

Breast cancer diagnostics: CSCE screening using the BioNumerics® software

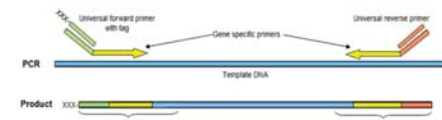
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INTRODUCTION: Conformation sensitive capillary electrophoresis (CSCE) is a sensitive method for mutation scanning (e.g. BRCA1/2 mutation detection). The method, more rapid and cheaper than full gene sequencing, uses electrophoretic mobility differences between homoduplex and heteroduplex DNA. Its successful application with fluorescently labeled DNA in a capillary electrophoresis system has increased the resolution over traditional gel electrophoresis systems and improved the sensitivity. We present a software platform for automated high-throughput mutation analysis based on CSCE on capillary sequencers from Applied Biosystems (AB).



PCR of BRCA1 exon 11



Pooling of PCR products with multiplexing

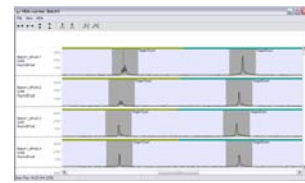
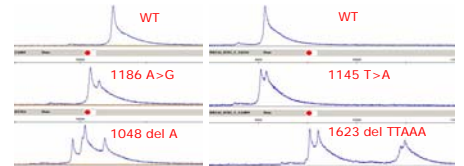
Example of a universal primer panel

- FAM: Fluorescent primers can be used to label any fragment with any colour enabling flexibility in design of multiplex assays.
- T7 promoter: Other utility can be built into tags for specific assays e.g. T7 promoter for MALDI-TOF based mutation screening.
- GC clamps: GC clamps can be used for simple optimisation in techniques such as dHPLC.
- Unlabeled primers for bi-directional/diopoly terminator sequencing.



Separate using capillary sequencer

METHODOLOGY: PCR will result in amplicons of max. 500bp, fluorescently labeled (e.g. FAM, VIC, NED and PET) and possibly multiplexed with a minimum size separation of 40 bp. At the end of the PCR program, a suitable heteroduplexing is performed. PCR products can be pooled and separated by fluorescent labels, size or both. In practice pools consisting of four differently sized fragments for each of the four fluorescent labels, run in up to 96 capillaries, are feasible. Suitable sequencers are the AB 3730(xl), 3100 or 3130, using the 36 or 50 cm arrays with 5% Conformational Analysis Polymer (CAP), using 4M urea and 7.5% sucrose.



ABI sequence files are immediately imported. CSCE Curves window after first search for relevant peaks

DATA PROCESSING: Automatic batch import of AB .FSA files based on a file naming strategy and using the BioNumerics® software, enables high throughput processing. BioNumerics® provides an **adapted database environment** to store all imported data and takes care of all data management activities. The software offers a **proper analysis tool for the mutation detection**: Single peaks are identified automatically as targets, even if multiplexed, using a pre-set area of the trace with various user-adjustable tolerance settings.

Fast, sensitive and reliable **peak matching** is done using a proprietary algorithm that uses five user-adjustable curve parameters, that discriminates 'wild type samples' (mostly a single peak with a sharp leading edge and a somewhat more extended tail) from 'heterozygous mutants' (mostly having a split peak signal or differing by small changes in peak shapes). Per PCR product, polymorphic variants can be defined, or WTs can automatically be traced by the software assuring easy accommodation of the software to any lab specific protocol or target.

Results of automatic peak matching are displayed in a clear overview report with color indication of reference peaks, positive matches, mismatches, failed peaks and problem cases requiring verification: click and zoom functions allow easy on-screen evaluation of match parameters.

Final results are stored and reported. Feedback connection with LIMS, and automatic learning from stored analyses through a statistics function that inventories all average parameters and its limits for each target are possible.

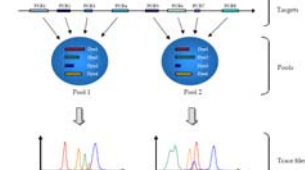
Currently validation of the technology by Eurogentest is being carried out. This validation includes the BioNumerics® software.

Automatic import of batched .FSA files

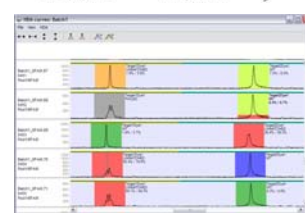
Automatic analysis of WT versus mutant curves

Evaluation report & result approval

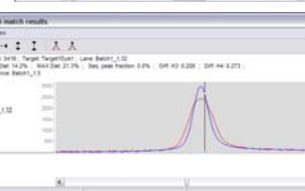
Final report & database construction



Schematic overview illustrating the relation between PCR targets, pools and trace files in a typical CSCE experimental setup. In this example, only one target gene is used per dye in the same pool.



The manual curve parameter calibration window, gives you full control on the search criteria for each target.



Automatic search based on statistics improves performance.

Sample	Summary	Origin	Preparation of Sample	Sample Name	TargetID#1	TargetID#2
#265	Controlled	Greenwood Hospital	2005-06-10	0265.F1	P. Jones	UNMATCHED
#266	Controlled	Greenwood Hospital	2005-06-10	0266.F1	P. Jones	UNMATCHED
#267	Controlled	National Health Lab	2005-05-16	0267	J.H. Hub	UNMATCHED
#268	Controlled	National Health Lab	2005-05-16	0268	J.H. Hub	UNMATCHED
#269	Controlled	National Health Lab	2005-05-21	0269	J.H. Hub	UNMATCHED
#270	Controlled	National Health Lab	2005-05-14	0270	J.H. Hub	UNMATCHED
#281	Controlled	Greenwood Hospital	2005-06-10	0281.F2	P. Jones	UNMATCHED
#282	Controlled	Greenwood Hospital	2005-06-10	0282.F2	P. Jones	UNMATCHED
#283	Controlled	Greenwood Hospital	2005-06-10	0283.F1	P. Jones	UNMATCHED



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