KRAS Mutation Detection by Compact Sequencing Compared with Real-Time Detection PCR and Pyrosequencing in the Routine Clinical Laboratory

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Background

Patients with colorectal cancer (CRC) and *KRAS* mutations do not benefit from treatment with monoclonal antibodies to epidermal growth factor receptor (EGFR) [1-3]. Therefore, *KRAS* mutation detection has been introduced into clinical practice. Several clinical laboratory methods have been established such as direct sequencing, pyrosequencing, real-time detection PCR (RTD-PCR), which show varying detection limits and differing grades of automation [4,5].

Compact sequencing is a primer extension reaction, which uses PCR-amplified DNA and primers immobilized on a solid surface followed by hybridization of extended products in automated fashion (Fig. 1). This method can be applied to *KRAS* mutation detection.

The aim of this study was to validate the performance of the compact sequencing assay (hybcell Onco plexA-1-01) for *KRAS* mutation detection in CRC tissue samples for use in the clinical routine laboratory in comparison with RTD-PCR (TheraScreen® *KRAS* Mutation Kit) and pyrosequencing (Diatech Pharmacogenetics *KRAS* Kit).

Material & Methods

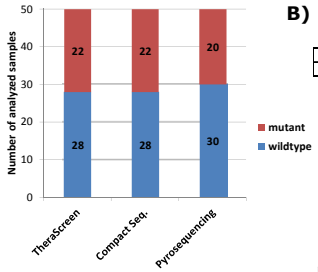
Back-up FFPE samples (50) from CRC patients were used in blinded fashion. DNA was extracted from the samples by using the QIAamp DNA FFPE Tissue Kit (Qiagen). DNA assay in-put was titrated, the detection limit for the G12V mutation was determined and repeatability was tested.

The FFPE samples were assessed by the three assays for the presence of mutations in *KRAS* codons 12 and 13.

All experiments were performed according to the manufacturers' recommendations.

Results

A)

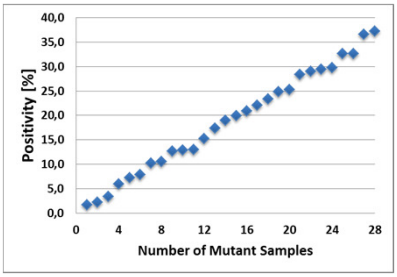


B)

	Compact Sequencing	%	Thera-Screen	ΔCP	Pyro-Sequencing
Sample 1	12 D	2,2	12 D	6,04	wt
Sample 2	12 D	1,7	12 D	5,32	wt

Figure 2: Inter-assay comparison
A) Comparison of 50 samples analyzed with the three different assays. Results were identical except for two mutant samples, which were missed by the pyrosequencing method.
B) Results obtained for two discrepant samples.

Figure 3: Range of mutant DNA detection
28 samples were tested positive for either 12C (Gly12Cys), 12D (Gly12Asp), 12V (Gly12Val) or 13D (Gly13Asp). The obtained positivity of the samples spanned a wide range of mutant tumor DNA ranging from 1,7% to 37,2%.
Detection limit as determined by a cell culture dilution series was found to be at 0,7% for G12V mutant DNA (data not shown).



- **Repeatability:** Wildtype DNA as well as mutant DNA (G12V) were analyzed 10 times each showing identical results, respectively. Mean percentage of mutant DNA samples was 29%±2,7%.
- **DNA input titration:** A dilution series of 1200-600-300-150-75-37,5 ng of input DNA was analyzed. Minimum detectable amount of DNA was 75 ng. 300 ng of genomic DNA was used in routine measurements.

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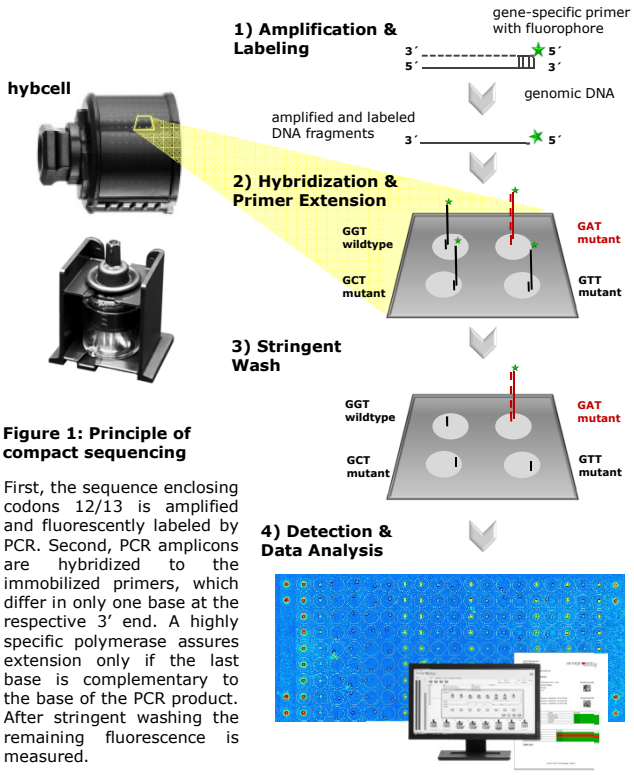

Figure 1: Principle of compact sequencing

First, the sequence enclosing codons 12/13 is amplified and fluorescently labeled by PCR. Second, PCR amplicons are hybridized to the immobilized primers, which differ in only one base at the respective 3' end. A highly specific polymerase assures extension only if the last base is complementary to the base of the PCR product. After stringent washing the remaining fluorescence is measured.

Conclusions

- Results for *KRAS* mutation detection by compact sequencing are in excellent agreement to assays already in clinical use such as the TheraScreen® assay or pyrosequencing assays.
- The detection limit is below 1%.
- Due to the high degree of automation, compact sequencing is a fast and easy assay suitable for daily laboratory use with the little hands on time of 30 min.

Literature

1) Amado, R.G. et al., *J. Clin. Oncol*. 26 (2008): 1626-1634.
2) Karapetis, C.S. et al., New England J. Med. 359 (2008): 1757-1765.
3) Roberts, P.J. et al., *J. Clin. Oncol*. 28 (2010): 4769-77.
4) Weichert, W. et al., *J. Mol. Diagn*. 12 (2010): 35-42.
5) Tsiatis, A.C. et al., J. Mol. Diagn. 12 (2010): 425-432.

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