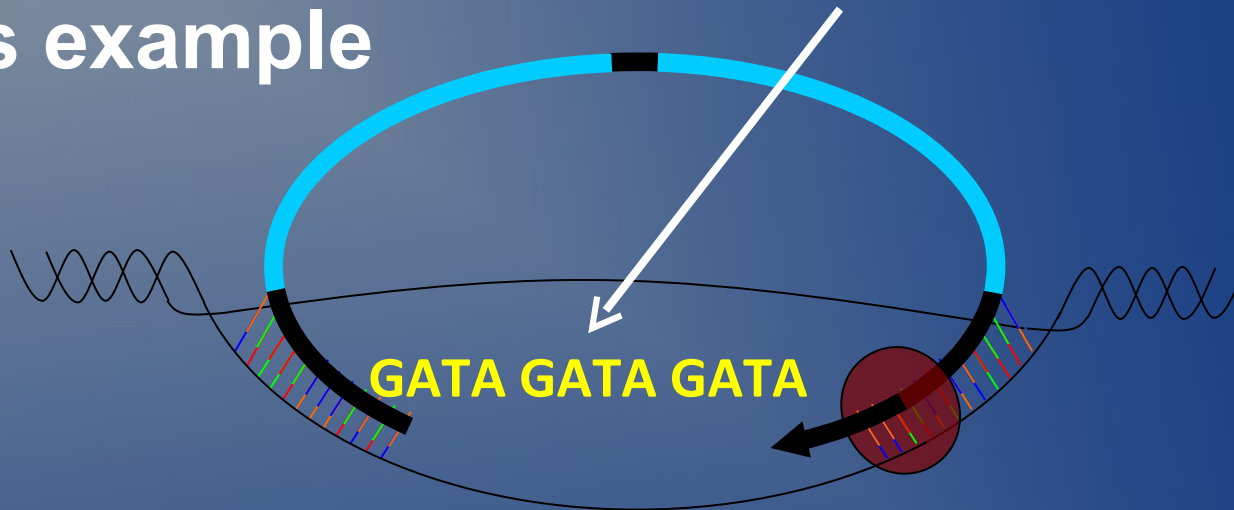
- A spontaneous **branch migration assay** to remove labeled single-stranded regions of DNA
- Branch migration assay distinguishes the **heterozygous** alleles
- No “**cross hybridization**” between the targets

Connector Inversion Probe (CIP)



CIPer and Trinucleotide Threading

Extension of STR region by sequence based three nucleotides e.g. G A T in this example



- STR Specificity can be achieved by using three nucleotides
- Non-specific product will not extend due to absence of 4th nucleotide (dCTP in above example)

CIP assay's Advantages

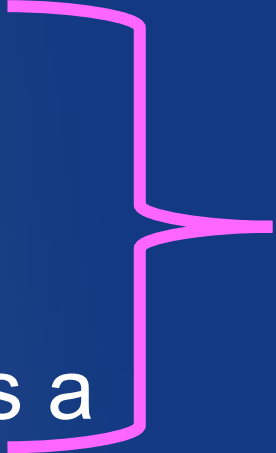
- 1) Larger multiplex capability due to addition of two universal primer sites with each STR
- 2) Multiplex capability to analyze miniSTRs and degraded DNA
- 3) Selective and specific amplification due to lack of 4th nucleotide

Pyrosequencing

Sequencing by synthesis

Why not sequencing approach for STR analysis?

Limitations

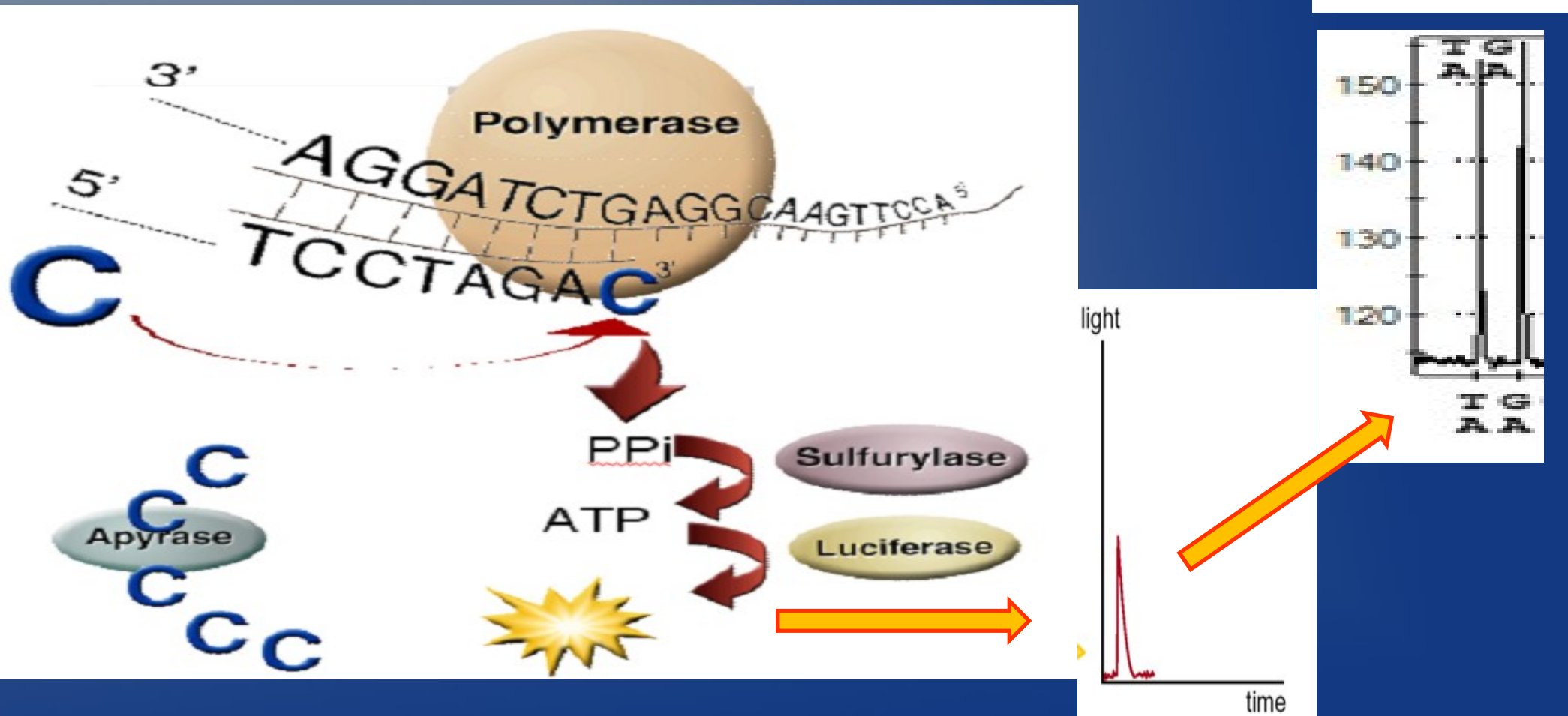
- 1) Issue of Multiplexity of markers
 - 2) Issue of cost
 - 3) Sequencing of Heterozygous samples is a challenge
- 

Pyrosequencing provide a solution for these challenges

Dyad Pyrosequencing

Dyad Pyrosequencing strategy :

Dispensation of two dNTPs instead of one to shorten the analysis time



Pyrosequencing

It is cost effective method compare to Capillary Electrophoresis

No need for dye labeled primers

No need for allelic ladder

Multiplex PCR product

16 loci can be divided in to different wells and sequence-specific primers can be used

Heterozygous sample

Can be sequenced by template tailoring

DNA template tailoring to sequence heterozygous sample

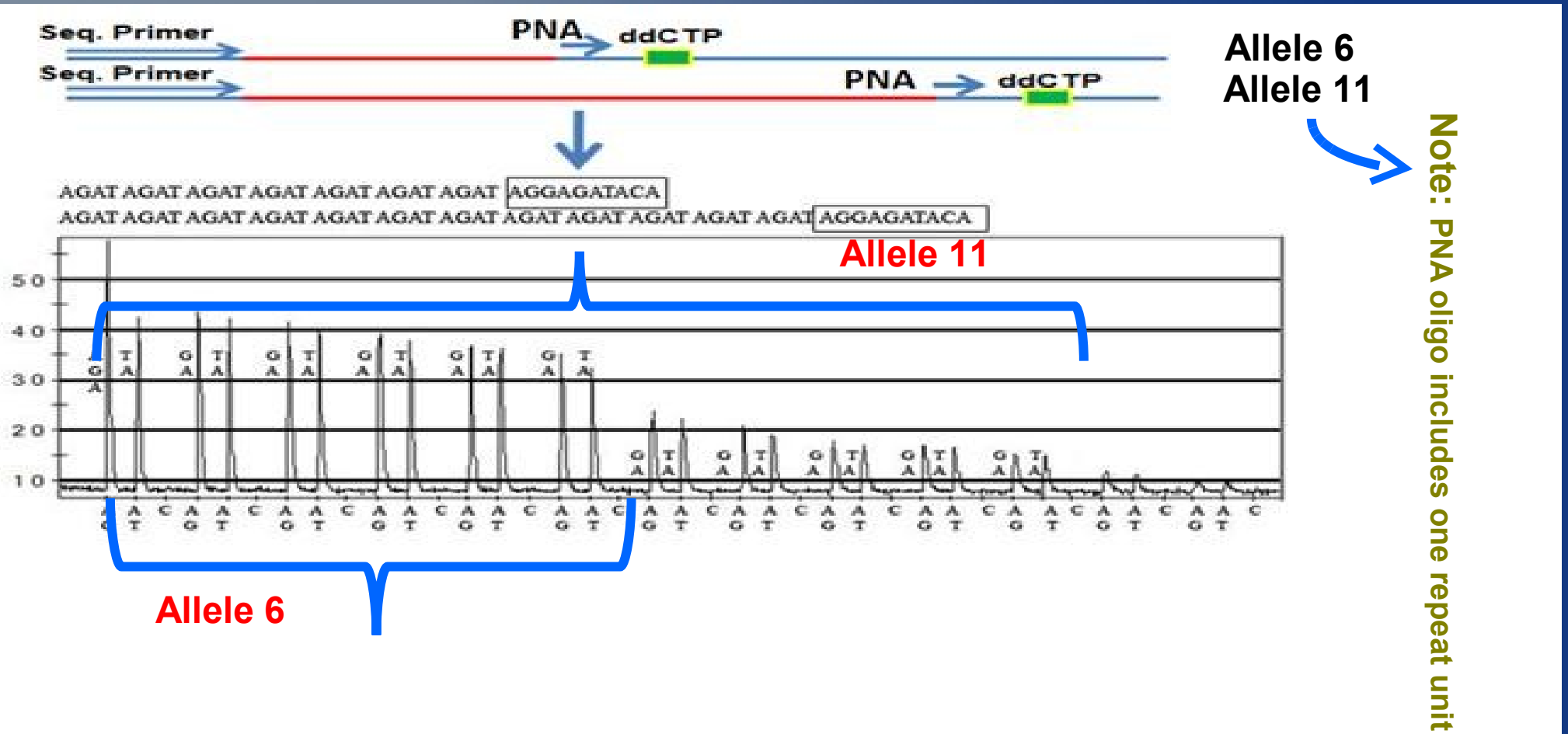
Template tailoring

Termination of polymerization at downstream flanking region of STRs

- 1) Terminator nucleotide (ddNTP)
- 2) Peptide Nucleic Acid (PNA)

Concept of template tailoring (heterozygous sample)

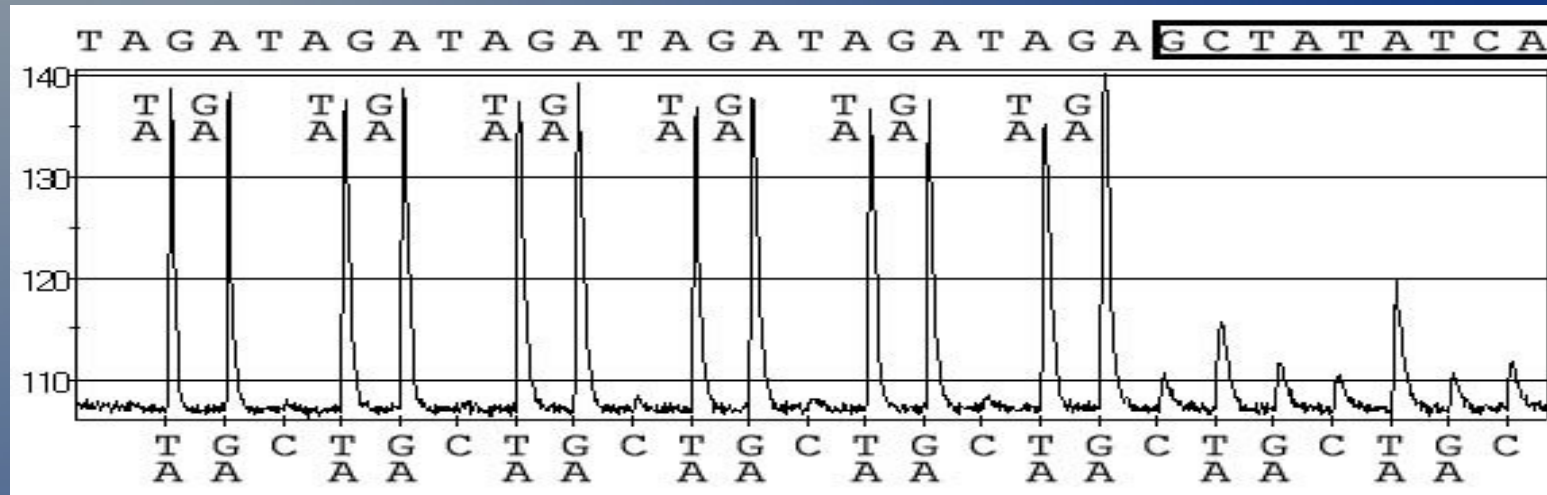
3: Template tailoring using PNA and ddCTP



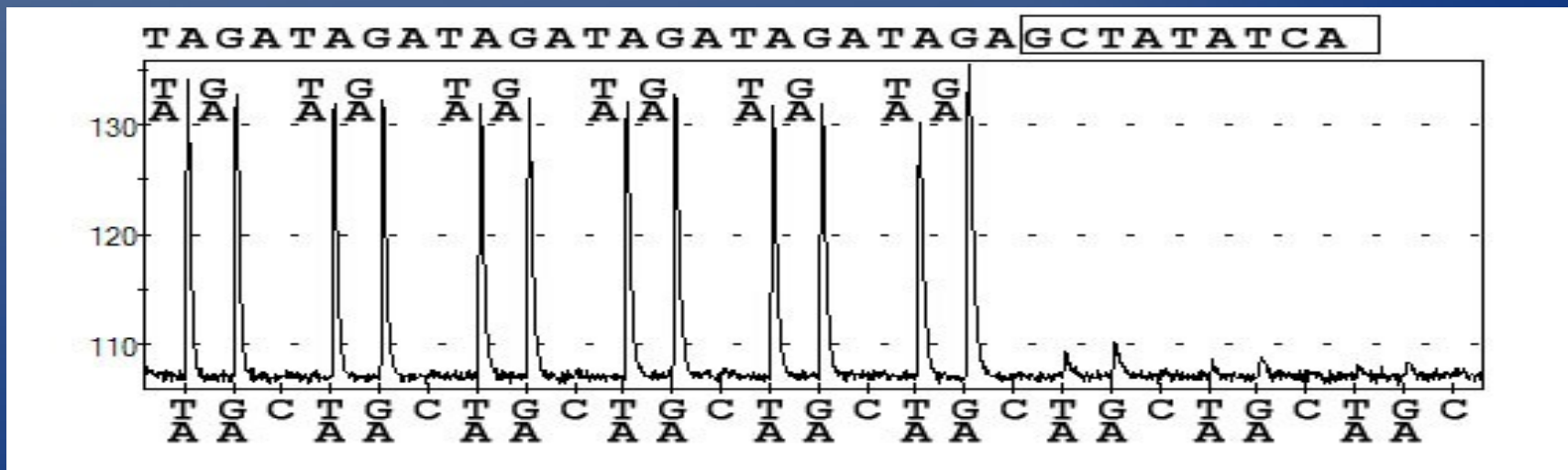
GATA172D05 (Homozygous Sample)

Terminating nucleotide has advantage

Template tailoring by PNA **Genotype 6,6**



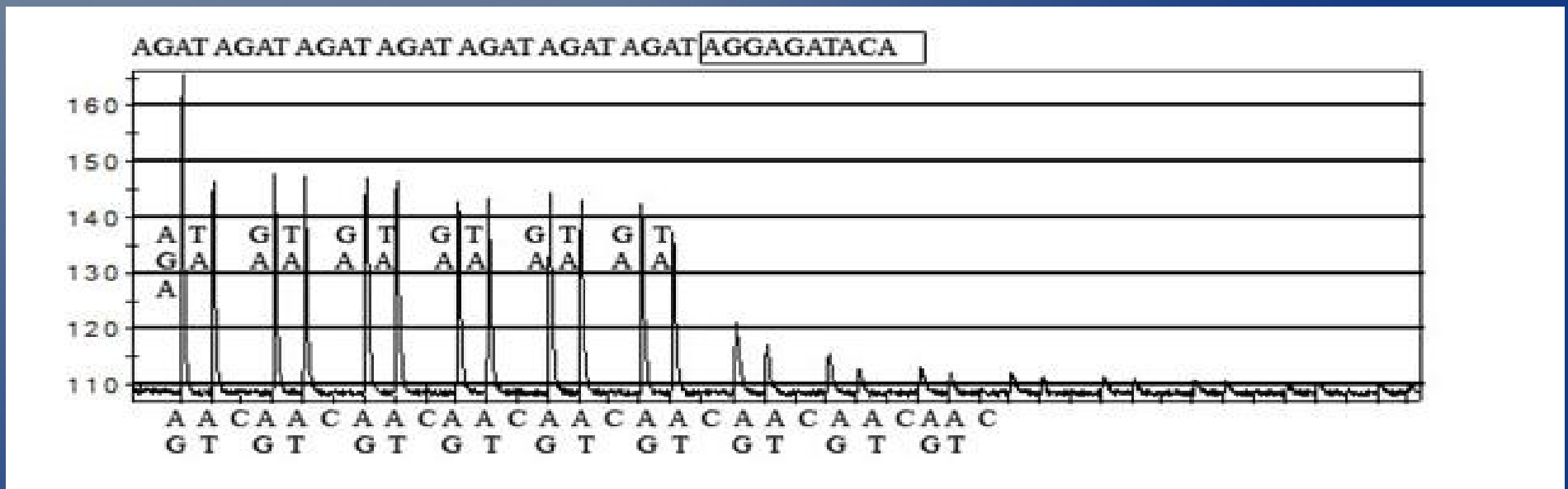
Template tailoring by PNA + ddCTP **Genotype 6,6**



GATA31E08

PNA has advantage (when 4th possible nucleotide for terminating is not at the start of flanking region of STR)

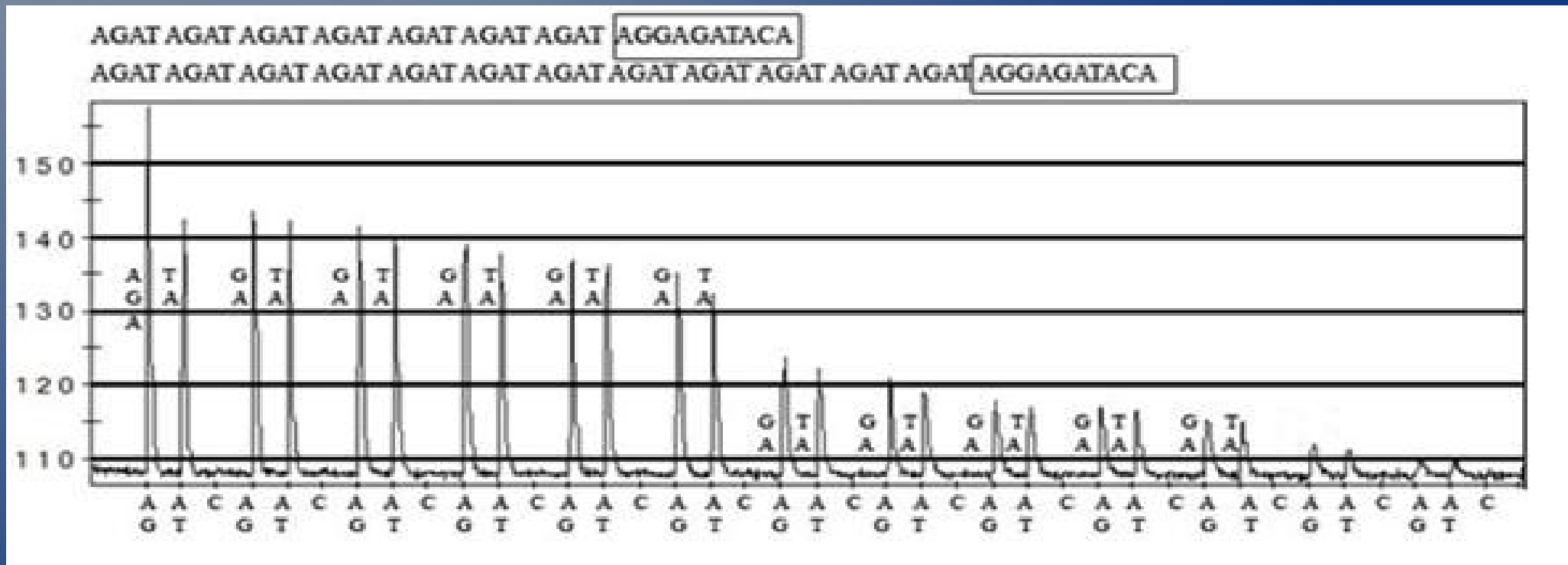
Homozygous sample



GATA31E08

PNA has advantage (when 4th possible nucleotide for terminating is not at the start of flanking region of STR)

Heterozygous sample



Genotyping of 15 samples by combinatorial Pyrosequencing

Sample	GATA172D05	Sample	GATA31E08
01	6,6	01	13,13
02	10,10	02	11,11
03	10,10	03	7,7
04	8,10	06	9,9
06	10,10	08	10,10
08	12,12	09	12,12
09	11,11	Ak3	10,13
10	6,9	Ak5	9,12
Ak2	6,12	Ak6	9,12
Ak4	8,8	Ak8	9,12
Ak5	10,12	Ak9	9,10
Ak8	11,12	Ak11	7,12
Ak9	10,12	Ak12	7,7
Ak10	11,11	Ak13	7,10
Ak15	8,8	Ak19	11,12

Confirmation of genotypes by ABI Genetic Analyzer 3100

Conclusions

- **A simple, flexible and cost effective system (No fluorescent dye labeling, No allelic ladder)**
- **Pyrosequencing method demonstrates an approach to sequence Heterozygous STR**
- **Dyad Pyrosequencing generates unique DNA pattern, shorten the analysis time, and increase the read-length**
- **These assays can be used for other applications such as monitoring “engrafting”**

Future Directions

Pyrosequencing of 16 Autosomal STRs (CODIS)

- Amplification of 16 STRs in multiplex format using Connector Inversion Probe (CIP) / Trinucleotide Threading (TNT)
- Sequence-specific primer for individual marker sequencing

Pyrosequencing of SNPs

- Multiplex amplification of 50 SNPs
- Sequence-specific primer for individual SNP

Use CIP/TNT for sample preparation for next generation sequencing platforms

Apply for funding to achieve these objectives

Acknowledgements

Akram Sial

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Eric Brown

Eveline Farias-Hesson

Andy Mak

Adam Rogers

Dr. Steven Lee



Peptide Nucleic Acid (PNA)

Repeating N-(2-aminoethyl)-glycine units linked by amide bonds

Strong hybridization kinetics

Klenow can't displace PNA oligonucleotide bound to complementary DNA

Used PNA oligonucleotide to terminate polymerization at flanking region of STR

