

Abstract

Pyrosequencing is a DNA sequencing technology based on the sequencing-by-synthesis principle that has many applications including rapid genotyping of a broad spectrum of bacteria [1, 2]. Despite the increased use of Next Generation Sequencing for studying microbial diversity, pyrosequencing remains a cost-effective solution for genotyping short DNA stretches within bacterial genomes, hence providing rapid genetic assessment of specified microbial antibiotic resistance mechanisms [3, 4].

In some cases, molecular typing may require to genotype multiple DNA stretches, each carrying a resistance determinant. Several pyrosequencing primers could be used simultaneously, with overlapping primer-specific pyrosequencing signals as main issue.

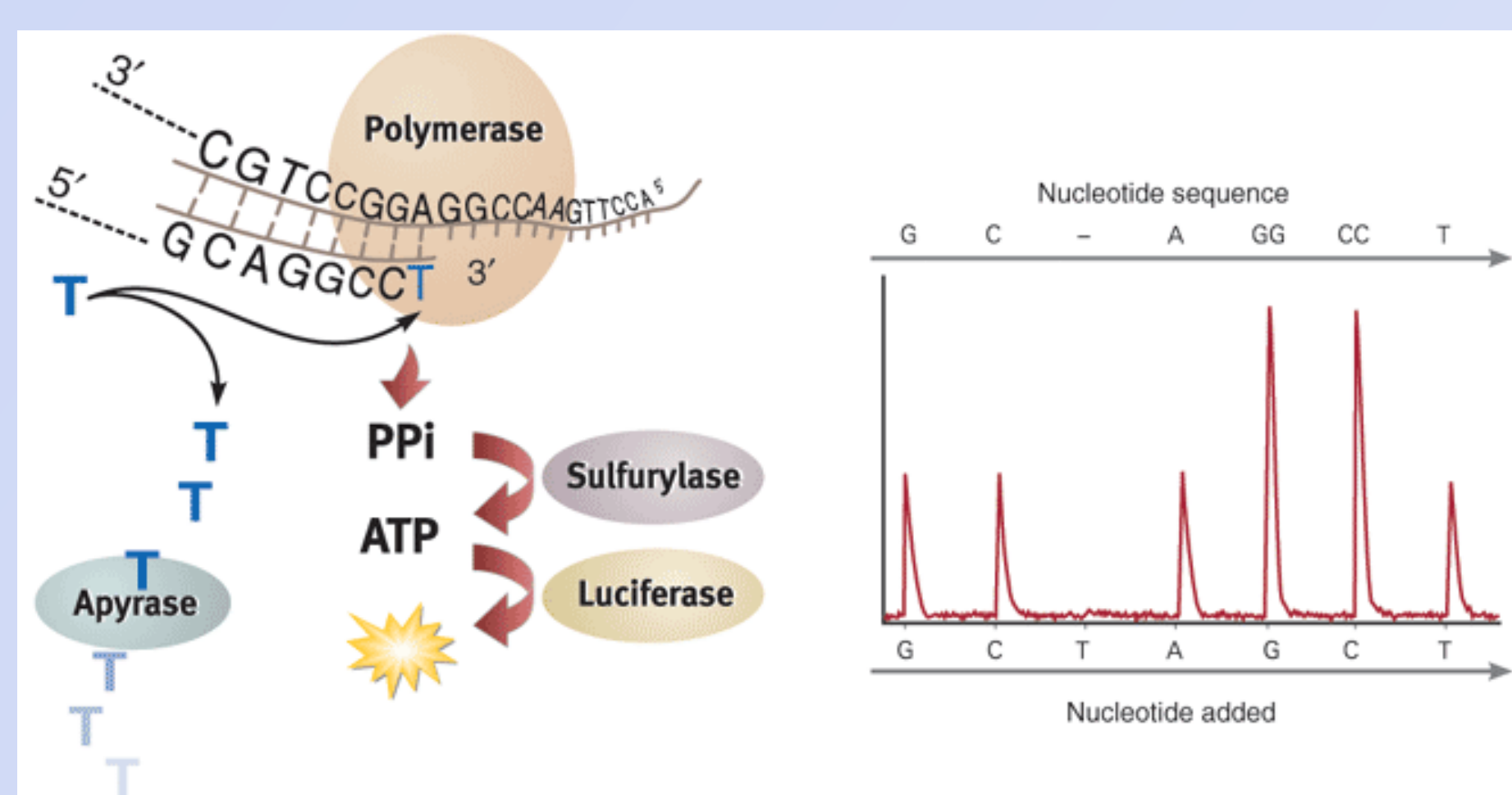


Figure 1: The principle of pyrosequencing.

Source: England et al. Pyro Q-CpGTM: quantitative analysis of methylation in multiple CpG sites by Pyrosequencing, Nature Methods, 2005

Method

Multiplex pyrosequencing Method:

- ♦ 1: Optimal nucleotide dispensation order.
- ♦ 2: Compilation of a pyrosequencing signal dictionary.
- ♦ 3 Application of a new algorithm: AdvISER-PYRO [5].

Bacterial material:

As a proof of concept, this new signal processing method was applied on DNA extracted from 4 confirmed ESBL clinical bacteria (i.e. 3 species: *E. coli*, *K. pneumoniae*, *E. cloacae*) and 4 (3 resistant and 1 sensitive) reference strains (ATCC700603, ATCC35218, DSM22313 and DSM22314). The method was used to generate and analyze quintuplex pyrosequencing signals from the simultaneous pyrosequencing of 11 unique SNPs distributed through 3 and 2 genomic regions of TEM and SHV beta-lactamase resistance genes [6], respectively.

strain	SHV 179	SHV 238	TEM 104	TEM 164	TEM 238
ATCC700603	GCGACA	CTGCCAAGC	TTGAG	ATCGTT	CCGGTG
ATCC35218	GCGACA	CTGCCAAGC	TTGAG	ATCGTT	CCGGTG
DSM22313	GCGACA	CTGCCAAGC	TTGAG	ATCGTT	CCGGTG
DSM22314	GCGACA	CTGCCAAGC	TTGAG	ATCGTT	CCGGTG
R021	GCGACA	CTAGCAAGC	TTGAG	ATCGTT	CCGGTG
MMA55	GCGACA	CTAGCAAGC	TTGAG	ATCGTT	CCGGTG
BS031	GCGACA	CTGCCAAGC	TTGAG	ATCGTT	CCGGTG
BS035	GCGACA	CTGCCAAGC	TTGAG	ATCGTT	CCGGTG

Figure 2: 11 SNPs in 5 Distant genomic regions

Results

A perfect concordance (100%) was found when comparing quintuplex pyrosequencing assay, monoplex pyrosequencing and Sanger sequencing.

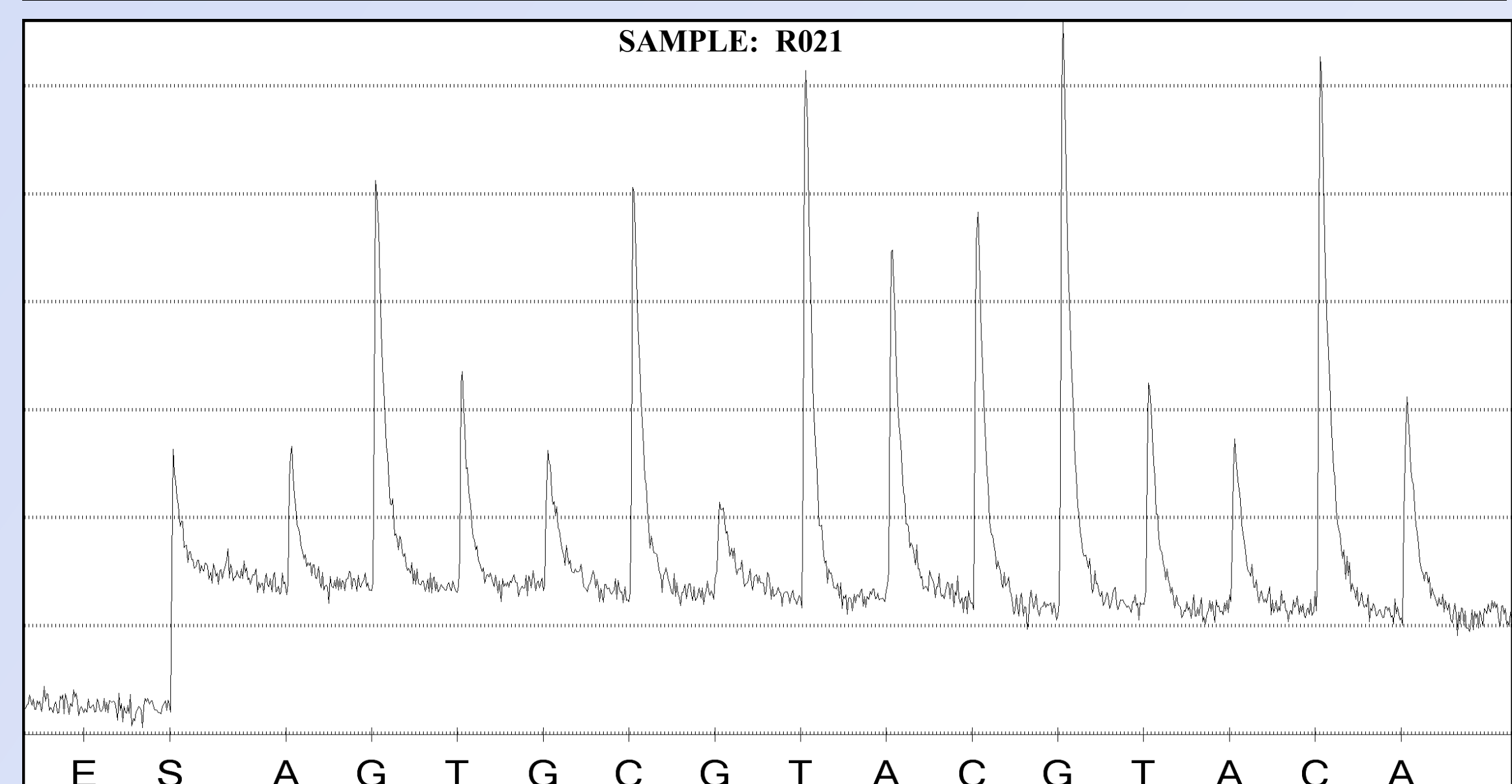


Figure 3: Multiplex pyrosequencing signal

SIGNAL PROCESSING :

$$\sum_{i=1}^n \left(y_i - \sum_{j=1}^p \beta_j x_{ij} \right)^2 + \lambda ||\beta_j||_0$$

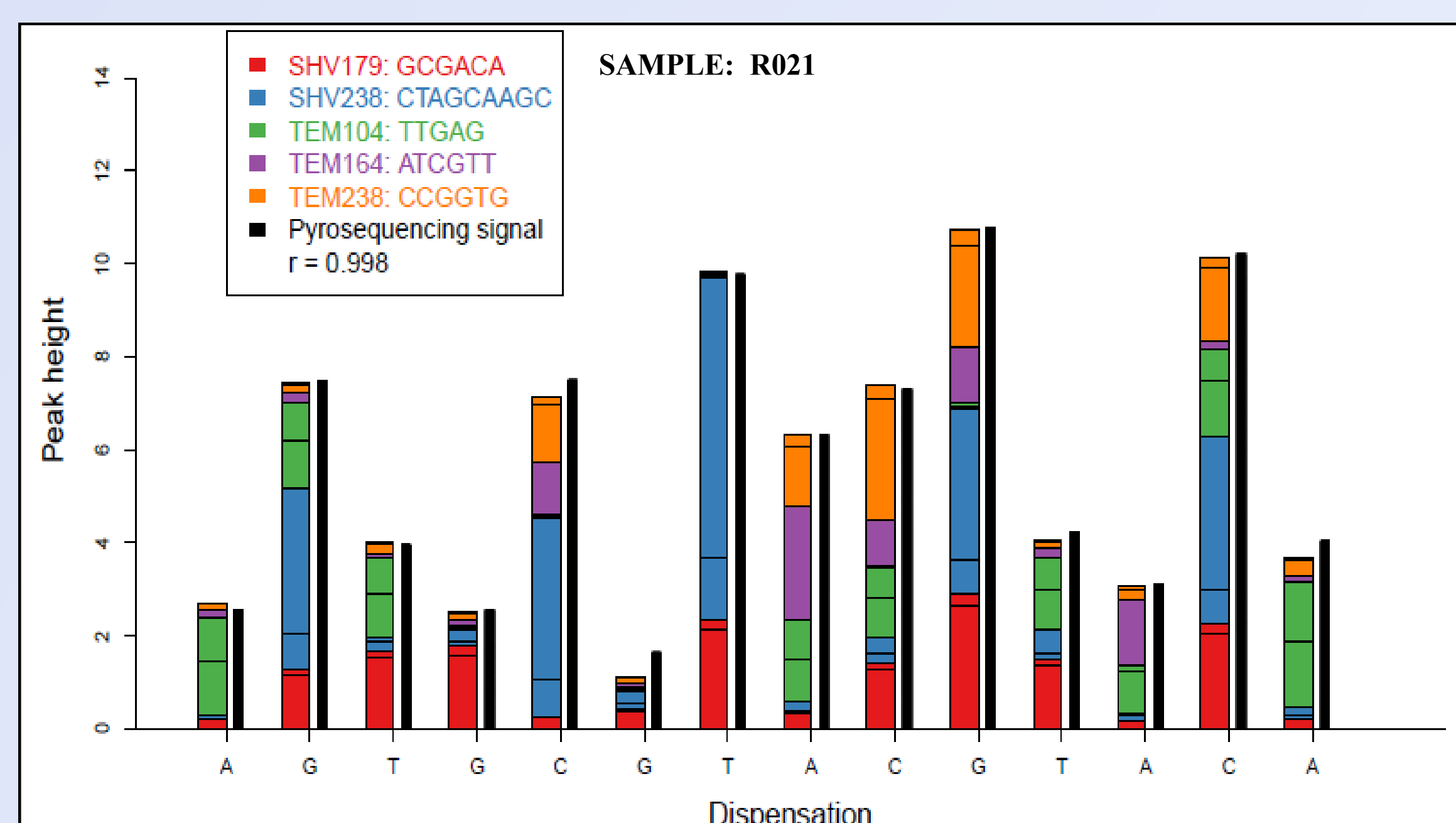


Figure 4: Simultaneous identification of 11 SNPs in 5 distant genomic regions within a single pyrosequencing signal

Conclusions

Quintuplex pyrosequencing enables to lower the global turnaround time of bacterial resistance identification, decreases substantially analytical reagent costs while providing reliable results.

These advantages are highly relevant when assessing clinical infections from natural or criminal origin in field condition, as well as for resistance genotyping of bacterial DNA bank (European Biodefense Laboratory Network, EDA).

References

1. Ronaghi, M., Pyrosequencing sheds light on DNA sequencing. *Genome Res*, 2001. 11(1): p. 3-11.
2. Ronaghi, M. and E. Elahi, Pyrosequencing for microbial typing. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2002. 782(1-2): p. 67-72.
3. Amoako, K.K., et al., Rapid detection and antimicrobial resistance gene profiling of *Yersinia pestis* using pyrosequencing technology. *J Microbiol Methods*, 2012. 90(3): p. 228-34.
4. Deccache, Y., et al., Development of a pyrosequencing assay for rapid assessment of quinolone resistance in *Acinetobacter baumannii* isolates. *J Microbiol Methods*, 2011. 86(1): p. 115-8.
5. Ambroise, J., et al., AdvISER-PYRO: Amplicon Identification using Sparse Representation of PYROsequencing signal. *Bioinformatics*, 2013. 29(16): p. 1963-1969.
6. Cohen Stuart, J., et al., Rapid detection of TEM, SHV and CTX-M extended-spectrum beta-lactamases in *Enterobacteriaceae* using ligation-mediated amplification with microarray analysis. *J Antimicrob Chemother*, 2010. 65(7): p. 1377-81.