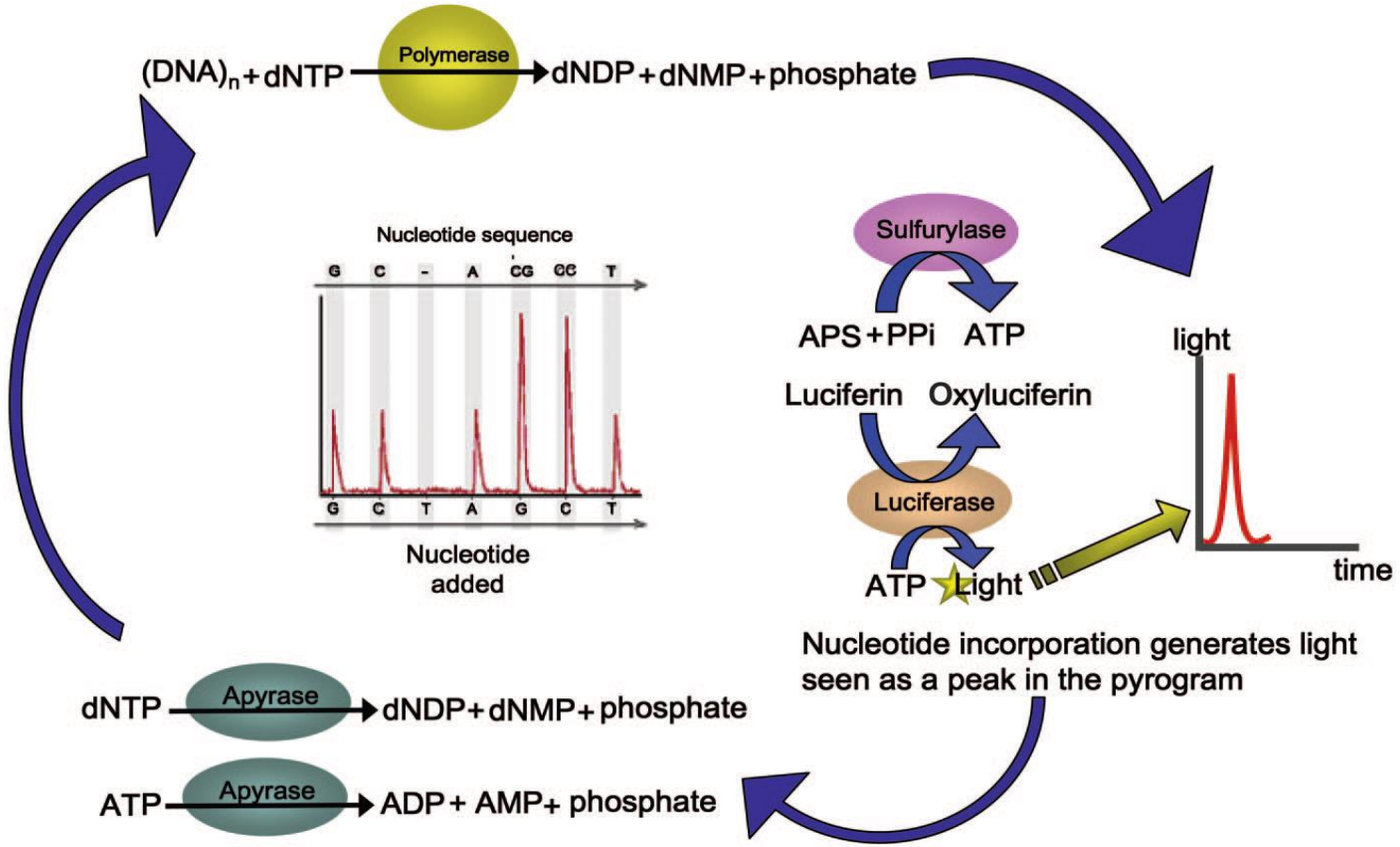
Pyrosequencing

- Pyro: Greek Puro = fire
- Light sequencing
- Uses:
- Detection of SNP's
- Analysis of difficult 2ndary structures
- Tag sequencing
- Microbial typing
- Resequencing

Principle of Pyrosequencing



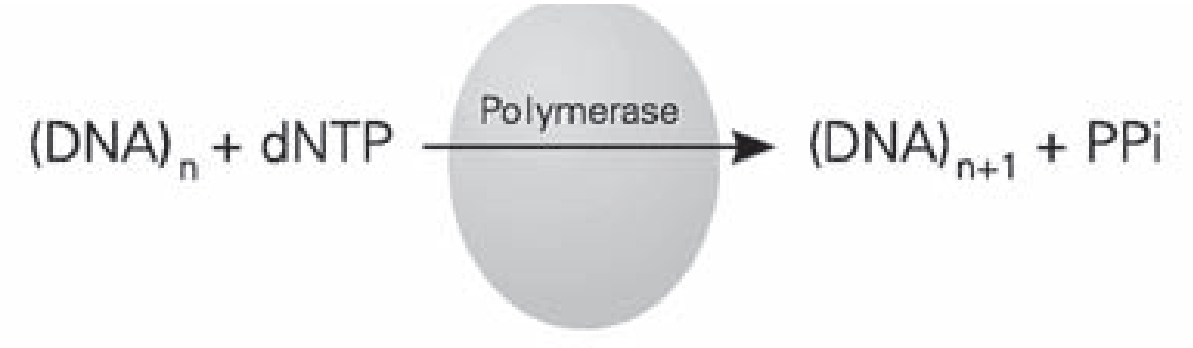
http://www.pyrosequencing.com

Step 1

- A sequencing primer is hybridized to a single stranded, PCR amplified, DNA template
- Incubated with the enzymes, DNA polymerase, ATP sulfurylase, luciferase and apyrase, and the substrates, adenosine 5′ phosphosulfate (APS) and luciferin

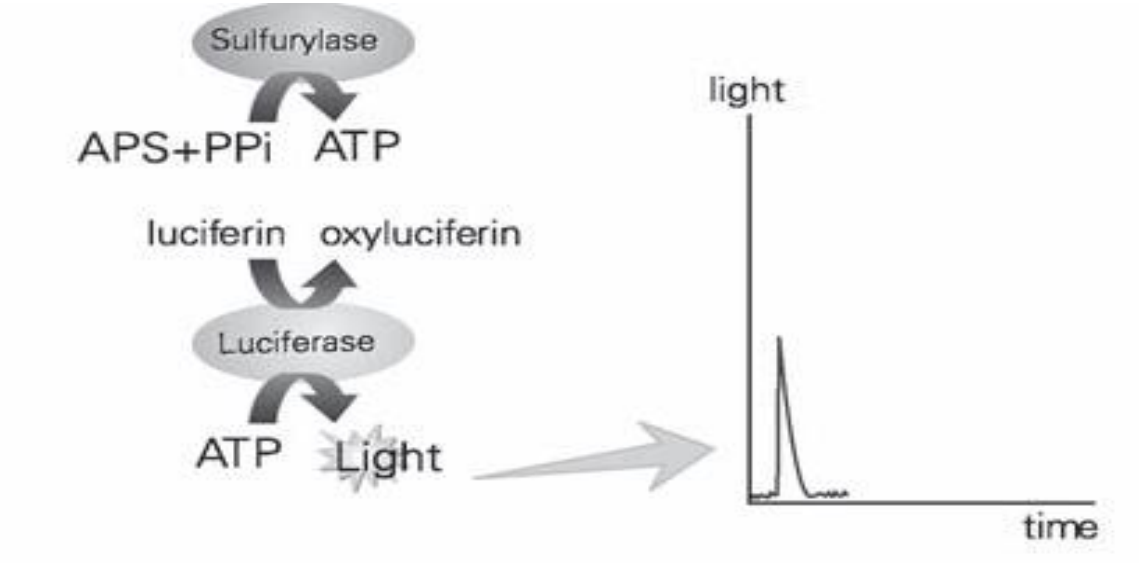
Step 2

- The first of four dNTP's is added to the reaction
- DNA polymerase catalyzes the reaction
- Incorporation of the dNTP into the DNA strand, if it is complementary to the base
- Release of pyrophosphate (PPi)



Step 3

- ATP sulfurylase quantitatively converts PPi to ATP in the presence of APS
- This ATP drives the luciferase mediated conversion of luciferin to oxyluciferin that generates visible light
- The light produced in the reaction is detected by a CCD camera and seen as a peak in a Pyrogram™

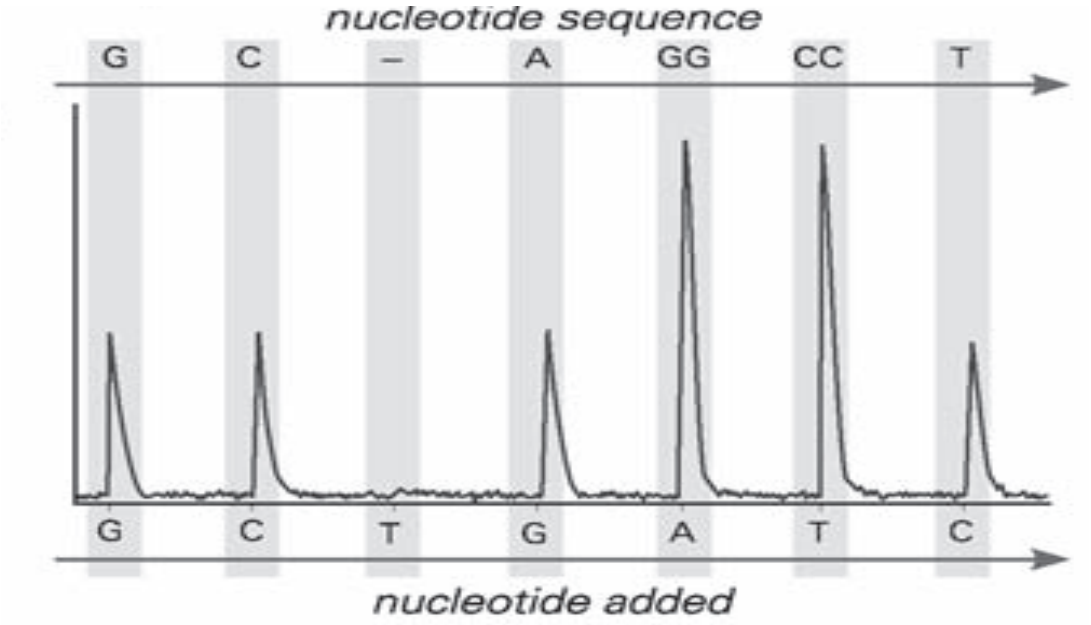


Step 4

- Apyrase continuously degrades ATP and unincorporated dNTPs
- This switches off the light and regenerates the reaction solution
- The next dNTP is then added

Step 5

- Addition of dNTPs is performed one at a time
- The nucleotide sequence is determined from the signal peaks in the Pyrogram
- Deoxyadenosine alfa-thio triphosphate is used as a substitute for the natural dATP



Applications of Pyrosequencing

Genotyping of Single-Nucleotide Polymorphisms

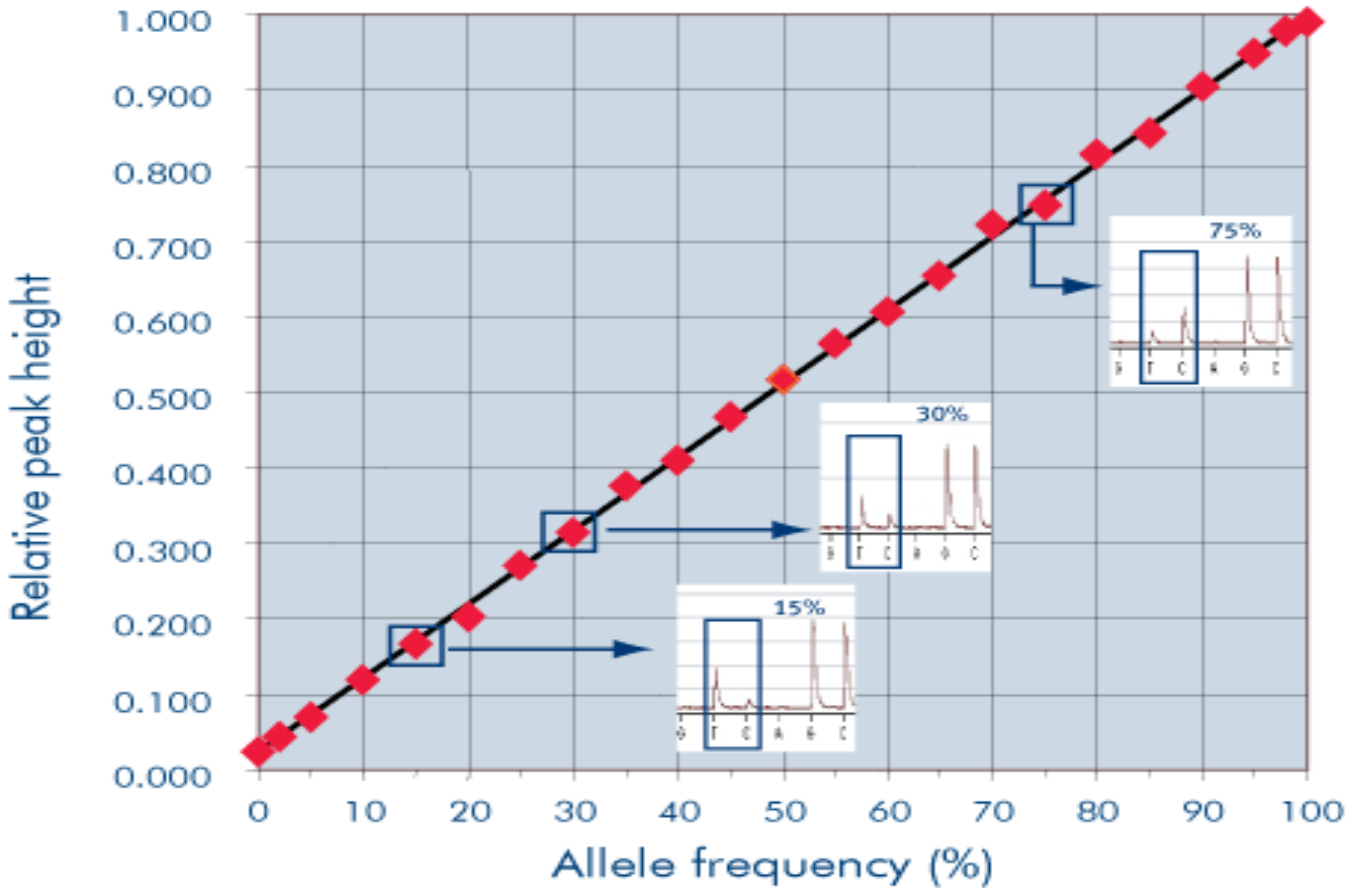
- As pyrosequencing signals are very quantitative it is possible to use this strategy for the studies of allelic freq.
- Determination of the phase of SNPs when they are in the vicinity of each other allowing the detection of haplotypes

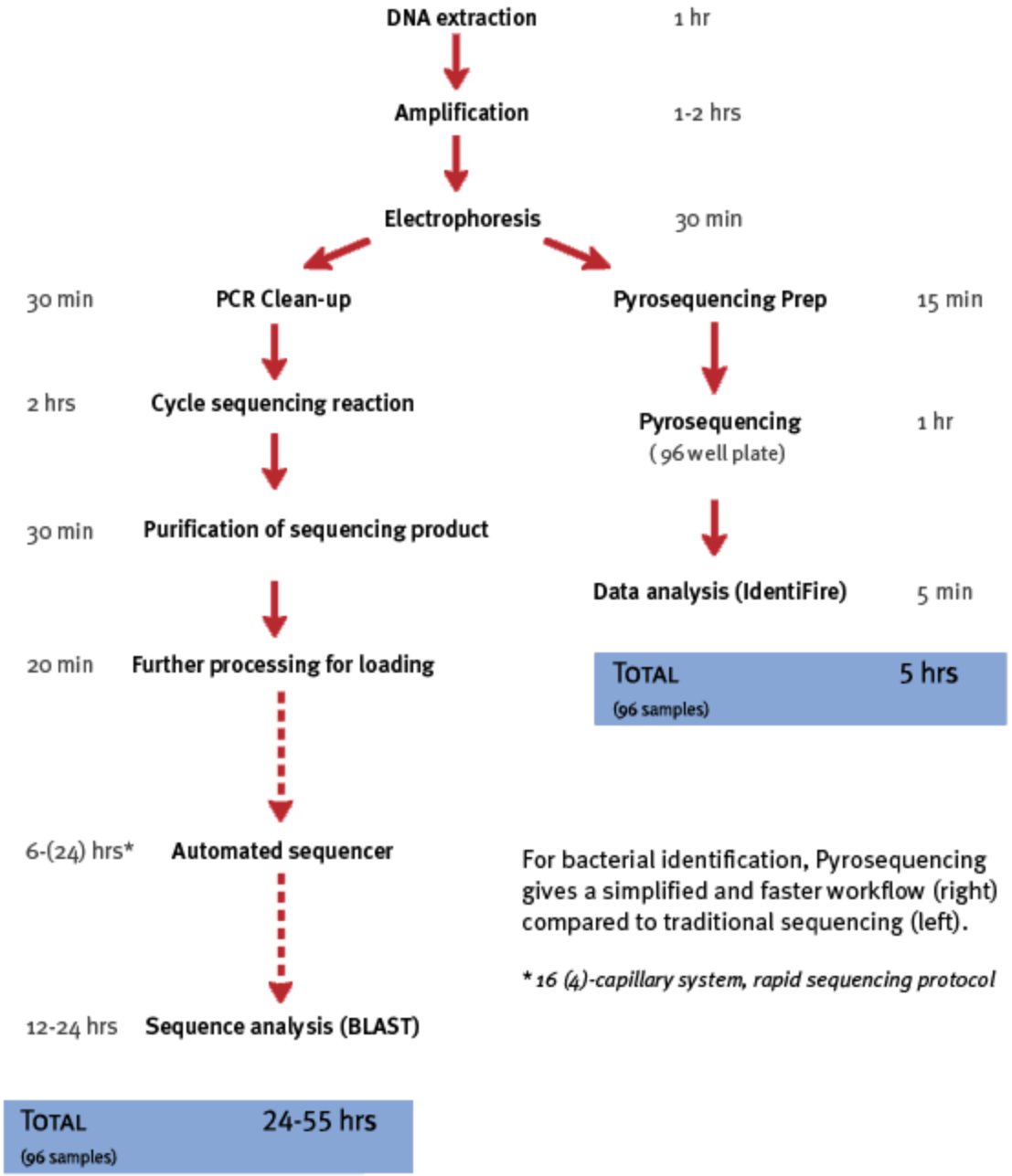
Microbial Typing

- Analyzing a sequence between 20–100 nucleotides on 16S rRNA gene, it is possible to taxonomically group different bacteria

Resequencing

- Accurate quantification of the mutated nucleotides
- Longer read length than de novo sequencing
- Programmed dispensing


Relative peak height
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
10
20
30
40
50
60
70
80
90
100
Allele frequency (%)
75%
30%
15%



For bacterial identification, Pyrosequencing gives a simplified and faster workflow (right) compared to traditional sequencing (left).

** 16 (4)-capillary system, rapid sequencing protocol*

Tag Sequencing

- 98% of genes in a human cDNA library could be uniquely identified by sequencing a length of 30 nucleotides
- Pyrosequencing was used to sequence this length for gene ident.
- Pyrosequencing offers high-throughput analysis of cDNA libraries because 96 samples can be analyzed in less than one hour

Analysis of Difficult secondary structures

- Hairpin structures are common features in genomic material
- conventional DNA sequencing usually gives rise to DNA sequence ambiguities seen as "run-off" or compressions
- Pyrosequencing was successfully applied to decipher the sequence of such regions

References

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