Pyrosequencing分析儀的原理及應用

(A New Light on High-throughput SNP Analysis)

博微生物科技股份有限公司

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Presentation overview

- Core technique of the pyrosequencing method
- Instrumentation
- Applications
  - SNP Analysis (SNP)
  - Allele Quantification (AQ)
  - Sequence Analysis (SQA)
  - CpG methylation

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The pyrosequencing method

- **solution for applied DNA analysis**

- Sequence based technology
- Accurate
- Simple and robust
- No labels or gels
- Real-time results

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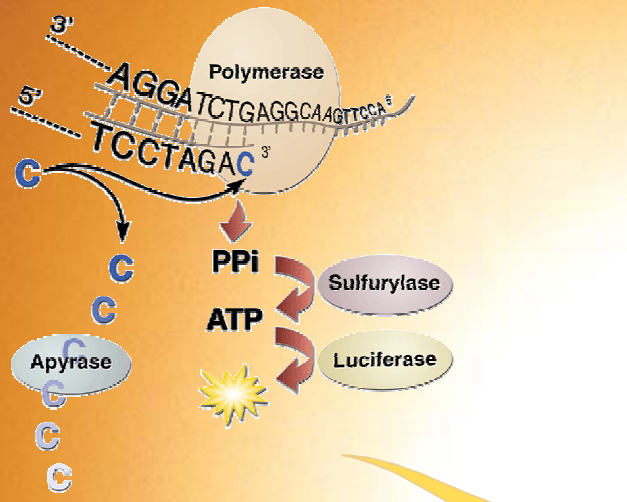
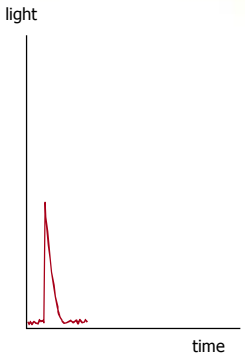
The pyrosequencing method

- detection of the light





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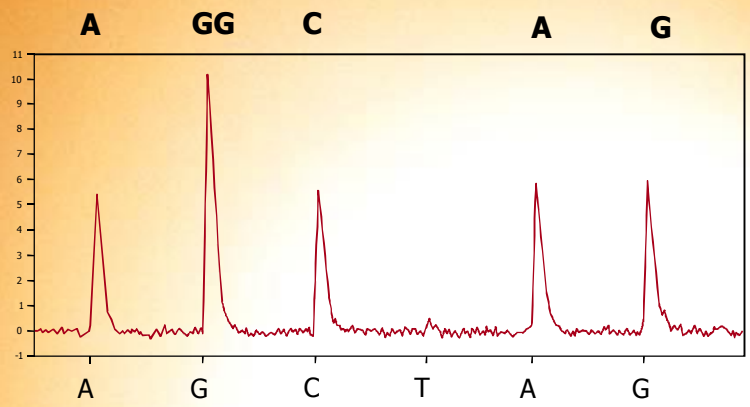
The pyrosequencing method

- nucleotides dispensed sequentially



the sequence in this pyrogram™ is AGGCAG

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Instrumentation

- PSQ™ 96

- Automatic dispensation of reagents
- 96 well format
- CCD camera
- Processes
  - 500 samples per hour
  - 4500 samples per day

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Instrumentation

Vacuum Prep Tool

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Applications

- **software modules for PSQ96**

- SNP Analysis (SNP)
- Allele Quantification (AQ)
- Sequence Analysis (SQA)

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Applications - SNP Analysis

- SNPs as genetic markers

- Single Nucleotide Polymorphisms are isolated single base variations in the genome
- Occur every 500-1000 bases along the 3 billion bases of the human genome
- The most common form of genetic inter-individual variation
- The major source of phenotypic variability between individuals

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Applications - SNP Analysis

- Pyrosequencing™ for SNP analysis

SNP Discovery	SNP Confirmation	Allele Frequency	SNP Relevance	SNP Diagnostics
New SNPs • Sanger • In silico • etc...	Verify SNP as true SNP	Frequency of SNP in populations	Validate SNP as marker for phenotype	Utilization of SNP markers

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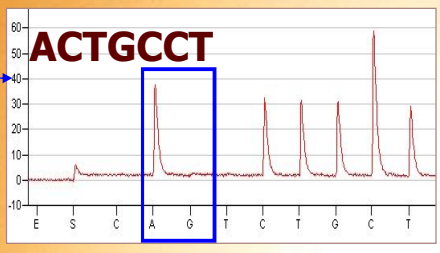
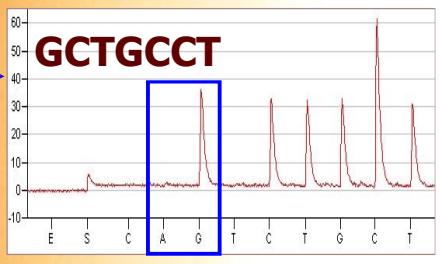
Applications - SNP Analysis

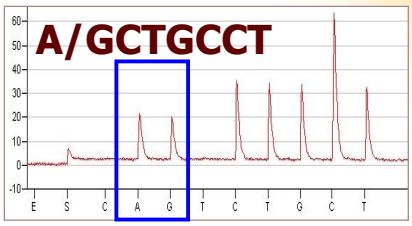
-**Clearly distinguish heterozygotes and homozygotes**

Homozygotes





Heterozygote



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Applications - SNP Analysis

- multiplex genotyping by pyrosequencing™

..analysis of more than one SNP per well

- Reduces cost per genotype
- Increases efficiency
- Increases speed ...of genotyping studies

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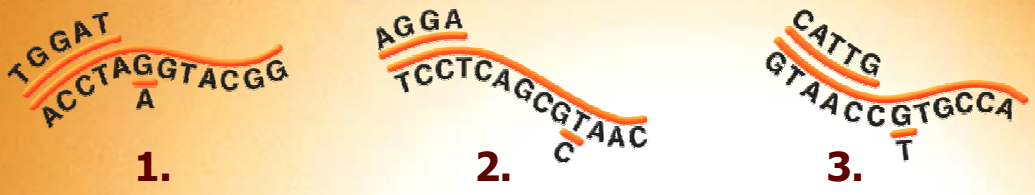
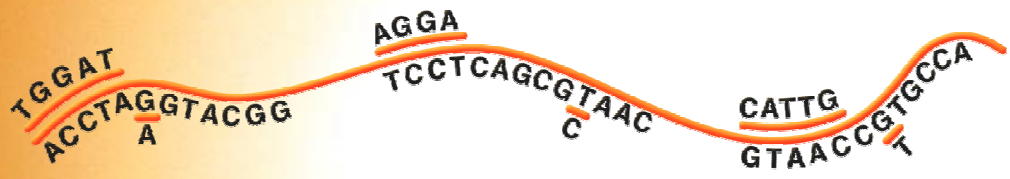
Applications - SNP Analysis

- principle of multiplex genotyping by pyrosequencing™

SNPs located on different fragments….



…or on the same



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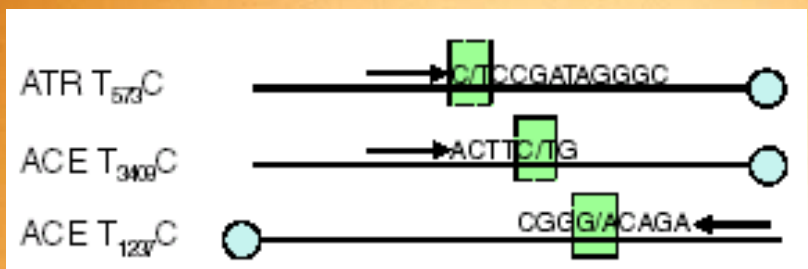
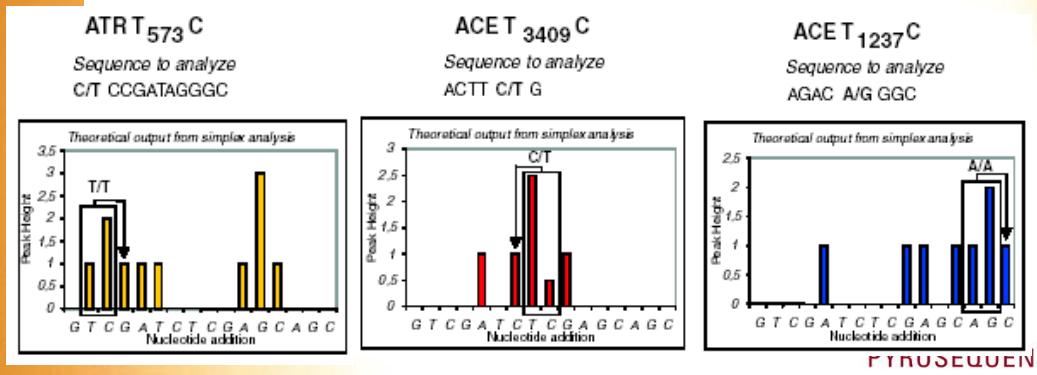
Applications - SNP Analysis

- principle of multiplex genotyping by pyrosequencing™





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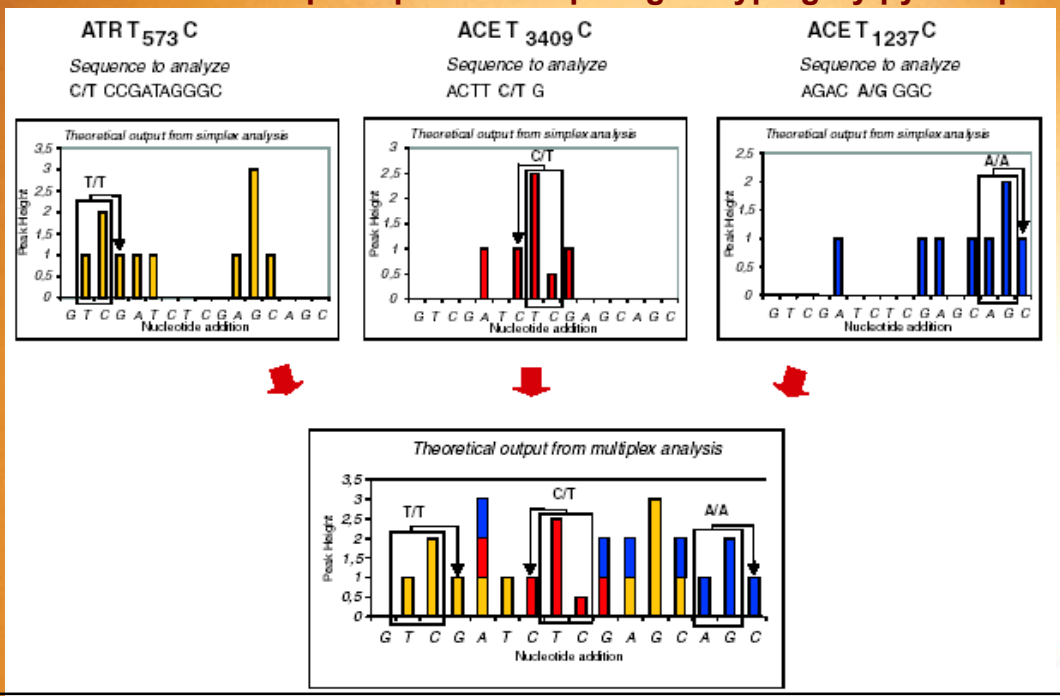
Applications - SNP Analysis

- principle of multiplex genotyping by pyrosequencing™



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Applications - allele quantification

- pooling DNA samples

..influence efficiency and cost of SNP studies

- Reduction in number of analyses
- Reduced costs (reagents and labor)
- Less genomic material required

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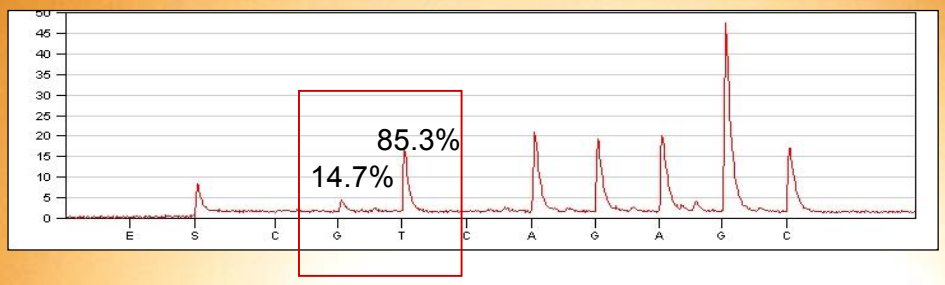
Applications - allele quantification

- result from Karolinska Institute



SNP 1: **1126 individuals**
SNP Software AQ: **G: 14.7% T: 85.3%**
Expected: **G: 15% T: 85%**

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Summary

The strength of Pyrosequencing

- Many applications
- Accurate
- Sequence confirmation (compared to yes/no)
- Fast, 96 genotypes in 10 minutes
- Easy, little hands-on and short optimization time
- 96-well format – easy to automate
- Automatic scoring of the results

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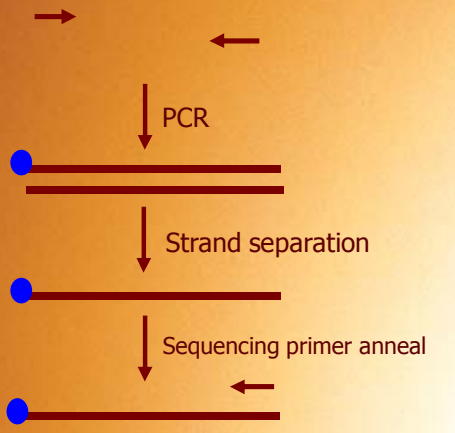
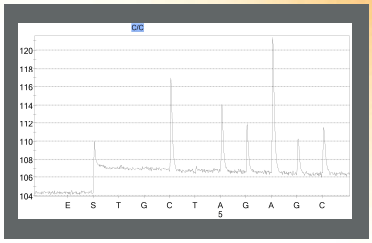
SNP analysis

- working with the PSQ™ 96





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SNP analysis

- **Sample preparation (PCR)**

1.PCR products design

2.PCR primer design- **Biotinylated PCR primer** →

← Biotin

3.PCR reaction

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SNP analysis

- Sample preparation

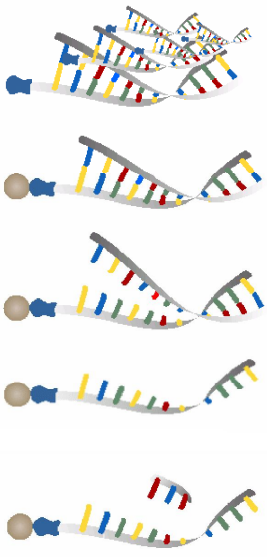
1. Amplify relevant region by PCR (preferentially 80 - 200 bp), one primer biotinylated

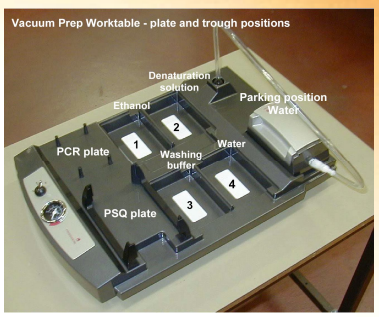
2. Immobilize biotinylated PCR products onto streptavidin-coated beads

3. Separate strands by denaturation in NaOH

4. Wash /neutralize the immobilized strand

5. Anneal sequencing primer





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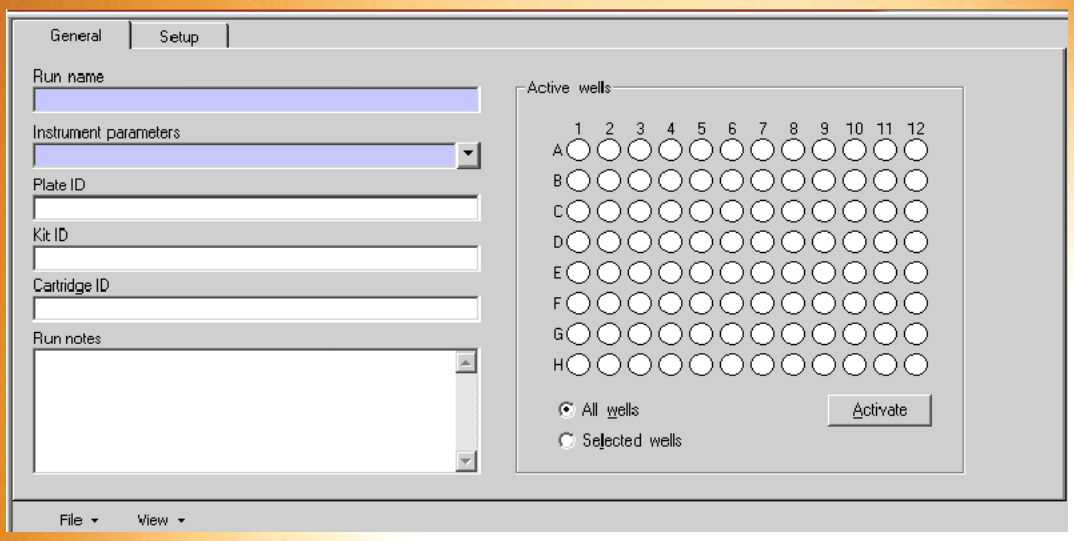
SNP analysis

- entering SNP information



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SNP analysis

- **working with the PSQ™ 96**

1. Prepare samples
2. Insert samples in PSQ™ 96
3. Insert reagent cartridge (enzymes, substrate, nucleotides)
4. Start run

sequence automatically scored

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Pyrosequencing™ for analysis of DNA methylation

As easy as PCR
As detailed as Sanger

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Pyrosequencing™

- Quantification of individual sites
- Fast and simple
- Built-in QC for the bisulfite treatment
- Analyse any CpG sites you like

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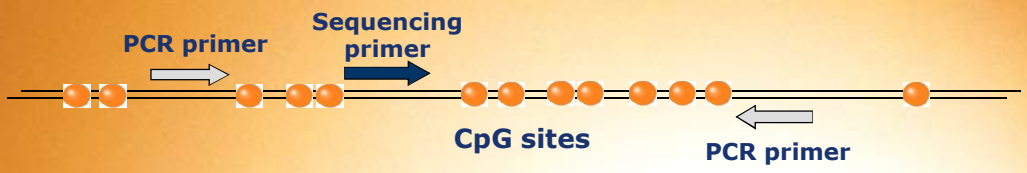
CpG assay design



- All primers are located in non-variable regions, in between CpG sites
- Enables analysis of several adjacent CpG sites with one sequencing primer

  Freedom in positioning of the sequencing primer

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CpG methylation analysis

Bisulfite conversion of DNA

PCR

~ 2h

Sample Prep

~ 15 min

Pyrosequencing

~ 10-100 min

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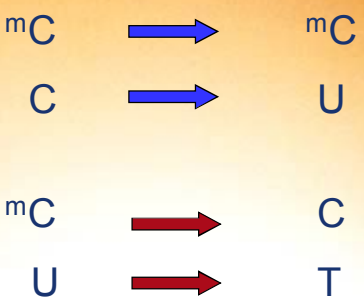
CpG methylation analysis with Pyrosequencing

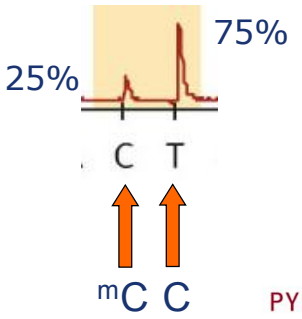
1. Bisulfite conversion



2. PCR amplification

3. Pyrosequencing

Degree of methylation is analyzed as a "C/T SNP" in the software



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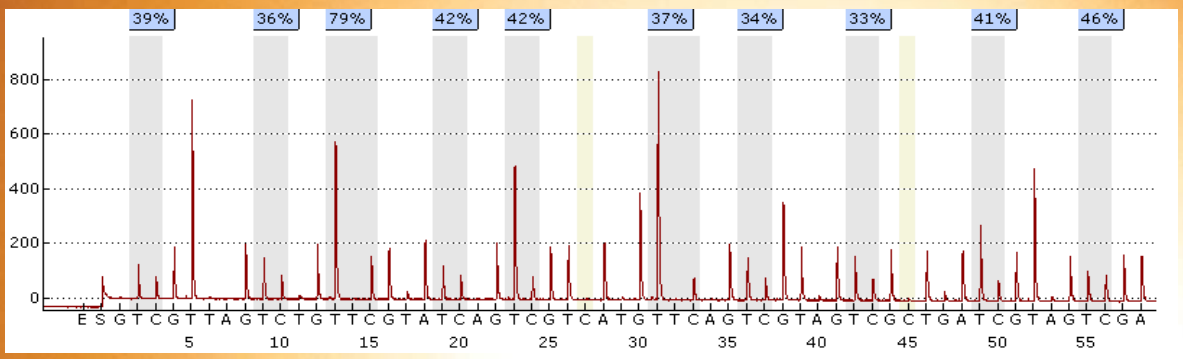
Pyrosequencing assay results

Individual degrees of methylation in adjacent CpG sites are shown in sequence context



The sequence context confirms that the assay worked properly

C/TGTTTTGC/TGTTTC/TGAC/TGTTC/TGTAGGTTTTC/TGC/TGGTGC/TGTA TC/TGTTTGC/TGATTTGG

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Benefits of Pyrosequencing for CpG methylation analysis

- Quantitative analysis of multiple consecutive sites
- Flexible assay design
  - *Forward – reverse/ Upper – lower*
  - *Flexible primer positioning*
- Built-in Bisulfite treatment control
- Excellent Performance
  - *Accuracy*
  - *Precision*
  - *Reproducibility over time*
- Fast results

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