

MODERN TECHNIQUES IN GENE RESEARCH: PROSPECTS AND APPLICABILITY FOR THE DETECTION OF GENE DOPING

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Possible methods and approaches for doping to enhance athletic performance

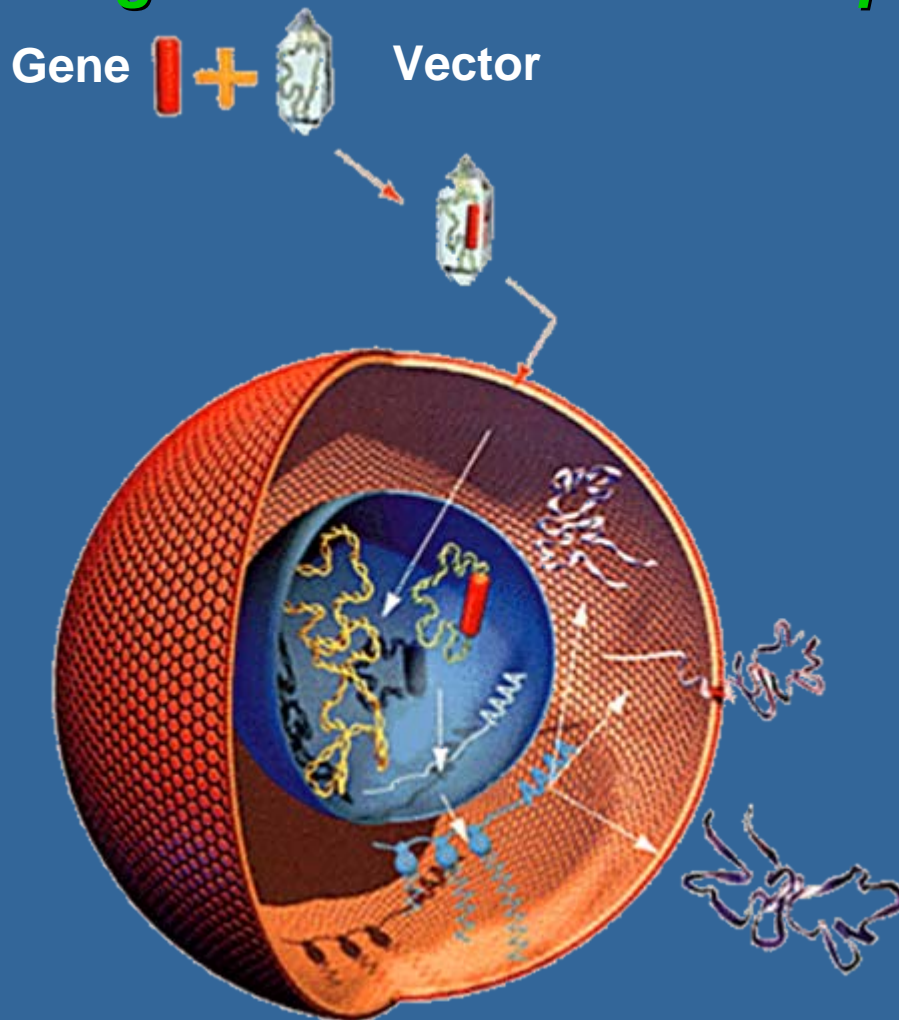
-Gene Therapy

*-Targeting of Signaling Pathways and Gene
Regulatory Elements*

-Introduction of small interfering RNA

Gene therapy -

the transfer of genetic material into a cell, tissue, or whole organ, with the goal of curing a disease or at least improving the clinical status of a patient.

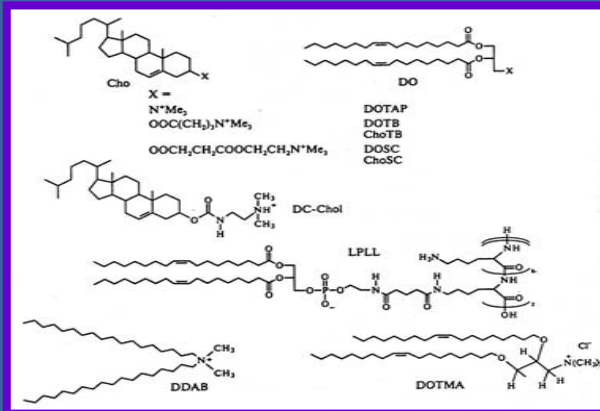


Gene Therapy:

- Hereditary disorders
- Multifactorial diseases
- Vaccination
- Gene doping

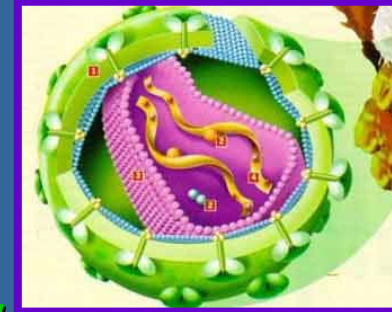
Gene transferring methods

Nonviral vectors



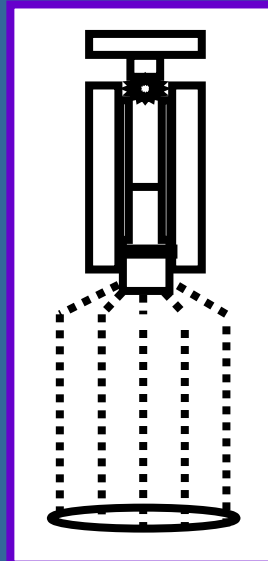
Liposomes
Polymers
Proteins
Peptides

Viral



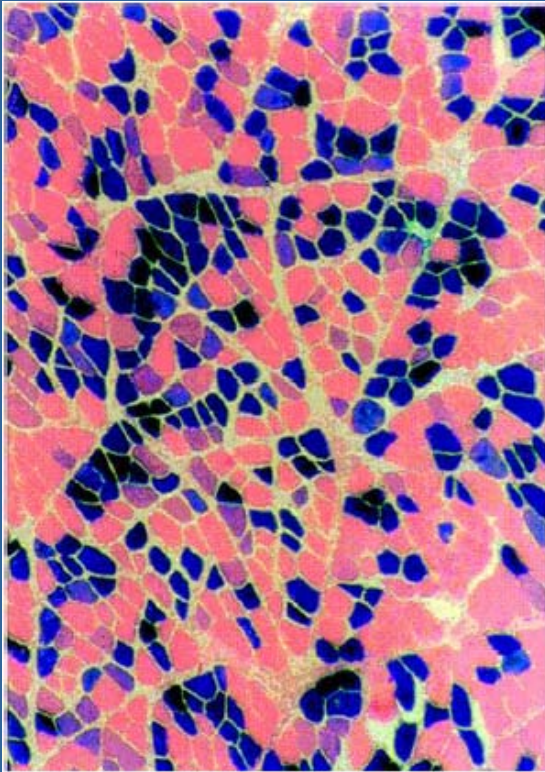
Adenoviruses
Retroviruses
Adenoassociated
viruses

Physical



Electroporation
Ballistic transfection

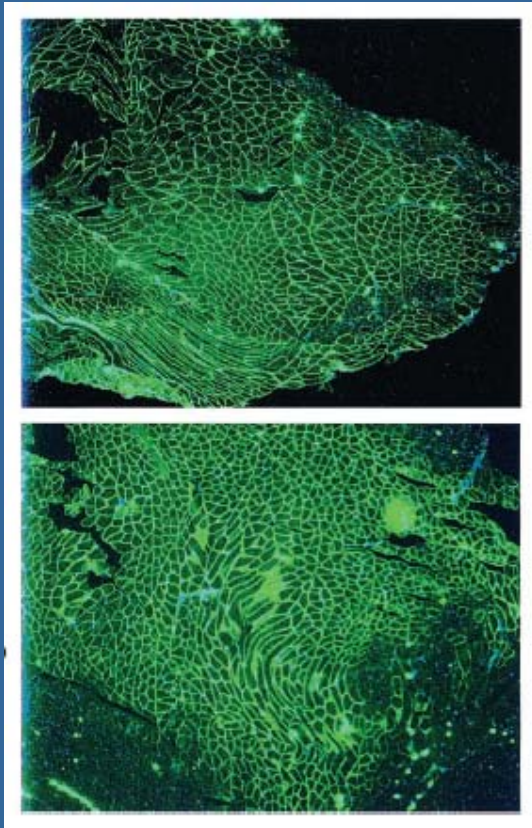
Efficient expression of naked DNA (*lacZ*) delivered intraarterially to limb muscles of nonhuman primates



Expression efficacy < 30% cells

Zhang et al. Efficient Expression of Naked DNA Delivered Intraarterially to Limb Muscles of Nonhuman Primates // Hum. Gene Ther. 2001. V. 12. P. 427–438.

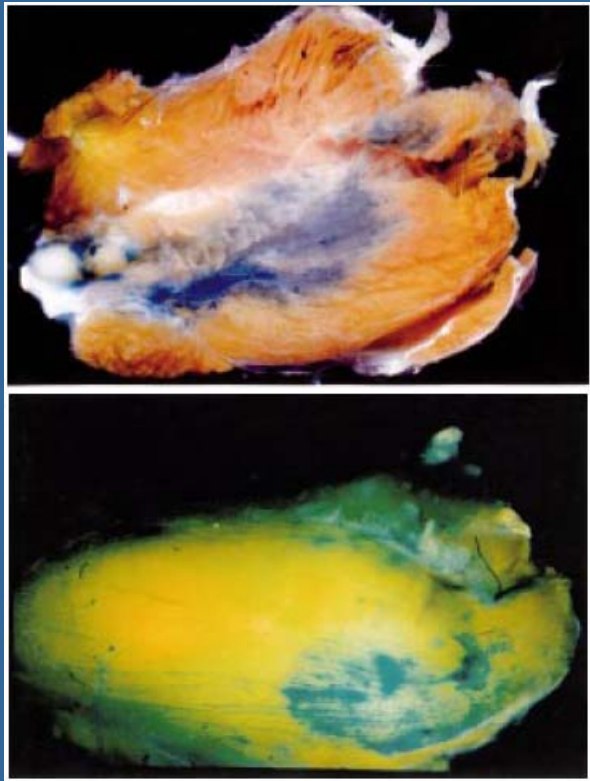
Efficient expression of adeno-associated virus vector carrying human minidystrophin genes in mdx mouse model



Expression efficacy < 88% cells

Wang et al. Adeno-associated virus vector carrying human minidystrophin genes effectively ameliorates muscular dystrophy in mdx mouse model // PNAS. 2000. V. 97. P. 13714–13719.

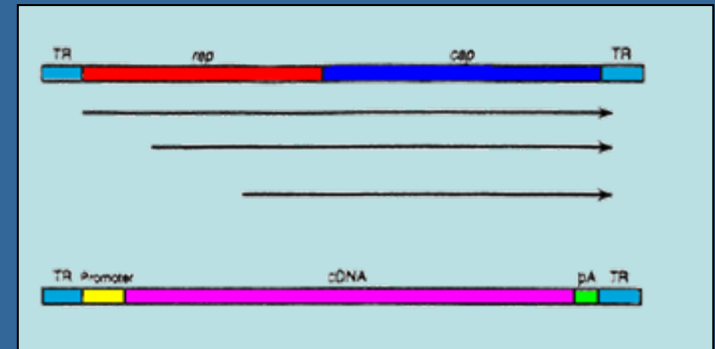
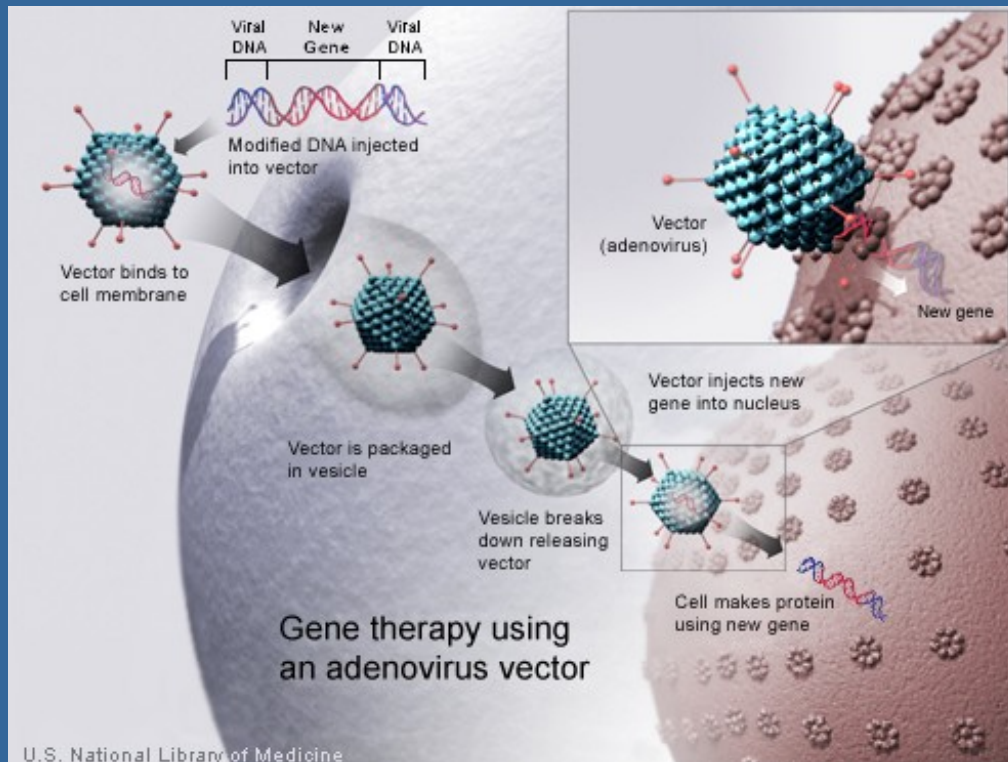
Efficient expression of *lacZ* and human minidystrophin genes delivered by polymer microspheres to mdx mouse muscles



Expression efficacy < 18% cells

Baranov et al. Local and distant transfection of mdx muscle fibers with dystrophin and LacZ genes delivered in vivo by synthetic microspheres // Gene Ther. 1999. V. 6. P. 1406–1414

Gene therapy and vector construction



Adenoassociated virus construction

<http://ghr.nlm.nih.gov/handbook/illustrations/therapyvector>
<http://www.microbiologybytes.com/virology/peel/peel3.html>

Drug targeting of signaling pathways, genome regulatory elements etc.



Wild type

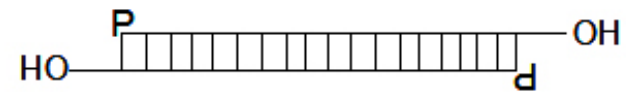
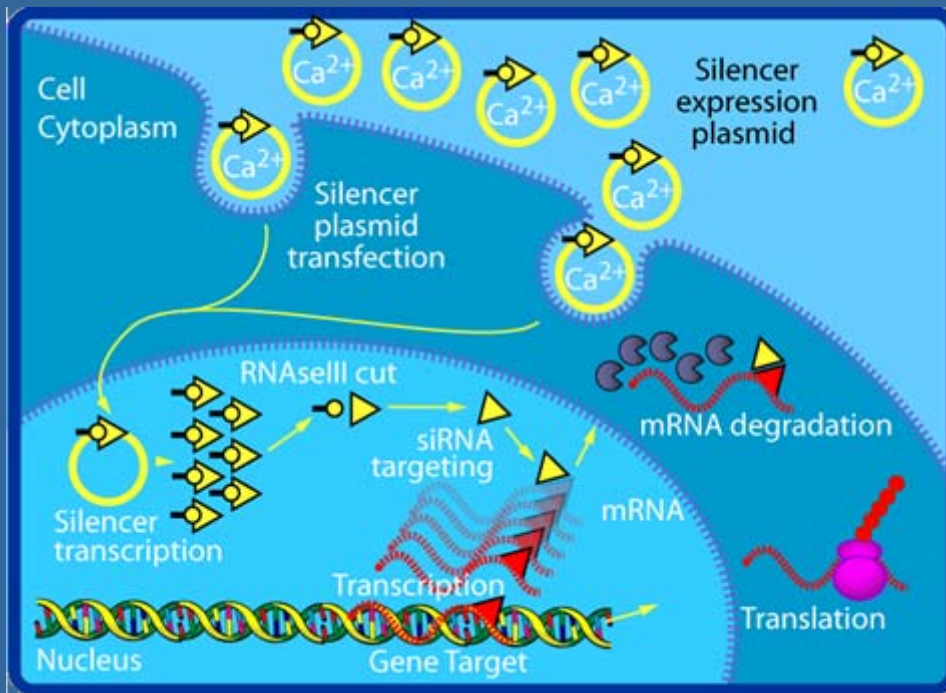


*Myostatin
knockdown*

Lee S-J Quadrupling Muscle Mass in Mice by Targeting TGF- β Signaling Pathways. // PLoS ONE 2007 2(8): e789.

Small interfering RNA

- *20-25 nucleotides*
- *double-stranded RNA molecules*
- *inhibition of gene expression*



Schematic representation of a siRNA molecule: a ~19-21basepair RNA core duplex that is followed by a 2 nucleotide 3' overhang on each strand. OH: 3' hydroxyl; P: 5' phosphate.

http://en.wikipedia.org/wiki/Small_interfering_RNA

Genes of Interest:

- *Myostatin (MSTN)*
- *Erythropoietin (EPO)*
- *Insulin-like growth factor I (IGF1)*
- *Peroxisome proliferator-activator receptors (PPARs)*
- *Other genes (which associated with enhancement of athletic performance)*

What are the motifs that can be used as objects for genetic doping detection?

- *A) sequence of vector elements*
- *B) sequence of cDNA (foreign DNA)*
- *C) expression profiling*
- *D) antibody for foreign proteins or constructions*
- *E) complex approaches*

Problems of detection

- *Low concentration of target DNA/RNA sequences in the sample*
- *Difficulty of regulatory elements analysis (promoters, enhancers, silencers, terminators, exon-intron boundaries)*
- *Possible use of new target genes and new methods of transfer*
- *Detection of end-product is impossible*
- *Low stability of the material*

Requirements for antidoping gene analysis:

- *Simplicity of sample collection*
- *Non-invasive (desirable)*
- *Sufficient sample quality*
- *Opportunity to divide the sample for 2 identical tests*

Which biological material can be used for the molecular genetic analysis?

- *Blood*
- *Urine*
- *Saliva*
- *Biopsy*

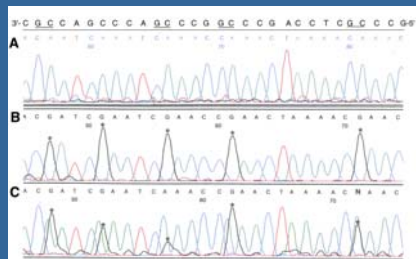
The Best Method – direct analysis of DNA sequence

- *Possibility to detect primary sequences*
- *Opportunity to analyze regulatory elements (promoters, enhancers, silencers, terminators, exon-intron boundaries)*
- *Simplification of the analysis (decreasing number of steps)*
- *DNA is more stable than RNA*
- *Direct method of gene doping detection*

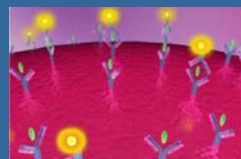
MODERN TECHNIQUES OF DNA ANALYSIS

PCR +

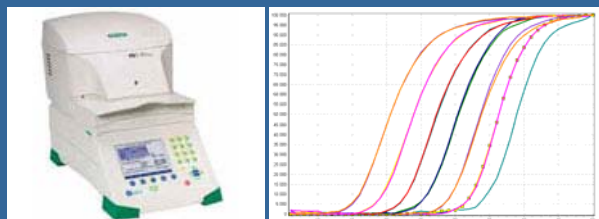
Sequencing



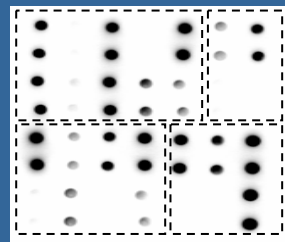
FCBS (xMAP)



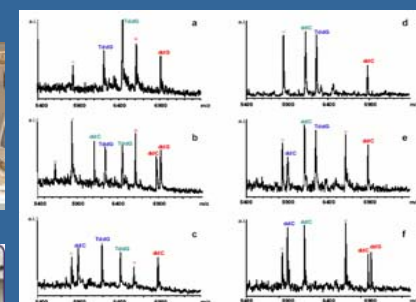
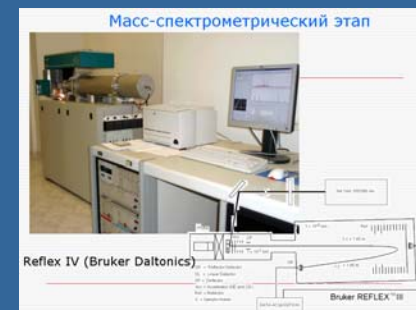
Real-Time PCR



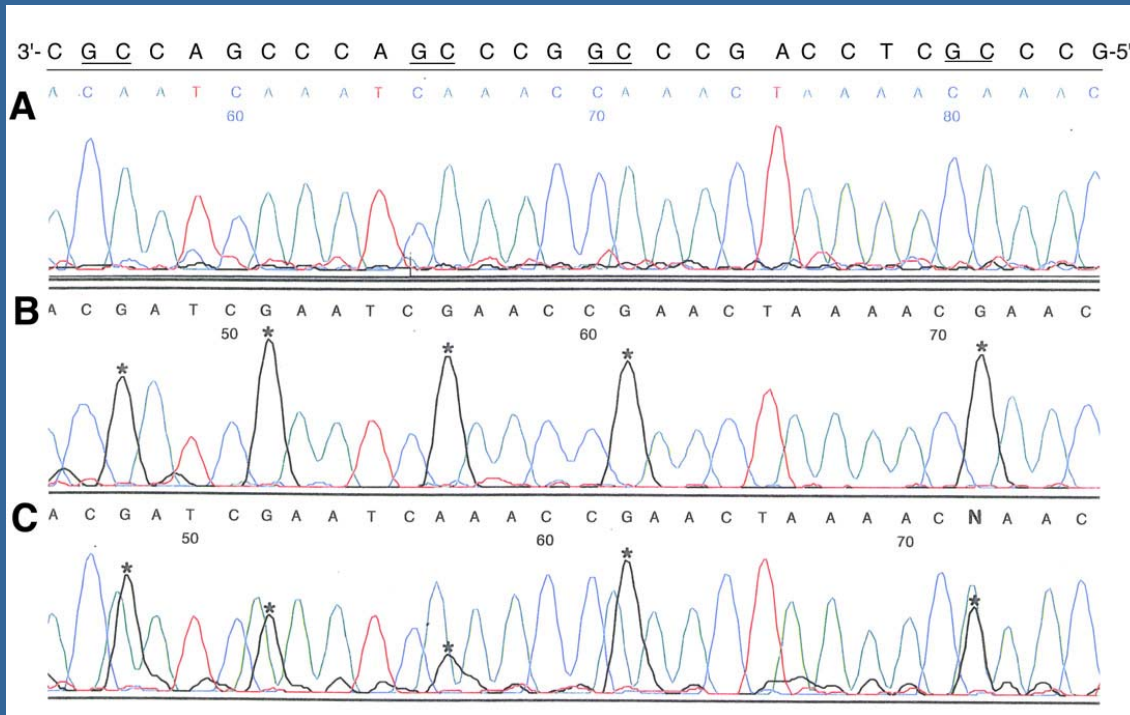
Microarray



MALDI-TOF mass spectrometry



Sequencing



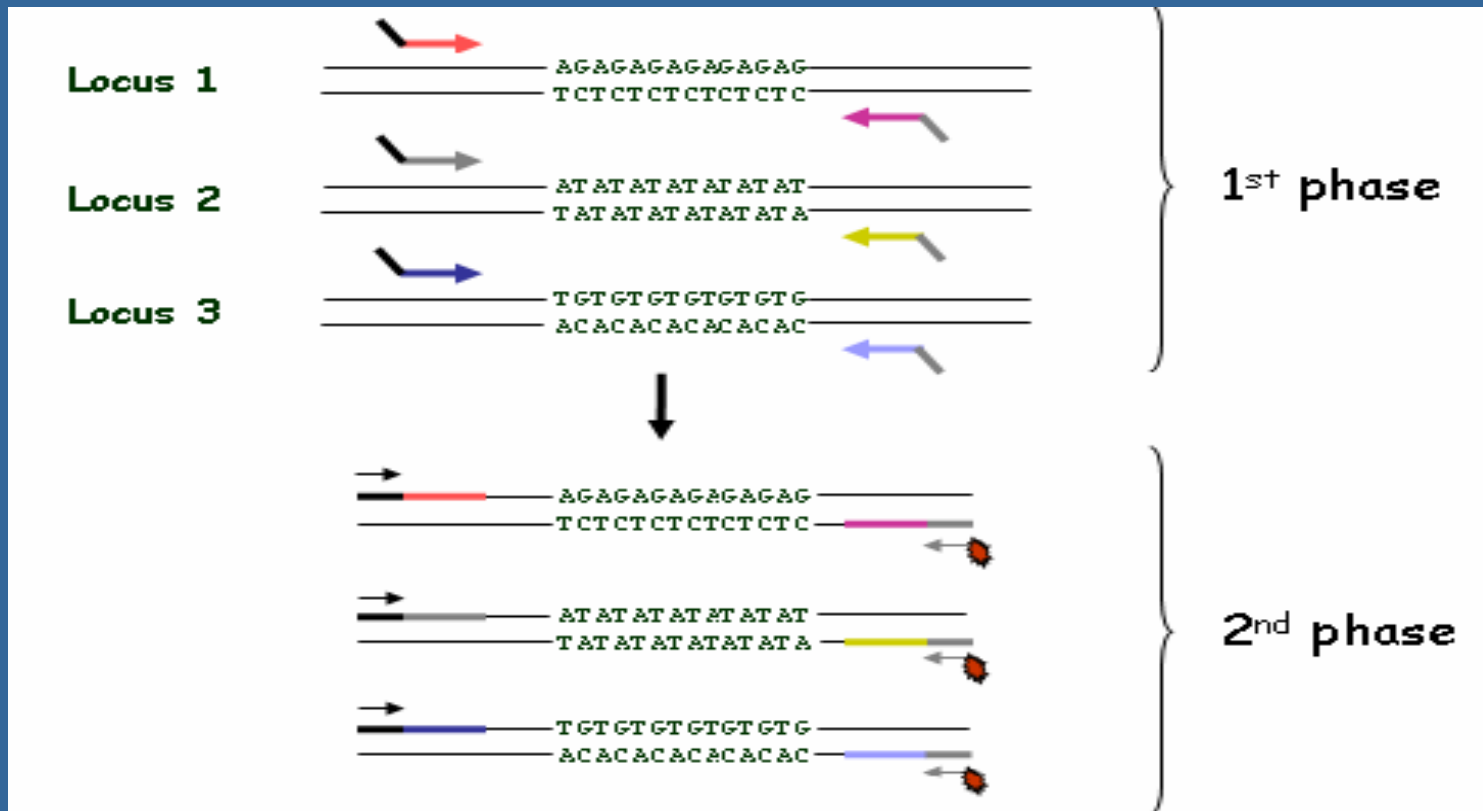
Advantages:

1. High accuracy and reproducibility of result
2. Duration of the analysis about 1 day

Lacks:

Insufficient sensitivity

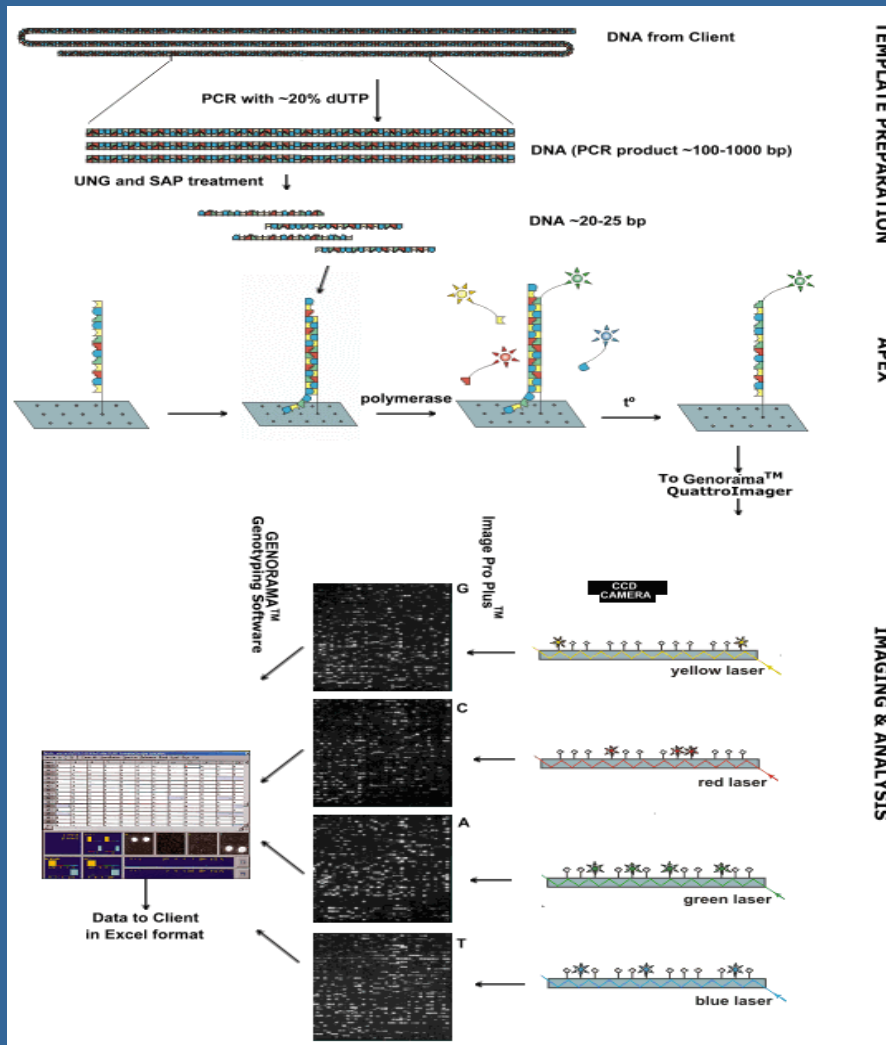
Universal primers for PCR



M.J. Hayden, T.M. Nguyen & K.J. Chalmers Multiplex-Ready Markers: A technique for high-throughput, low-cost genotyping on an automated DNA fragment analyser.

S. Parachini, B. Arredi, R. Chalk and C. Tyler-Smith. 2002. Hierarchical high-throughput SNP genotyping of the human Y chromosome using MALDI-TOF mass spectrometry. *Nucleic Acids Res.*6

Microarray (Single-based extension)



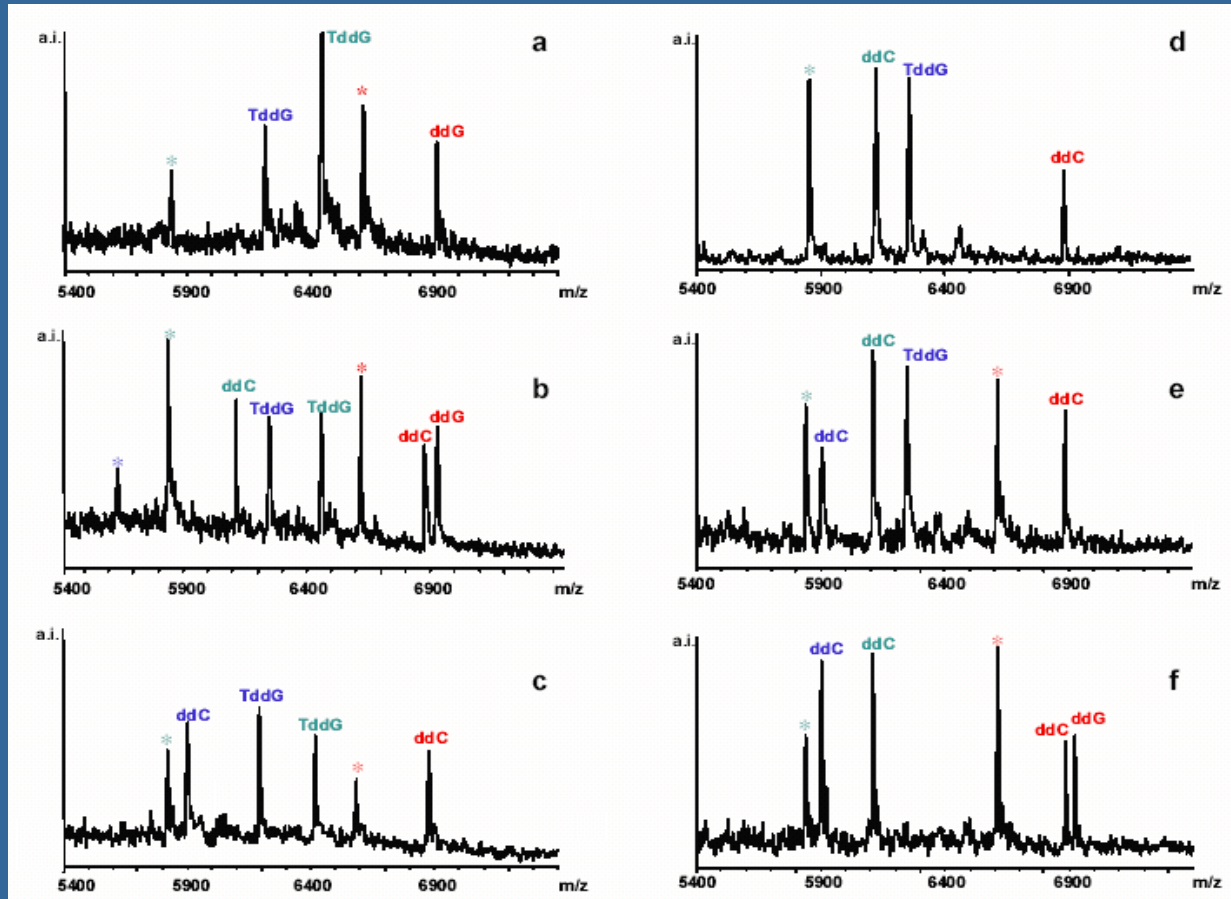
Advantages:

1. High accuracy and reproducibility of result
2. Duration of the analysis about 2-4 hours

Lacks:

Sensitivity in quantification of PCR product

MALDI-TOF analysis



Advantages:

1. High accuracy and reproducibility of result
2. Duration of the analysis about 1-3 hours

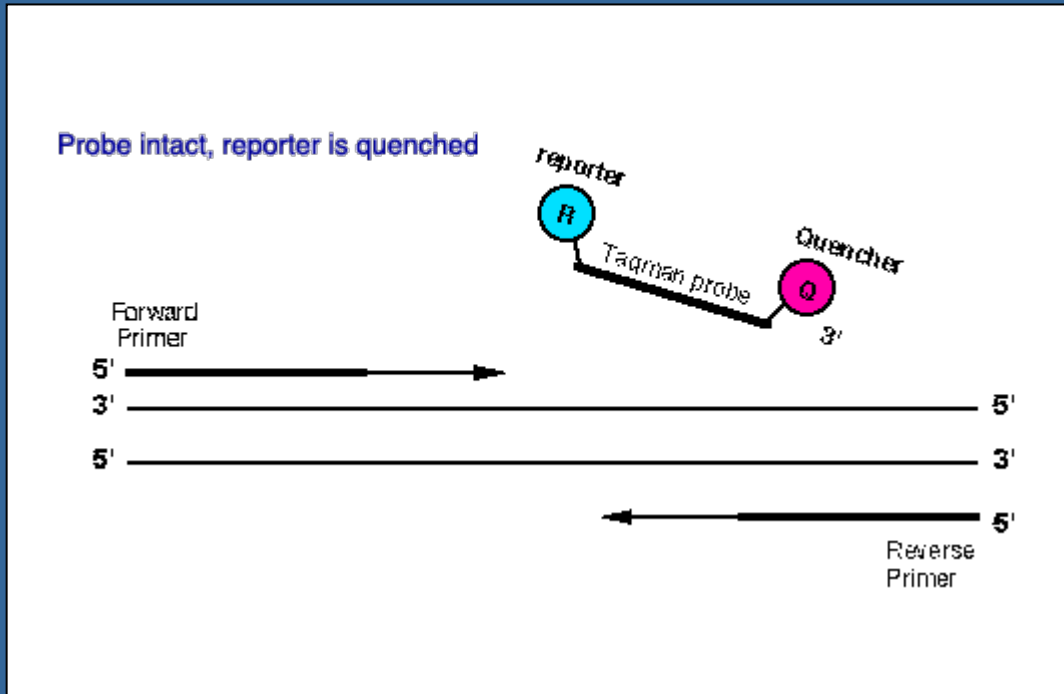
Lacks:

Special education of personnel

ApoE and A2M genotyping by MALDI-TOF mass spectrometry for determination of Alzheimer disease risk (<http://www.daltonics.bruker.com>)

Real-time PCR

(TaqMan)



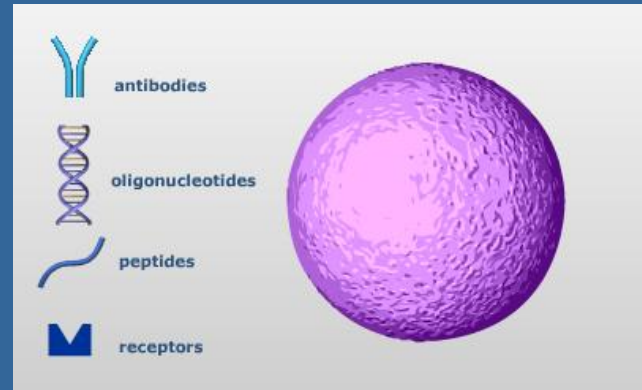
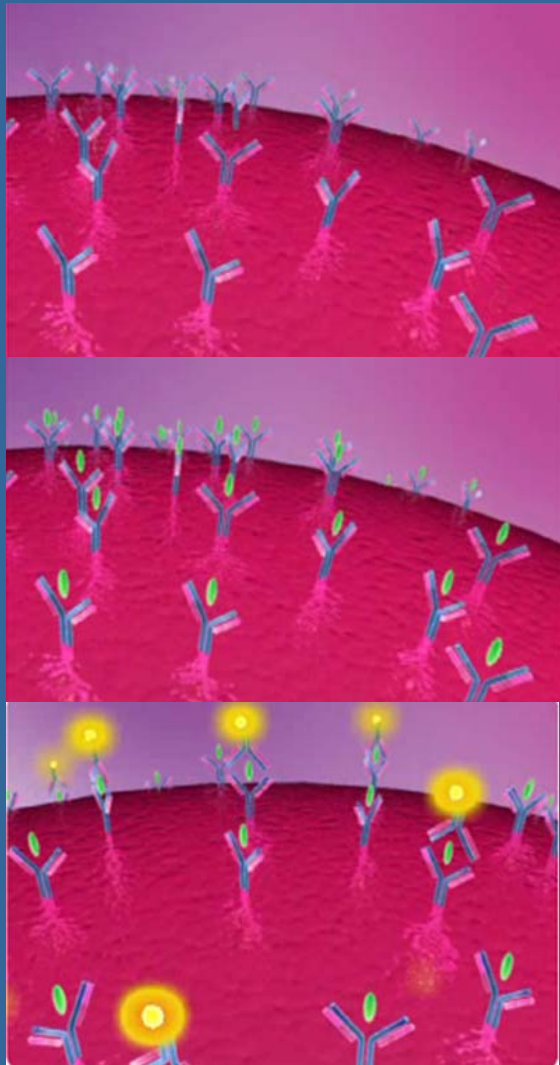
Advantages:

1. High accuracy and reproducibility of result
2. Duration of the analysis about 1-2 hours

Lacks:

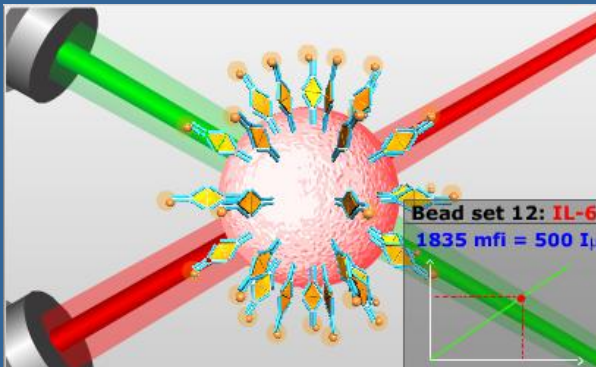
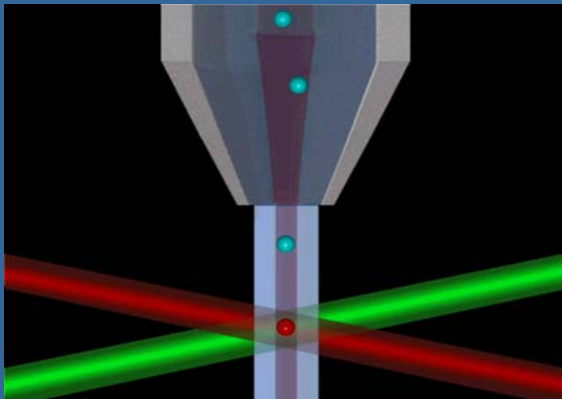
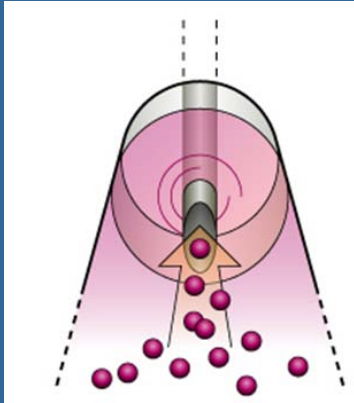
Sensitivity in quantification of DNA

Flow cytometry based systeme (xMAP)



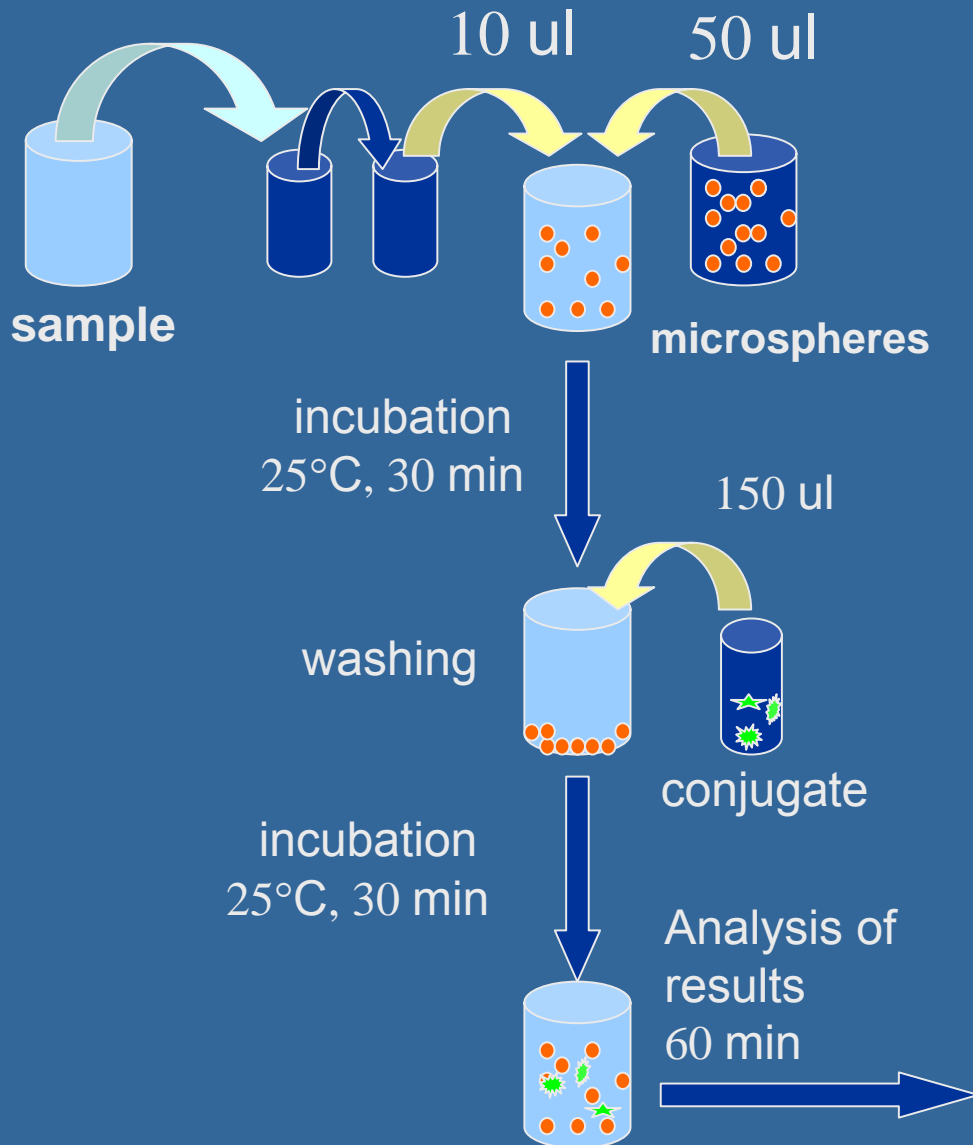
- **Microspheres are covered by reagents (oligonucleotides or antibodies)**
- **The sample is added to microspheres**
- **Fluorescent reagent is added**

xMAP



- Automatic fence from cells.
- Lasers are used to detect fluorescent dyes - the red laser is used for classification of particles (microspheres), and the green laser for the reception of analytical signal.
- Multiplex analysis for each set of microspheres is possible.

xMAP (scheme of analysis)



+ 2 positive controls
+ 1 negative control

Acknowledgments

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