Optimization of High Resolution Melting Analysis for Detection of KRAS Gene Mutations

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Abstract

Background: Mutations of the KRAS oncogene occur in a variety types of human tumors. By assessing the mutation status of KRAS, clinicians can predict patient response to anti-EGFR therapy such as cetuximab (Erbitux®) or panitumumab (Vectibix®) in patients with metastatic colorectal cancer. The aim of this study was to optimize a real-time PCR method followed by high resolution melting analysis (HRM) in a single step for detection of most common mutations within the KRAS gene. Methods: Seven DNA samples with predefined KRAS mutations and 19 tumor samples from patients with metastatic colorectal cancer were used. KRAS mutation detection was performed by direct sequencing as well as HRM. Optimization was performed using touchdown PCR and co-amplification at lower denaturation-temperature PCR. Results: All DNA samples were successfully analyzed with direct sequencing and HRM. Moreover, the improved amplification efficiency and sensitivity was achieved using optimized PCR run protocol. Conclusion: HRM is a simple, inexpensive and reliable method for mutation detection within KRAS. By applying HRM as prescreening method would help reduce labour, time and costs.

Keywords: Sanger sequencing, COLD-PCR, Touchdown- PCR, cetuximab, panitumumab
Introduction
Cancer, being one of the leading causes of death, claims lives of millions of people worldwide. According to the world health organization 13% of all deaths (7.9 million) were caused by cancer in 2007 and rising number of cancer deaths are thought to continue.\(^1\)

Cancer is a multi-step process, initiating with alterations of DNA that are acquired overtime or are inherited. These alterations include mutations of several important genes that are involved in cell growth, survival and proliferation. Accumulation of genetic mutations leads to progression of normal cells becoming highly dysplastic and finally progress into an invasive cancer.

Treatment of cancer includes resection of tumor, chemotherapy, radiotherapy or targeted therapy.

KRAS, the human homolog to the \textit{Kirsten rat} sarcoma viral oncogene, belongs to a family of RAS genes and encodes for a guanosine triphosphate-binding protein which is an intracellular signal transducer \cite{1-4} and plays an important role in several signaling pathways, one of which is downstream of epidermal growth factor receptor (EGFR), activating the mitogen-activated protein kinase (MAPK) cascade affecting cell growth, proliferation and survival. KRAS is active when bound to guanosine triphosphate (GTP) and becomes inactive when GTP is hydrolyzed (with the help of its intrinsic GTP-ase activity and catalyzing proteins such as GTP-ase activating proteins (GAP) and guanine nucleotide exchange factor (GEF) and replaced with guanosine diphosphate (GDP) \cite{1} (figure 1).

\textbf{Figure 1} The activation and inactivation of KRAS is regulated upon binding of guanosine triphosphate (GTP) and guanosine diphosphate (GDP). These reactions are catalyzed with the help of guanine nucleotide exchange factor (GEF) and GTP-ase activating proteins (GAP).\(^2\)

Mutations in KRAS gene cause substitutions at codon 12 and 13 in exon 2, which account for the majority of them, and less commonly at codon 61 in exon 3 \cite{5,6}. These substitutions result in

\(^1\)http://www.who.int/mediacentre/factsheets/fs297/en/ (2010-08-30)
\(^2\)See reference \cite{7}. Permission granted from Wolters Kluwer Health provided by Copyright Clearance Center
conformational alterations, leading to altered GTP-ase activity that consequently leaves the protein in a constitutively active state, independent of stimulating signals regulated through EGFR [7]. The frequency of mutations in KRAS is relatively high in several different types of human tumors. Pancreatic cancer (58%), cancer of the large intestine (33%), biliary tract (31%) small intestine (20%) and lung cancer (17%) are few among others that top that list.

Colorectal cancer is the second most common type of cancer in men and women. Colorectal cancer initiates with an alteration of the phenotype in the colorectal epithelium that in approximately 90% of cases further develops into adenomatous polyps. These polyps in time become a high-dysplastic adenoma and finally progress into an invasive cancer. Along this path comes the oncogenic mutation of KRAS that with other genetic alterations drives the progression into cancer and metastasis.

Monoclonal antibodies targeting EGFR such as cetuximab (Erbitux®) and panitumumab (Vectibix®) have been developed and are utilized in combination with chemotherapy or as monotherapy to treat patients with metastatic colorectal cancer [2,7]. Binding of these monoclonal antibodies to the extracellular domains of EGFR inhibit its downstream signaling, thus tumor growth and progression are inhibited. However, when EGFR signaling is dysregulated due to mutations in KRAS, there is no effect of these antibodies. An illustration of this is shown in figure 2. Benefits of these EGFR-targeted therapies have been shown to be restricted to patients with colorectal tumors not harboring any mutations in KRAS (wild-type-type KRAS) [4,7]. Hence testing for mutations within KRAS has become extensively important in patients with metastatic colorectal cancer to guide treatment, as activating mutations in KRAS are associated with poor prognosis and no response to EGFR-targeted therapy [1,3,7].

3 http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=bygene&ln=KRAS&start=1&end=189&coords=AA:A (2010-08-30)
Figure 2 The signaling pathway of EGFR (epidermal growth factor receptor) (A). Anti-EGFR has its function trough inhibition of signals downstream EGFR, effecting cell growth, survival and proliferation (B). Mutations within KRAS gene cause the KRAS protein to become constrictively active (indicated red), independent of signals trough EGFR, thus excessive cell growth and proliferation is not inhibited by anti-EGFR (C)

To accurately assess the mutational status of KRAS requires assays that can provide specificity and high sensitivity. One most commonly used method is the Sanger direct sequencing [3-5]. The main advantage of it is that it allows you to detect any existing mutations in the DNA sequence but it lacks in sensitivity. Another method, frequently used in clinical laboratories, is the allele specific amplification using TheraScreen DxS KRAS-muation kit (QIAGEN, Hilden, Germany). It is based on a technique that combines PCR ARMS (amplification refractory mutation system) and Scorpions to detect the 7 most common mutations in codon 12 and 13 within KRAS. Nonetheless, the disadvantage of this kit is that it misses those mutations that are less frequent.

A more recently developed method, High resolution melting (HRM) analysis has gained a lot of attention. HRM analysis is a post-amplification method and is based on polymerase chain reaction (PCR) melting curve technique. In HRM analysis the melting property of DNA is used to detect any existing alterations or variation in the DNA sequence. Prior to HRM the sequence of interest is amplified using real-time PCR (rt-PCR) in the presence of a fluorescent dye that

binds specifically to double-stranded (ds) DNA. The amplicons are then melted through heating at a very slow rate. When the temperature is low the DNA form duplexes which emit strongly. As the temperature is raised and the melting temperature of the amplicons is reached, the DNA duplexes melt to single-stranded (ss) DNA consequently fading in fluorescence emission. The fluorescence signals is then plotted against the temperature, generating a melting curve that is characteristic to each different DNA sequence, thus, variation in the DNA sequence can easily be detected. Since HRM provides no identity to the mutations detected, it is required that samples are further analyzed by direct sequencing for confirmation.

The demand of PCR-based methodologies that provide higher resolution and enrichment of minority alleles has led to development of methods such as touchdown and COLD-PCR (co-amplification at lower denaturation-temperature PCR). Touchdown PCR is defined by applying varying annealing temperature to the PCR cycles. At the early stage of amplification the annealing temperature is set above the projected optimal $T_m$ (melting temperature) of the primers used and decreased with, in this case, 0.5°C for each subsequent cycle until it reaches a temperature lower (touchdown) than the $T_m$ of the primers where the remaining amplification steps are carried out. This will help to produce more specific amplicons that will later during amplification serve as templates, thus, generating more specific product at final [8].

COLD-PCR is based on the idea of enriching target allele in mixtures of abundant wild-type allele by using the critical denaturation temperature ($T_c$) of the target sequence. During PCR cycling the annealing temperature set to a level where a cross-hybridization between mutant and wild-type allele is allowed, forming homo-heteroduplexes. These duplexes are less stable and require lower temperature in order to denature. This is especially utilized in COLD-PCR. The amplification cycling is initiated with high denaturation temperature and slowly decreased to a level, permitting the homo-heteroduplexes to be denatured and further amplified where the homoduplexes are not denatured, hence not amplified efficiently. This would result in greater amplification of target mutant allele [9].

The aim of this study was to optimize and evaluate HRM as prescreening method for KRAS mutations in clinical samples. We used different designs in terms of selection of suitable primers and PCR condition templates to favor specific amplification of target DNA and accurate melting
profiles generated by HRM. We experimented with different types of PCR, namely touchdown and COLD-PCR.

**Materials and methods**

**Materials**

Materials used for the purpose of this study comprised of 7 positive controls, consisting of DNA samples earlier confirmed positive for following mutations: Gly12Asp, Gly12Ala, Gly12Val, Gly12Ser, Gly12Arg, Gly12Cys, and Gly13Asp. A DNA sample from healthy unaffected tissue with wild-type-type KRAS was used as standard control. In addition, 19 incoming tumor samples from patients with metastatic colorectal cancer were also genotyped with both HRM and direct sequencing. These were collected as clinicians requested KRAS analysis to predict response to anti-EGFR therapy. Patient samples used for detection of KRAS mutations consisted of formalin-fixed paraffin-embedded (FFPE) tissue blocks. All samples were registered at arrival and were coded with laboratory sample identification.

Evaluation and validation of an analytical method for clinical use is done as a part of the quality work in a clinical laboratory. Since this study was by definition not a research study, ethics approval was not acquired and therefore not obtained. Yet, patient samples were handled anonymously where clinical parameters as well as patient identification could not be linked to the DNA samples.

Before DNA extraction a hematoxylin eosin (H&E) stained section of each tissue block was reviewed by a pathologist in order to assess the amount tumor cells in relation to non-neoplastic, inflammatory and necrotic cells based on morphological characteristics on H&E-stained slide. Only paraffin-embedded tissues containing at least 25% tumor cells were considered eligible for downstream applications.

Once the paraffin blocks were reviewed they were cut in three 4µm thick serial sections of which the last one was stained with hematoxylin eosin (H&E) for an estimation of the amount of tumor cells remaining in the paraffin block. This helps to further check that representative amount of tumor cells are present in the collected sections.

The first two sections were collected in a 1.5 mL tube and deparaffinized through a short incubation in 1 mL xylene, vortexing and centrifugation at maximum speed (20 000xg) for 1 min
in which the supernatant was removed by pipetting. The sections were then washed with 1 mL ethanol (99.5%), vortexed shortly and centrifuged at maximum speed for 1 min, and the supernatant was removed. The samples were then dehydrated using Thermo Savant Speedvac DNA110 system (Thermo Scientific, Waltham, MA, USA). In cases of poor tumor tissue available relative to surrounding non neoplastic tissue, manual macro-dissection was performed to enrich tumor cell content. For manual macro-dissection the paraffin blocks were sectioned as described above only this time the sections were left to dry on the slide. The area with tumor tissue were separated from the non-tumor area using a scalpel and collected in 1.5 mL tube. In order to locate the exact position of tumor cells, an H&E slide had to be available with the tumor section correctly marked by a pathologist.

Genomic DNA extraction and quantification
Extraction of genomic DNA was performed using QIAamp DNA FFPE tissue kit (QIAGEN, Hilden, Germany) in accordance with protocol provided by the manufacturer. The deparaffinized tissue was digested with proteinase K (QIAGEN) in presence of digestion buffer ATL (QIAGEN) and incubated at 56°C overnight on a thermoshaker to ensure complete lysis of tissue. The incubation step was followed by heating the sample at 90°C for 1 h allowing proper digestion of the remaining unlyzed tissue and inactivation of proteinase K. DNA was then isolated using QIAamp MinElute column (QIAGEN). DNA yield and purity was checked with a NanoDrop spectrophotometer (ND-1000, Thermo Scientific, Waltham, MA, USA). The extracted genomic DNA was then stored at -20°C until further mutation analysis with HRM and Sanger sequencing.

High resolution melting analysis
Real-time PCR with high resolution melting analysis (HRM) was performed using LightCycler 480 II platform (Roche Diagnostics GmbH, Mannheim, Germany). All generated HRM data was analyzed using the supplied Gene Scanning Software (Version 1.5.0). A total of 20 µL reaction mixture was prepared in duplicate for each sample, composed of a 1X HRM master mix (QIAGEN), 0.7 µM primer mix and approximately 30 ng genomic DNA. A duplicate of non template control (NTC) was also included for each run to detect potential contamination. All reactions were performed on a 96-well plate.
Primers used, flanking the significant mutations at codon 12 and 13 on exon 2, were designed using the online version of Primer3 software (v. 0.4.0)\(^5\) producing amplicons 90bp of size. The different PCR templates that were experimented with are listed in table 1.

### Table 1 The different PCR conditions that were used.

<table>
<thead>
<tr>
<th>PCR template</th>
<th>cycling conditions</th>
<th>45 cycles</th>
<th>95°C</th>
<th>5 min</th>
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<tbody>
<tr>
<td><strong>Standard</strong></td>
<td>activation</td>
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<td></td>
<td>denaturation</td>
<td></td>
<td>95°C</td>
<td>15 s</td>
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<tr>
<td></td>
<td>annealing &amp; extension</td>
<td></td>
<td>60°C</td>
<td>1 min</td>
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<tr>
<td></td>
<td>HRM</td>
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<td>75-95°C</td>
<td>0.02°C/sec 25 acquisition/°C</td>
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<td><strong>Touchdown PCR</strong></td>
<td>activation</td>
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<td>5 min</td>
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<td></td>
<td>denaturation</td>
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<td>95°C</td>
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<tr>
<td></td>
<td>annealing &amp; extension</td>
<td></td>
<td>65-60°C</td>
<td>1 min 0.5°C/cycle step</td>
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<tr>
<td></td>
<td>hold</td>
<td></td>
<td>65°C</td>
<td>5 min</td>
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<tr>
<td></td>
<td>HRM</td>
<td></td>
<td>65-90°C</td>
<td>0.02°C/sec 25 acquisition/C</td>
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<tr>
<td><strong>COLD-PCR</strong></td>
<td>activation</td>
<td></td>
<td>95°C</td>
<td>5 min</td>
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<td></td>
<td>denaturation</td>
<td></td>
<td>95°C-80</td>
<td>5 s 0.5C/cycle step</td>
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<tr>
<td></td>
<td>annealing &amp; extension</td>
<td></td>
<td>63°C</td>
<td>45 s</td>
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<td></td>
<td>hold</td>
<td></td>
<td>63°C</td>
<td>4 min</td>
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<td></td>
<td>HRM</td>
<td></td>
<td>63-85°C</td>
<td>0.02°C/sec 25 acquisition/°C</td>
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<tr>
<td><strong>Final</strong></td>
<td>activation</td>
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<td>95°C</td>
<td>5 min</td>
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<td>denaturation</td>
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<td>annealing &amp; extension</td>
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<td>63-85°C</td>
<td>0.02°C/sec 25 acquisition/°C</td>
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Abbreviations: PCR - polymerase chain reaction. COLD - co-amplification at lower denaturation temperature. HRM - high resolution melting.

**Direct Sanger Sequencing**

DNA samples that were going to be sequenced were first amplified using the conventional PCR (ABI 9700, Applied Biosystems, Foster city, CA, USA). A duplicate total of 50 μL reaction mixture was prepared for each sample comprising of 1x PCR buffer (Applied Biosystems), 0.2 μM dNTP mix, 0.3 μM of each forward and reverse primer, 2 units of ampliTaq Gold (Applied

\(^5\) [http://frodo.wi.mit.edu/primer3/](http://frodo.wi.mit.edu/primer3/), (2010-08-30)
Biosystems), 30 ng genomic DNA. Primers used were designed flanking the significant mutations at codon 12 and 13 on exon 2, producing an amplicon size of 225bp. The cycling conditions consisted of an initial incubation for 10 min at 95°C for enzyme activation and denaturation of DNA, followed by 40 cycles of denaturation at 95°C, annealing at 60°C and extension at 72°C 30 s per step. One cycle of a final extension was performed at 72°C for 7 min. The PCR-product was purified using ChargeSwitch PCR Clean-up Kit (Invitrogen, CA, USA) and collected in 1.5 mL tubes and stored in the 4°C until use.

Using the same set of primers, the PCR products were sequenced in the ABI 9700 thermal cycler (Applied Biosystems). The reaction mixture consisted of buffermix (BigDye sequencing kit, Applied Biosystems), premix (BigDye sequencing kit, Applied Biosystems), 3 µL PCR product and 0.3 µL primer mix. Sequencing conditions were set as follows; 1 cycle enzyme activation at 96°C for 5 min, 25 cycles of denaturation at 96°C for 10 s, annealing at 50°C for 5 s and extension at 60°C for 2 min and 30 s.

After Sanger sequencing the sequenced DNA was precipitated as follows; addition of 5 µL 125mM EDTA and 60 µL 99.5% ethanol. The mixture was transferred to 1.5 mL tubes and centrifuged at 11000xg for 20 min and the supernatant was removed. The DNA was then washed in 300 µL of 75% ethanol, centrifuged at 11000xg for 10 min and the supernatant was removed. Samples were dried using the Thermo Savant Speedvac DNA120 system (Thermo Scientific) at high rate for 3 min. Fifty microliter of formamide was added to the tubes and they were kept in dark for approximately 1 h. Twenty microliter of each sample was transferred to a 96-well plate and run in ABI 3130xl genetic analyzer (Applied Biosystems). The electropherogram was reviewed in the mutation surveyor software (Soft Genetics, State College, PA, USA).

**Results**

Patient samples admitted to molecular testing for KRAS status consisted of colon tumor (n=11), rectal tumor (n=5), liver metastasis (n=2) and sigmoid tumor (n=1).

An adequate amount of DNA was obtained from all the patient samples and KRAS mutation analysis was successfully performed by direct sequencing.
In the present study, an overall of 16% (3/19) of the patient samples were tested positive for mutation in KRAS of codon 12 and 13 by direct sequencing. All the patient samples were reanalyzed by HRM analysis with the positive controls using the different PCR templates. The results were compared with those of direct sequencing.

*High resolution melting analysis*

The standard PCR template and HRM setup generated false positive results. The melting profiles generated with this template are shown in figure 3. The red colored curves are indicating samples with mutant KRAS and blue curves are indicating samples with wild-type KRAS. The melting curves indicated an abundant formation of homoduplex of both wild type and mutant allele which is shown by their typical shape.

**Figure 3** This figure shows the melting curves for the samples (7 positive controls, 3 patient samples and sample with wild-type type) run with standard PCR and HRM template. The fluorescence signals are plotted against the temperature. The melt curve patterns indicate that there was formation of homoduplex of both mutant and wild-type alleles. Red colored curves are indication of positive results for KRAS mutation and blue colored patterns are indication of samples with wild-type KRAS.
The varying annealing temperatures set up for the touchdown template generated improved results. The negative samples were correctly grouped as wild-type and the melting curves for the samples with true mutation were readily distinguished from those of wild-type type. When homo-heteroduplex is formed between the wild-type and mutant DNA template, it produces a duplex that is less stable than the homoduplex. They are more readily denatured when temperature is raised. Thus melting curve for the homo-heteroduplexes appears earlier during temperature raise than for those of homoduplex. This is shown in figure 4.

![Normalized and Shifted Melting Curves](image)

**Figure 4** Melting curves for samples run with the touchdown template. Curves for the positive samples are displaced from the blue ones (wild-type), indicating formation of homo-heteroduplex between wild-type and mutant DNA template.

COLD PCR with gradually decreasing denaturation temperature down to 80°C did not result in successful amplification of target DNA sequence (data not shown). The fluorescence signals during amplification were very low and very late, generating a plot that was not fully interpretable.

In the final template we were able to set the PCR and HRM conditions up to a working one. Melting curves generated with the final template were correctly shaped for samples with wild-type and mutant DNA template respectively. Applying an optimal annealing temperature of 63°C
during the whole PCR run followed by melting curve produced the best resolution to distinguish between the wild-type and mutated samples.

![Melting curves for the five patient samples run with the final PCR and HRM template. One of the samples was tested positive for mutation in KRAS by direct sequencing which is readily revealed here.](image)

**Figure 5** Melting curves for the five patient samples run with the final PCR and HRM template. One of the samples was tested positive for mutation in KRAS by direct sequencing which is readily revealed here.

Patient samples earlier tested with the different PCR and HRM templates were reanalyzed using the final template (table 1). Two of the samples that were tested negative for mutations in KRAS by direct sequencing, showed positive by HRM study. They were later run with the TheraScreen Dxs kit and confirmed positive for Gly12Ser (GGT>AGT) and Gly12Arg (GGT>CGT) mutations.

**Discussion**
Tumor samples submitted to molecular testing in clinical settings are many times comprised of specimens with heterogeneous cellular content were the amount of tumor cells are limited in relation to the surrounding apoptotic, inflammatory and non-neoplastic cells. Methods used for
testing of samples of such nature require a sensitivity that can handle a low number of mutant templates to be detected.

Direct sequencing, one of the most common methods used in detection of mutations in KRAS, is advantageous in detecting mutation when there is one. However, its low sensitivity may not make it the best method of choice, as it requires at least 25% mutant allele to be present in background of wild-type allele [10]. Analyzing samples that are based on tissues that are preserved in formaldehyde and embedded in paraffin blocks are less suitable for sequencing, as these conditions result in more degraded DNA. Using direct sequencing that requires longer DNA templates is another aspect of this method not being the most appropriate one. The sensitivity of the real-time PCR kit, TheraScreen KRAS mutation kit, has been demonstrated to be much higher than that obtained from direct sequencing. Limitations of this kit include the inability to detect mutations other than those targeted by the designed primers, thus less commonly accruing mutations in KRAS would not be detected in this case and would result in false negativity [10]. Another disadvantage is the high cost of it.

Starting off with the standard template, we could see that the primers that were designed were amplifying the target sequence. However, the HRM melting profiles that were gained for many of the samples were spurious. The main reason could be due to the relatively low annealing temperature set during amplification. As primers tend to bind less specifically when annealing is set at low temperature it could generate bi-products. Thus, low annealing temperature could also have contributed to the inhibition or minimum permission of mutant and wild-type allele to hybridize and form homo-heteroduplexes.

To achieve the highest possible annealing temperature, touchdown PCR was performed. Touchdown PCR is generally applied to amplify a broad range of primer sets with different annealing temperatures. However, through a touchdown protocol one can obtain the optimal annealing for primer pairs in a single reaction. Our results that were obtained using the touchdown template were thus improved. From the results that were gained, we were able to derive that the optimal annealing temperature for the primers to produce more specific amplicons
was at about 63°C. In addition, a final-extension step may have helped in the generation of more complete amplicons for further melt curve analysis.

Recently, Li et al. [9] presented a study in which they were able to modify a standard PCR by reducing the denaturating temperature to enrich TP53 mutation alleles in a wild-type allele background. The results were promising. Another study presented by Zuo et al. [11], where COLD-PCR were applied in detection of mutations within KRAS in both fresh and FPPE tumor tissue. The results confirmed that COLD-PCR provides increased sensitivity for mutation detection in specimens with low neoplastic cell content. Our primary attempt to perform a COLD-PCR template did not allow enough products to be formed. This may have been caused either too low denaturating temperature or the minimum time required during this step. Theoretically when homo-heteroduplexes are formed they require lower denaturation temperature due to their instable nature in comparison to homoduplexes. Thus when denaturation temperature was slowly lowered we expected a more specific amplification of target template. The main reason for failure may have been the reduced denaturation temperature was set to 80°C which is much below what Li et al. recommended.

HRM, a newly presented method, has been shown to be a promising approach in quality and cost-effective assessment of mutation detection in clinical settings. HRM has been applied to detect somatic mutations within EGFR and KRAS gene in non-small-cell lung cancer. It was shown that screening of samples for mutations prior to sequencing resulted in time and costs savings [5]. In a similar study, the ability of HRM to detect mutations at codon 12 and 13 within KRAS was assessed using cell line and non-small-cell lung cancer specimens. The findings showed sensitivity level of 5-6%, especially when shorter amplicons are used [12]. Other than KRAS, HRM has been applied to detect mutations within BRAF and PIK3CA in colorectal cancer which reproduced similar results [13].

Reviewing patient samples admitted for KRAS status-testing during the previous years (2008-2009) in our laboratory, showed that KRAS mutation was confirmed in 38-40% in all the samples. If HRM had been used to scan all the samples for KRAS mutation, it would have led to time and cost savings for approximately 60% of them. Adding the fact that correct and accurate detection of mutation in KRAS is essential, necessitates the use of efficient and reliable assays.
Activating mutations in KRAS are associated with poor prognosis and overall survival. Patients suffering of metastatic colorectal cancer treated with anti-EGFR such as cetuximab have shown improved overall survival [14]. However, these benefits has shown to be restricted to tumors with wild-type KRAS, hence the great importance in stratifying patients that would benefit from treatment and those who would not and might suffer severe side-effects.

In this study we were able to construct a real-time PCR and HRM template that generated more specific, accurate reproducible results. This approach will save turn-around time in which every oncologist will receive a faster result from molecular pathology laboratory. In addition, the cost-benefit advantage of KRAS screening will have a positive impact on society. Further research with larger material is however required to validate the sensitivity and robustness of HRM. Serial dilutions with known mutation content will help to determine sensitivity of HRM. Since new mutations such as BRAF and PIK13CA will also affect decision on tailored therapy, the HRM method will be an attractive platform for multiplex mutation detection.

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References


