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# Development of a Comprehensive Mutational Panel as an Effective Tool for Personalized Diagnostic of Medullary Thyroid Carcinomas

# Abstract

Medullary Thyroid Carcinoma (MTC) originates from mutations in Calcitonin-Producing Parafollicular C cells of the thyroid, is a rare malignancy, accounting for 3-4% of all thyroid carcinomas. It occurs in a hereditary form (HMTC, 25%) or in a sporadic form (SMTC, 75%). The prognosis for patients with MTC is poor, as the tumor metastasizes at early stages; and the only curative therapeutic option so far is radical surgery. Genetic analysis helps identify inherited cases at a stage where prophylactic surgery can be offered to carriers of such mutations to prevent the disease. This approach may also be used to determine better treatment options for patients who are already diagnosed with MTC.

The goal of this project was to develop the most comprehensive mutational panel for the detection of clinically relevant mutations in clinical MTC samples. A total of 143 mutations in 8 human genes were selected from numerous papers and public databases and included into the MTC mutational panel. The assay design was carried out using Sequenom's online design tools. The final file comprised from 115 assays corresponding to 143 mutations included in the MTC panel will be further processed using the SEQUENOM® Mass-ARRAY iPLEX® platform for DNA genotyping of clinical samples by the cancer research scientists at the Abramson Cancer Center of the University of Pennsylvania.

# Introduction

Medullary Thyroid Carcinoma (MTC) that originates from calcitonin-producing parafollicular cells of the thyroid gland is a rare malignancy accounting for 3-4% of all thyroid carcinomas. It was first characterized by John B. Hazard in his paper entitled "Medullary (solid) carcinoma of the thyroid—a clinicopathologic entity" (Hazard et al. 1959). The prognosis for patients with MTC is very poor as the tumor metastasizes at early, and since the tumor's average age of onset is  $21 \pm 6$ , the only realistic curative therapeutic option so far is radical surgery (Alvandi et al. 2011).

MTC occurs in two forms: hereditary (HMTC in 25% of all cases) and sporadic (SMTC in 75% of all cases) (Jimenz et al. 2008). In 1961, John H. Sipple described the association between MTC and pheochromocytoma, an association that's known as multiple endocrine neoplasia type 2 (MEN 2) syndrome (Jimenz et al. 2008). There are two sub-types of MEN 2 syndrome, MEN 2A that is found in 20-50% of HMTC cases and only in 5-20% of HMTC cases when together with hyperparathyroidism. MEN 2B subtype is much more aggressive then MEN 2A and occurs in 50% of cases alongside marfanoid habitus and with mucosal and digestive neurofibromatosis (OMIM 162300). Familial MTC (FMTC) is not associated with any of the MEN syndromes, and it is least aggressive of the three HMTC (OMIM 155240). Genetic analysis has helped identify inherited cases at a stage where prophylactic surgery can be offered to carriers to prevent the disease. Activating mutations of the *RET* proto-oncogene are associated with the pathogenesis of MTC and have been demonstrated in nearly all hereditary and in 30-50% of SMTC cases (Cakir et al. 2009). Only 60% of SMTC cases were successfully attested

to mutations, and the rest remain unclassified. In the same time the SMTC is equally aggressive as its HMTC version.

Since gene mutations are the main suspect of cause of most types of cancer, mutational profiling of clinical tumor samples becomes very important as a guide for tumor classification, potential prediction of the patient outcomes and treatment options (). Matrix-Assisted Laser Desorption/Ionization – Time of Flight (MALDI-TOF) mass spectrometry (MS) of DNA is in broad use for targeted single nucleotide polymorphism (SNP) and somatic mutations genotyping studies. Detection of somatic mutations requires a higher level of sensitivity than most standard SNP genotyping methods. Whereas germline and other genetic mutations are simply classified as heterozygote or homozygote alleles, somatic mutations, due to the fact that they are present in a portion of cells (typically in tumor cells surrounded by normal cells) require quantitative mutation frequency assessment.

MALDI-TOF MS is used as the base for the commercially available Sequenom MassARRAY platform for mutations genotyping. The Sequenom approach can detect mutations even if they are present only in 5% of the cell population and can give quantitative information on each mutation (<u>www.sequenom.com</u>).

The multiplex reaction (iPLEX) assay in this method is a single base primer extension assay. First, PCR amplifying fragments of about 100 base pairs (bp) with primers flanking the mutation is conducted in a multiplex reaction for several products. Next, extension primers designed immediately adjacent to the mutation site prompt extension by one nucleotide depending on the template sequence. The difference in mass between extended products allows distinction of wild-type and mutant alleles (Gabriel S, et al, 2009; Millis M. 2011) (Figure 1).

The prime objective of this project was to develop a most comprehensive mutational panel to date for the detection of clinically relevant mutations in MTC samples. To this end a total of 143 mutations in 8 human genes were selected from numerous peer-reviewed publications as well as from the available public databases to be included into the MTC mutational panel. The selection criteria were based on the coding mutations (mutations that occur in the coding area of the genes) that were reported to occur in MTC patients and considered functionally relevant. Some of the mutations included into the MTC mutational panel (such as *BRAF* gene V600E mutation) were also described in other types of thyroid cancer, but most of the mutations were unique to MTC (such as all *RET* gene mutations). To this end a Mutational Assay Panel was designed for MALDI-TOF MS genotyping encompassing the most significant genes in this disease: total of 143 mutations in RET, BRAF, KRAS, HRAS, SDHB, SDHD, VHL and CDKN1B organized in the file consisting of 115 assays. The assay design was carried out using Sequenom's online design PreXTEND tools (ProxSNP and (https://www.mysequenom.com/Tools) and Assay Design software (v. 3.1)).

The developed assay file is fully compatible with the SEQUENOM® Mass-ARRAY iPLEX® software and will be further used for DNA genotyping of clinical tumor samples.

**FIGURE 1**. A representative case of MALDI-TOF based genotyping. The Figure shows the MassARRAY spectrum for a NRAS mutation (c. 182 A>G) for which there is either a

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wild-type allele (A) or mutation (G). This figure has been transcribed from the publication by Ricarte-Filho et al. 2009.



# **Materials and Methods**

The list of mutations for the MTC mutational panel was generated based on results of annotation of scientific publications (source: PubMed database

(http://www.ncbi.nlm.nih.gov/pubmed/)) and screening of public biological databases

including the Catalogue Of Somatic Mutations In Cancer (COSMIC) Database

(http://www.sanger.ac.uk/genetics/CGP/cosmic/), University of Utah MEN2 Database

(<u>http://arup.utah.edu/database/MEN2/MEN2\_display.php</u>) and National Cancer Institute Database

(http://www.cancer.gov/cancertopics/pdq/genetics/medullarythyroid/HealthProfessional/Table4).

Human DNA sequences that flanking mutations selected were retrieved from COSMIC

database, UCSC Genome Browser (http://genome.ucsc.edu/) and formatted with

DNASTAR Lasergene software (v. 9). The assay design was carried out using

Sequenom's online design tools (ProxSNP and PreXTEND

(https://www.mysequenom.com/Tools) and Assay Design software (v. 3.1)).

### **Results and Discussion**

#### Data Collection

As a first step, the list of genes mutations described in MTC was prepared. To date, 98% of mutations found in hereditary MTC belong to *RET* (Rearranged During Transfection) gene. *RET* proto-oncogene encodes one of the receptor tyrosine kinases, cell-surface molecules that transduce signals for cell growth and differentiation (OMIM 164761). RET mutations differ in the aggressiveness of the MTC (Abraham11\_6–8), for example, RET mutations at amino acid position 918 and 883 are considered to be responsible for the most aggressive types of MTC (Cakir et al. 2009). They are found in over 95% of the MEN 2B cases and in most of the RET mutations of SMTC. (http://emedicine.medscape.com/article/1744824-overview). Overall, the mutation in position 918 is found in over 50% of the classified MTC cases. RET mutations in amino acid positions 609, 611, 618, 623, 630 and 634

((<u>http://emedicine.medscape.com/article/1744824-overview</u>) are responsible for over 90% of MEN 2A and FMTC cases. Two percent of HMTC remain unclassified, with outlying mutations in genes such as Succinate Dehydrogenase (*SDHB* and *SDHD*), being found. Their contribution to MTC pathogenesis remains unknown.

SMTC is much more ambiguous then its hereditary counterpart, as only 40-60% of known SMTC cases can be attested to mutations. While being found in almost all known cases of HMTC, *RET* gene is found in only 20-40%

(http://emedicine.medscape.com/article/1744824-overview) of SMTC. Mutations in genes such as *BRAF*, *KRAS*, *VHL*, *HRAS* and *CDKN1B* have also been found in SMTC, but altogether they only amount to 10-20% of the known cases of SMTC.

The selection criteria for the Mutational Panel were based on coding mutations that were reported to occur in MTC and are considered to be functionally relevant. The genes examined are represented in Table 1, and the complete list of mutations that was included in the panel is available in Table 2. The mutations were collected from Biological databases as well as a wide range of online publications found through Public Medical Database (PubMed). A grand total of 143 mutations that occur in MTC were included in the Mutational Panel.

Next, fragments of gene sequences (typically 200-250 bp in length) containing mutations selected were collected in the Excel file. To specify a mutation in the DNA sequence the following format was used:

1) For a single nucleotide variation

catc[A/T]tggt

2) For deletion

catc[TTC/--]tgggt

3) For insertion

catc[--/TTC]tgggt

#### Assay Development

The Excel file was converted to a .txt format, to be used as an input file for the ProxSNP, which, through the connection to SNP database, inspects the nucleotide sequences for any polymorphisms, and if too many potential polymorphisms are found, then the sequence is considered inadequate and the program rejects it, the reason being that software cannot find appropriate sequences for primer design s. The output file of ProxSNP is at the same time an input file for PreXTend, a program that highlights sequences that are suitable for the design of primers (a fragment of DNA that can serve as a starting point for DNA synthesis) and makes sure that there will be no crosshybridization between them.

Subsequently, the output file of PreXTend was used as an input file for MassARRAY Assay Design Software, which in turn generated the Array File, compatible with Sequenom Software. The Array File includes sequences for two PCR primers (Primers that have 5' tag nucleotide sequences attached), the Unextended Extended Primers (UEP) and their mass, two Extended Primers and their mass; and additional information about each of 115 assays (which correspond to 143 mutations). This file contains 18 iPLEXs (W1-W18). The number of assays in each iPLEX varies at intervals of 1 to 15 (see Figure 2)

# Conclusion

The prime objective of this project was to develop a most comprehensive mutational panel for the detection of clinically relevant mutations in MTC samples. The mutational panel developed and delivered by this applicant will be further processed using the SEQUENOM® Mass-ARRAY iPLEX® platform and ultimately used for mutations profiling of the clinical MTC samples by the cancer research scientists at the Abramson Cancer Center of the University of Pennsylvania.

# FIGURE 2

UNIL         TEM         SIP_D         Del-CPP         INF-DEP         AMP_LEUP_CONF MP_CONF MP_CONF MP_CONF MP_CONF MP_CONF MP_DEP         WP_DPB         UPP_DPB         UPP_D		A	в	С	D	E	F	G	Н	1	J	K	L	M	N	0	P	Q	R	S	
2 UM PLEX RET_V38C_A0 ACOTTOCATGACTOTOCATGA ST 7:1 80 92.5 98.8 4 F 9133 CTCCTCA 4 9488 SCTCTCA 4 9488 SCTCTCA 4 9498 SCTCTCA	1	WELL	TERM	SNP_ID	2nd-PCRP	1st-PCRP	AMP_LEN	UP_CONF	MP_CONF	Tm(NN)	PcGC	PWARN	UEP_DIR	UEP_MASS	UEP_SEQ	EXT1_CA	ALEXT1_MASS	EXT1_SEQ	XT2_CALL	. EXT2_MASS	E)
3. M         PLEX         Storb LINGR_0.0         ACOTTORATE_CONDATE         8         88         90         52.2         91.1         R         5568.1         100100CTA         5502.2         100100CTA         10010CTA	2	VV1	iPLEX	RET_Y806C_AG	ACGTTGGATG	ACGTTGGATGA	95	73.1	60	50.5	58.8	d	F	5137.3	CCTCCTC/	(A	5408.6	CCTCCTCA10	3	5424.6	C
4_W         PLEX         RET_0SS2_CAQOCTO         9988 al CAQOCTOA         9987 al CAQOTTOA         9977 al CA         9977 al CAQOCTA         9988 al CATCATTO         9977 al CAQOCTA         9988 al CATCATO         9977 al CAQOCTA         9978 al CAACTOTOA         9978 al CAACTOTOA         99788 al CAACTOTOA         9978 al CAACTOTOA	3	VV1	iPLEX	SDHD_H50R_AG	ACGTTGGATG	ACGTTGGATGO	88	98	60	55.2	61.1		R	5595.6	TGGTGGC	G	5842.8	TGGTGGCT	4	5922.7	T(
S, M         IPLE         REL VTRS. LOSS: _G1         ACCITOCATICACTIGATOT         B         94.6         B00         53.1         BSD         F         B000.9 OPACITICA         ESTVTS. A         ACCITOCATICACTIGATOT         BSD         SSD         F         B000.9 OPACITICA         ESTVTS. A         ACCITOCATICACTIGATOT         BES         D         F         B000.9 OPACITICA         ESTVTS. A         ACCITOCATICACTIGATOT         BSD         D         F         BSD         F         SSD         F         F         BSD         F         SSD         F         F         BSD         F	4	VV1	iPLEX	RET_V591I_GA	ACGTTGGATG	ACGTTGGATGT	99	88.3	60	60	68.4		R	5709.7	CCAGGCT	G	5956.9	CCAGGCTC	4	6036.8 (	C
6         IMP         PLEX         RET         SSE2_C         ACCITOCAGTGACTOGATGACTG         11         925         80         951         81         9164         6164         6164         11         ACTION           0         IMP         PLEX         RET         SSE2_C         ACTIONATIONATION         100         70         80         15         50         17         F         6041         11         AATOGAC         6941         31         70         6941         11         AATOGACTOCA         6941         31         70         6941         11         6941         14         6941         14         6941         14         6941         14         6941         14         6941         14         6941         6941         6941         6941         6941         6941         6941         6941         6941         6941         6941         6941         713         6941         713         6941         713         6941         713         6941         713         6941         713         6941         713         6941         713         6941         714         714         714         714         714         714         714         714         714         714	5	VV1	iPLEX	RET_G533C_GT	ACGTTGGATG	ACGTTGGATGT	96	94.6	60	55.3	63.2	D	F	6004.9	GGAGTG1	A	6276.1	GGAGTGTG	3	6292.1 (	G
T, Mu         PLEX         RET         SBML TAAATGOC         6413         TAAATGOC         6413         TAAATGOC         6413         TAAATGOC         6413         TAAATGOC         6413         TAAATGOC         6413         TAATGOC         6413         TAATGOC         6413         TAATGOC         6413         TAATGOC         6413         CAOCCTTAC         6813         CAOCCTTAC         7803         CATTAC         7803         CATTAC         7803         CATTAC         7803         CATTAC         7803         SpecaACC         78	6	VV1	iPLEX	RET_V778I_GA	ACGTTGGATG	ACGTTGGATGA	101	93.5	60	55.1	55	D	R	6164	GTGGTTG	G	6411.2	GTGGTTGA/	7	6491.1 (	G
B         IPLEX         RET_SOBALTCAA         ACOTTOGATAACOTTOGATOT         B08         C581/a         F         C681/a         CAACOTTOGATAACOTTOGATOT         C681/a         C677/a         C677/a <thc< td=""><td>7</td><td>VV1</td><td>iPLEX</td><td>RET_S922F_CT</td><td>ACGTTGGATG</td><td>ACGTTGGATGO</td><td>97</td><td>100</td><td>60</td><td>46.1</td><td>30</td><td></td><td>F</td><td>6204.1</td><td>TAAATGG</td><td>C</td><td>6451.3</td><td>TAAATGGAT</td><td>ſ</td><td>6531.2</td><td>T٨</td></thc<>	7	VV1	iPLEX	RET_S922F_CT	ACGTTGGATG	ACGTTGGATGO	97	100	60	46.1	30		F	6204.1	TAAATGG	C	6451.3	TAAATGGAT	ſ	6531.2	T٨
9         MPLEX         RET_S3940-F_CO/T         ACOTTOGATA ACOTTOGATA COTTOGATOR         9681 5 0           11         MPLEX         RET_S3940-F_CO/T         ACOTTOGATA ACOTTOGATOR         900 5         801 5 8         531 4         F         6584 3 (AADTOTC         6811 5 (AADTOTC)         7286 7 (ATTOTC)	8	VV1	iPLEX	RET_S686N_TCAA	ACGTTGGATG	ACGTTGGATGT	108	75.1	60	59.8	66.7	d	F	6343.1	CAGGCCT	AA	6614.3	CAGGCCTT 1	rC	6670.2 (	Ċ,
10         PIEX         RET_X30LCT         ACOTTOGATOGATOG         103         807         60         588         521 d         F         6778 4 OTGGAACC         728 5 OTGGAACC         7788 7 ATTGC	9	VV1	iPLEX	RET_S904C/F_CG/T	ACGTTGGATG	ACGTTGGATGT	95	87	60	45.4	33.3		F	6564.3	GAGATGI	С	6811.5	GAGATGTT	3	6851.5 (	G,
III M         PEEK         RET_V200M_GA         ACCTTOGATOGATO4         94         80.5         601         95.2         IF         66895 ADATCTTCA         72307 ADTTCTTO10         72467 JL           12 M         PEK         StPB_STEP_C         ACCTTOGATOGATOGATO5         52         M         F         7539 CTTATTTO1C         7488 DC         7738 F         600         613         602         F         7389 CCCCTATA         7788 F         7758 F         600         613         60         F         7389 CCCCTATA         7788 F         7758 F         600         613         60         F         7389 CCCCTATA         7788 F         7758 F         600         758 F         60         F         7389 C         7788 F         <	10	VV1	iPLEX	RET_T338I_CT	ACGTTGGATG	ACGTTGGATGO	103	90.7	60	59.8	59.1	d	F	6778.4	GTGGAAG	С	7025.6	GTGGAACAI	ſ	7105.5 (	G
12         IM         FLEX         SDME_SISE_TC         ACOTTOGATGACOTOGATGA         99         4         60         46.8         30.4 D         F         7758 CTATTTGC         7408 CTATTTGC         7408 CTATTTGC         7408 CCTATT         7768 D         CCTOATA         7788 D         CCTOATA         778 D         CTATTT         778 D         CTATTT         778 D         CTATTT         778 D         778 D         758 D <td>11</td> <td>VV1</td> <td>iPLEX</td> <td>RET_V202M_GA</td> <td>ACGTTGGATG</td> <td>ACGTTGGATGA</td> <td>94</td> <td>90.5</td> <td>60</td> <td>58.5</td> <td>52.2</td> <td>d</td> <td>F</td> <td>6959.5</td> <td>AGTTCTT</td> <td>A</td> <td>7230.7</td> <td>AGTTCTTG</td> <td>3</td> <td>7246.7</td> <td>A</td>	11	VV1	iPLEX	RET_V202M_GA	ACGTTGGATG	ACGTTGGATGA	94	90.5	60	58.5	52.2	d	F	6959.5	AGTTCTT	A	7230.7	AGTTCTTG	3	7246.7	A
13         IPLEX         IRET_0321R_0A         ACOTTOGATGACOTTOGATO         12         77.3         60         61.3         52.5 PH         R         7289.1         COCCTOA/G         758.8         COCCTOA/G         757.8         COCCTOA/G	12	VV1	iPLEX	SDHB_S163P_TC	ACGTTGGATG	ACGTTGGATGA	93	98.4	60	46.8	30.4	D	F	7159.7	CTTATTIC	С	7406.9	CTTATTIGAT	ſ	7486.8 (	C.
11         WI         PLEX         RET_DBIAGY_ACOT         ACOTTOGATO ACOTTOGATO         98         80         62         60         47         7800 § geccaACCC         7820 § geccaACCC         7821 § geccaACCC         7821 (g           15         MI         PLEX         RET_DBIAGY_ACOTTOGATO ACOTTOGATO         100         98.8         60         60.3         53.8 p         F         8080 2 (ACTOCTC         8307 (ACGOCAT         6307 (ACGOCAT)         6307 (ACCCAT)	13	VV1	iPLEX	RET_G321R_GA	ACGTTGGATG	ACGTTGGATGT	92	77.3	60	61.3	62.5	DH	R	7289.7	CCCCTGA	G	7536.9	CCCCTGAT	7	7616.8	C
IS         M         PLEX         RET_MeMB1_TC         ACOTTOGATO ACOTTOGATO         100         84.3         60         62.         60         d.8.3         6.7         7701         CCAGOCAT         7772.2         CCAGOCAT         7782.2         CCAGOCAT         5783.7         60.400.4         5583.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         5783.7         60.400.4         6783.7         70.400.4         6873.7         60.400.4         6783.7         70.400.4         6783.7         70.400.4         778.7         7783.7	14	VV1	iPLEX	RET_D631A/G/V_AC/G/T	ACGTTGGATG	ACGTTGGATGA	98	87.2	60	57.7	60	d	F	7580.9	gaccaACC	c	7828.1	gaccaACCC/	7	7852.1 (	g٤
16         VM         PLEX         RET_C6189V_CC         ACOTTOGATG ACOTTOGATG / 10         10         99.6         60.1         63.3         53.8 D         F         8080.2 (AACTCGCTC         837.4 (AACTCGTC)         6347.5 A           18         VQ         PLEX         RET_L6852_GC         ACOTTOGATG ACOTTOGATG / 94.6 (C)         96.3         55.3         55.4         55.8         55.4         75.8         60.4         60.7         63.3         70.60         65.33         70.60         62.7         63.3         70.60         65.3         57.9         D         F         558.0         70.7         65.3         57.9         D         F         558.0         70.7	15	VV1	iPLEX	RET_M848T_TC	ACGTTGGATG	ACGTTGGATGT	100	84.3	60	62	60	ds	R	7701	CCAGGCA	T	7972.2	CCAGGCAA	2	7988.2	Ċ(
17         V2         PLEX         RET_LES32-8330V_GOCD         ACOTTOGATIGACTOGO 199         98:03         653         554         658         647         IR         51804 (ARLACCE)C         5427.6         CATACCEGC         5427.6         CATACCEGC         5427.6         CATACCEGC         5427.6         CATACCEGC         5427.6         CATACCEGC         5533.7         CA         CATTOGATIGACTOTOGATIGA         98.7         B53.8         S18.0         R         S546.6         GCTACACC         S533.8         CCTACACC         S533.8         S533.8 <td>16</td> <td>VV1</td> <td>iPLEX</td> <td>RET_C618W_CG</td> <td>ACGTTGGATG</td> <td>ACGTTGGATGA</td> <td>100</td> <td>98.6</td> <td>60</td> <td>60.3</td> <td>53.8</td> <td>D</td> <td>F</td> <td>8060.2</td> <td>AACTGCT</td> <td>1C</td> <td>8307.4</td> <td>AACTGCTT</td> <td>3</td> <td>8347.5</td> <td>Ā</td>	16	VV1	iPLEX	RET_C618W_CG	ACGTTGGATG	ACGTTGGATGA	100	98.6	60	60.3	53.8	D	F	8060.2	AACTGCT	1C	8307.4	AACTGCTT	3	8347.5	Ā
18         V2         PLEX         RET_M655Q_CO         ACCTTOGATGACOTTOGATOT         94         87.1         65.3         51.4         58.8         I         D         Sessed	17	W2	iPLEX	RET_EL632-633DV_GCCG	ACGTTGGATG	ACGTTGGATGO	99	88.3	65.3	56	64.7	d	R	5180.4	GATCACC	CG	5427.6	GATCACCG	ЭC	5467.6	G.
19         V2         IPLEX         RET_D489N_GA         ACCITIOGATGACCITICGATOT         100         99.5         65.3         55.8         61.1         R         5546.5         0CCITAGACA         597.9         0           21         V2         IPLEX         RET_C259N_GA         ACCITICGATGACCITICGATGA         100         92.2         65.3         55.3         51.5	18	VV2	iPLEX	RET_H665Q_CG	ACGTTGGATG	ACGTTGGATGT	94	87.1	65.3	51.4	58.8	d	R	5346.5	GAGGAG.	i G	5593.7	GAGGAGA	2	5633.7 (	G.
20         VPLEX         RET_G691S_GA         ACCTTGGATGACGTGGATGA         97         97         65         55.5         57.9 ID         F         5880.7 GGTCAGCA         See1 JGGTCAGCTG         S97 J GCTAGCTG         S97 J GCTAGCTGGATGA	19	VV2	iPLEX	RET_D489N_GA	ACGTTGGATG	ACGTTGGATGT	100	98.5	65.3	53.8	61.1	D	R	5546.6	GCCTAGA	G	5793.8	GCCTAGAGA	7	5873.7	G
21         M2         IPLEX         RET_1278L_CA         ACOTTOGATGACOTTOGATG         100         92.2         65.3         63.9         79.9 DH         R         570.7 ICTCACC/C         5037.9 ICTCCACC/L         5037.9 ICTCCACC/L         5037.9 ICTCCACC/L         562.2 ICCCACC/L         5037.9 ICTCCACC/L         562.2 ICCCACT/L         562.2 ICCCACT/L         562.2 ICCCACT/L         562.2 ICCCATT/L         562.2 ICCCATT/L         562.2 ICCCATT/L         562.2 ICCCATT/L         563.9 ICTCCATC/L         562.2 ICCCATT/L         563.9 ICTCCATC/L         562.2 ICCCATT/L         563.9 ICTCCATC/L         573.9 ICTCCATC/L	20	W2	iPLEX	RET_G691S_GA	ACGTTGGATG	ACGTTGGATGA	97	87	65.3	51.5	57.9	D	F	5690.7	GGTCAGO	A	5961.9	GGTCAGCT	3	5977.9 (	G
22         W2         IPLEX         RET_J6S2M_CO         ACCTTOGATGACCTTOGATG         99         86.1         65.3         53.1         50 d         R         6175 (AGACATCIC         5422 (AGACATCIC         5503 (AGCAGAGC         550 (AGCAGACC)         5503 (AGCAGACC)         5503 (AGCAGACC)         5503 (AGCAGACC)         77033 (AGCAGACC)         7713 (AGCAGACC)         7713 (AGCAGACC)         7713 (AGCAGACC)         7713 (AGCAGACC)         7713 (AGCAGACC)         7723 (AGCAGACC)         773 (AGCAGACC) <td>21</td> <td>W2</td> <td>iPLEX</td> <td>RET_T278N_CA</td> <td>ACGTTGGATG</td> <td>ACGTTGGATGO</td> <td>100</td> <td>92.2</td> <td>65.3</td> <td>63.9</td> <td>78.9</td> <td>DH</td> <td>R</td> <td>5750.7</td> <td>CTCCACC</td> <td>C .</td> <td>6037.9</td> <td>CTCCACCA</td> <td>7</td> <td>6077.8 (</td> <td>Ċ.</td>	21	W2	iPLEX	RET_T278N_CA	ACGTTGGATG	ACGTTGGATGO	100	92.2	65.3	63.9	78.9	DH	R	5750.7	CTCCACC	C .	6037.9	CTCCACCA	7	6077.8 (	Ċ.
22         W2         IPLEX         RET_C520W_CO         ACOTTGGATGACOTTGGATG         65/3         52.3         55.0         F         6262.1         GAGGAGG         6543.3         GAGGAGG         6543.3         GAGGAGG         6543.3         GAGGAGG         6543.3         GAGGAGG         F         6262.1         GAGGAGGG         775.5         65.3         58.8         54.5         R         6806.4         GOTGGAGG         775.5         65.3         58.5         54.5         64.8         GOTGGAGGG         775.5         65.3         55.5         54.5         64.8         6606.4         GOTGGAGG         775.5         677.6         777.5         677.6         777.5         677.6         777.5         677.6         777.6         777.5         777.6         7	22	W2	iPLEX	RET_I852M_CG	ACGTTGGATG	ACGTTGGATGA	99	86.1	65.3	53.1	50	d	R	6175	GAGATCT	G	6422.2	GAGATCTG	2	6462.2	G.
24         W2         IPLEX         RET_0911D_0A         ACOTTOGATGACOTTOGATOC         104         83.9         65.3         49.8         33.1         R         63882   ATCCATTIO         66384   ATCCATTIO         675.4         675.3         ACOTTOGATGACOTTOGATOC         97.5         65.3         55.1         54.5         M         R         66884   AOGAGOC         7035.8         ACOTTOGATGACOTTOGATOC         97.5         65.3         55.1         54.5         M         R         66845   GATGACOT         773.15         AACTTOGATGACOTTOGATOC         97.8         65.3         55.1         54.5         M         R         66845   GATGACOT         773.17         GAAAACCCC         773.85         RT           28         W2         IPLEX         RET_E78D, OC         ACOTTOGATGACOTTOGATO         99         65.3         67.6         70.8         IN         770.8         INCCOCC         772.85         ITCCAAAGAC         778.8         ITC         770.8         INC         PLEX         RET_V791F, AT         ACOTTOGATGACOTTOGATO         99         65.3         67.7         78.0         R         770.9         ACOTTOGATGACOTTOGATO         99         78.5         59.5         67.7         70.0         A         ACOTTOGATGACOTTOGATO         99         78.5	23	W2	iPLEX	RET_C620W_CG	ACGTTGGATG	ACGTTGGATGO	100	97.1	65.3	52.3	55	D	F	6262.1	GAGGAG	(C	6509.3	GAGGAGG.	Э	6549.3	G.
25         W2         PLEX         RT 505R; OA         ACCTTGOATGACCTTGOATGA         97         57.5         66.3         95.8         54.5         Id         R         6808.4         AGGGOACG         7033.6         AGGGOACGA         7733.5         A           27         W2         PLEX         RET 5809.0         C         ACCTTGOATGACTGOATGACTGOATGA         99         98.6         65.3         49.9         39.10         R         694.2         COAAATCCT         7213.7         GAAATCCCC         722.8         IC           28         W2         PLEX         RET 7809.0         C         ACCTTGOATGACTTGOATGACTGOAGTGA         99         90.1         65.3         67.5         70.8         Dis         F         7018.6         ICAGAGAC         778.5         ICCCOACC         778.9         IT         778.	24	W2	iPLEX	RET_G911D_GA	ACGTTGGATG	ACGTTGGATGO	104	83.9	65.3	49.8	38.1		R	6389.2	ATCCATT	İG	6636.4	ATCCATTTA	7	6716.3 /	Ā
2E         VX2         PLEX         RET_05SBE_0A         ACCTTGOATGACCTTGOATGA         97         88         65.3         55.1         54.5         H         R         68442         GAAATCCT         7713.5         C           28         VV2         PLEX         RET_E7860 /C         ACCTTGOATGACCTTGGATG         99         98.6         65.3         52         60.9         D         F         7018.6         TCAGAGAC         728.5         TCAGAGACAC         728.5         TCAGAGACACACAC         728.5         TCAGAGACACACAC         728.5	25	W2	iPLEX	RET_E805K_GA	ACGTTGGATG	ACGTTGGATGA	97	57.5	65.3	58.8	54.5	d	R	6806.4	AGGGAG	G	7053.6	AGGGAGC	7	7133.5 /	Ā
27         V/2         PLEX         RET_R886W_CT         ACGTTOGATG ACGTTOGATG 4         99         98.6         65.3         49.8         39.1         P         69425         GAAATCCT         72137         GAAATCCCC         7228 SLC           29         W2         PLEX         RET_R886 V, CTAGAGAAC         ACGTTOGATG ACGTTOGATG         99         99.7         65.3         65.8         60.9         F         7188 STCGAGAGAC         7755 STCGAGAGAC         7755 STCGAGAGAC         7759 ST         7755 TCGAGAGAC         7755 ST         7018 STCGAGAC         7755 TCGAGAGAC         7755 TCGAGAGAC         7755 ST         775 ST         750 ST	26	W2	iPLEX	RET_G550E_GA	ACGTTGGATG	ACGTTGGATGA	97	89	65.3	55.1	54.5	dH	R	6846.4	GGTGGAG	G	7093.6	GGTGGAGA	7	7173.5	G
28         W2         PLEX         RET_E76BD_C         ACCITGOATG ACCITGOATO         91         957         653         652         609.D         F         7018.6         TCAGAGAC         7265.8         TCAGAGAC         7305.8         TCAGACAC         7305.8	27	W2	iPLEX	RET R886W CT	ACGTTGGATG	ACGTTGGATGA	99	98.6	65.3	49.9	39.1	D	R	6942.5	GAAATCO	т	7213.7	GAAATCCO	2	7229.8	G.
28         W2         IPLEX         CDM/HB_V/199G_TG         ACGTTOGATG/ACGTTOGATG/         99         90.1         65.3         67.6         70.8 bs         F         7468.8 ToCCGCGC         7758 ToCCGCGCT         7759.1 ToCCGCCGT         7879.1 ToCCGCCGT         7879.1 ToCCGCCGT         7879.1 ToCCGCCG         7559.1 ToCCGCC         7559.1 ToCCGCCG         7559.1 ToCCCCCG         7559.1 ToCCGC	28	W2	iPLEX	RET_E768D_GC	ACGTTGGATG	ACGTTGGATGT	91	99.7	65.3	62	60.9	D	F	7018.6	TCAGAGA	С	7265.8	TCAGAGAA	3	7305.8	τī
30         W2         IPLEX	29	W2	iPLEX	CDKN1B_V109G_TG	ACGTTGGATG	ACGTTGGATGA	99	90.1	65.3	67.6	70.8	Ds	F	7468.8	TGCCGGC	G	7756	TGCCGGCG1	•	7795.9	Ť
31         W2         IPLEX         RET_Y791F_AT         ACGTTGGATG/ACGTTGGATG         98         94.6         65.3         57.8         40.7 (d)         F         8147.3         ICAACCA/A         9418.5         ICAACCA/T         9474.4         17           32         W3         IPLEX         HRAS_G12C_GT         ACGTTGGATG/ACGTTGGATG         99         97.3         78.8         55.8         64.7 D         R         5051.3         ACCTCGA/A         5370.5         ACCACAA/A         5370.5         ACCATCAA/A         538.9         ACCATCAA/C         5388.9         JACCATCAA/A         603.1         8.47.4         42.1         R         5570.4         ACTCGAC/C         5389.9         JATCACACA/A         603.1         8.48         42.1         R         5704.7         ATTGCAC/C         599.9         ACTCCAC/A         6031.8         A           38         W3         IPLEX         RET_C5153_CCCT         ACGTTGGATG/ACGTTGGATG         98         96         78.8         47.4         42.1         F         5824.8	30	W2	iPLEX	HRAS A11/G12dup GC	ACGTTGGATG	ACGTTGGATGO	99	97.3	65.3	63.7	60	d	R	7602.9	GATGGTC	G	7850.1	GATGGTCA	2	7890.1	G.
32         PLEX         HRÅS_012_GT         ACGTTGGATG ACGTTGGATG         99         97.3         78.8         55.8         64.7         D         R         5051.3         ACCTCAGA         5322.5         ACCTCAGA         5329.5         ACCTCAGA         5329.5         ACCTCAGA         5370.5         ACCCAAAG         5322.5         ACCCAAAG         5370.5         ACCACAAG         5370.7         ATTGCACC         5399.9         ATTGCACTA         60031.8         A           38         W3         PLEX         RET_S0BCL         ACCTTGGATGACTTGGATGA         99.9         78.8         47.4         42.1	31	W2	iPLEX	RET Y791F AT	ACGTTGGATG	ACGTTGGATGT	98	94.6	65.3	57.9	40.7	d	F	8147.3	TCAACCA	A	8418.5	TCAACCACI	-	8474.4	τī
33         PLEX         RET_K666E_A         ACCTCGATG ACGTTGGATG         94         67.1         78.8         50.9         52.9         F         5099.3         ACCCACAA         5370.5         ACCACCAA(G         5370.5         ACCACCACA         5370.5         ACCACCACA         5370.5 <th< td=""><td>32</td><td>W3</td><td>iPLEX</td><td>HRAS G12C GT</td><td>ACGTTGGATG</td><td>ACGTTGGATGO</td><td>99</td><td>97.3</td><td>78.8</td><td>55.8</td><td>64.7</td><td>D</td><td>R</td><td>5051.3</td><td>ACTCTTG</td><td>G</td><td>5298.5</td><td>ACTCTTGC</td><td>-</td><td>5322.5</td><td>Ā</td></th<>	32	W3	iPLEX	HRAS G12C GT	ACGTTGGATG	ACGTTGGATGO	99	97.3	78.8	55.8	64.7	D	R	5051.3	ACTCTTG	G	5298.5	ACTCTTGC	-	5322.5	Ā
34         W3         PLEX         RET_R700_GA         ACGTTGGATG ACGTTGGATG         91         99.7         78.8         50.1         52.9         d         R         5170.4         ACCTCGA/G         5417.6         ACCTCGA/G         5478.6         CCTATGG/G         5478.6         CCTATGG/G         5478.6         CCTATGG/G         5478.6         CCTATGG/G         5478.6         CCTATGG/G         5478.6         CCTATGG/G         558.6         JACTCCA/C         568.7         JACCCA/C         568.7         JACTCCA/C         568.7         JACTCCA/C         568.7         JACTCCA/C         568.7         JACTCCA/C         568.7         JACTCCA/C         JACTCCA/C         JACTCCA/C         JACTCCA/C         JACTCCA/C         JACTCCA/C         JACTCCA/C         JACTCCA/C         J	33	VV3	iPLEX	RET K666E AG	ACGTTGGATG	ACGTTGGATGO	94	87.1	78.8	50.9	52.9	s	F	5099.3	ACCACAA	A	5370.5	ACCACAAC	3	5386.5 /	Ā
35       W3       IPLEX       RET_G11F_GT       ACGTTGGATG ACGTTGGATG /       101       96.1       78.8       53.3       55.6       d       F       5459.6       GCTATGG G       5746.8       GCTATGGCT       5786.7       G         36       W3       IPLEX       RET_C515S_GCCT       ACGTTGGATG ACGTTGGATG       98       99.4       78.8       55.3       66.7       0g       R       5598.6       GACTGCACT       5889.9       GACTGCACG       5889.9       GACTGCACG       5889.9       GACTGCACT       5889.9       GACTGCACG       5889.9       GACTGCACG       5889.9       GACTGCACT       6809.1       A       A       ACGTTGGATGACTTGGATGA       58.9       70.0       d       F       5849.9       CAGGCCCA       6221.1       CAGCTGCAGC       6409.2       TGGGGAGCC       6416.2       TG       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A	34	VV3	iPLEX	RET R770Q GA	ACGTTGGATG	ACGTTGGATGT	91	99.7	78.8	50.1	52.9	d	R	5170.4	ACTCTGA	G	5417.6	ACTCTGAC A	4	5497.5	Ā
36       W3       PLEX       RET_CS1SS_GCCT       ACGTTGGATG ACGTTGGATG1       98       94.6       78.8       55.3       66.7       Dg       R       5598.6       GACTGCA/CT       5889.9       GACTGCA/CGC       5885.9       GACTGCA/CGC       5885.9       GACTGCA/CT       5889.9       GACTGCA/CGC       5885.9       GACTGCA/CGC       5885.9       GACTGCA/CT       5889.9       GACTGCA/CGC       5885.9       GACTGCA/CT       5889.9       GACTGCA/CT       5889.9       GACTGCA/CT       6031.8       A         38       W3       IPLEX       RET_S0HK_GA       ACGTTGGATG/ACGTTGGATG/       98       96       78.8       47.4       42.1       F       594.9       CAGGCCC/A       6221.1       CAGGCCC/G       627.1       C.GGGCCC/A       621.1       CAGGCCC/G       623.7       IC         40       W3       IPLEX       RET_CS3HR_TC       ACGTTGGATG/ACGTGGATG/A       98       98.6       78.8       67.7       80 Dg       R       6129       TGGGGCA/C       621.1       CAGGCCC/A       621.1       CAGGCCC/A       650.3       CCTCACCT       6590.3       CCTCACCC/C       6563.3       CCTCACCC/A       6590.3       CCTCACCC/C       6563.3       CCTCACCC/A       6590.3       CCTCACCC/A       6590.4       C <td>35</td> <td>W3</td> <td>iPLEX</td> <td>RET C611F GT</td> <td>ACGTTGGATG</td> <td>ACGTTGGATGA</td> <td>101</td> <td>96.1</td> <td>78.8</td> <td>53.3</td> <td>55.6</td> <td>d</td> <td>F</td> <td>5459.6</td> <td>GCTATGG</td> <td>G</td> <td>5746.8</td> <td>GCTATGGC1</td> <td>-</td> <td>5786.7</td> <td>G</td>	35	W3	iPLEX	RET C611F GT	ACGTTGGATG	ACGTTGGATGA	101	96.1	78.8	53.3	55.6	d	F	5459.6	GCTATGG	G	5746.8	GCTATGGC1	-	5786.7	G
37       W3       PLEX       KRAS_Q61K_CA       ACGTTGGATG ACGTTGGATGT       96       99.9       78.8       48.8       42.1       s       R       5704.7       ATTGCACT       G       6031.8       A         38       W3       IPLEX       RET_E901K_GA       ACGTTGGATG ACGTTGGATGA       98       96       78.8       47.4       42.1       F       5524.9       ITGCCCCC       6072       TTGTCCCG/A       60961       1         39       W3       IPLEX       RET_V504LM_c 24130T/A       ACGTTGGATG ACGTTGGATG       93       63.3       78.8       59.8       701 d       F       5949.9       ITGCCCCC       6022       TTGTCCCG/A       60961       1         40       W3       IPLEX       RET_C531R_TC       ACGTTGGATG ACGTTGGATG       96       99.2       78.8       60.8       77.1 kD       6192       TGGGGACT       6400.2       TGGGGAGCC       6416.2       TC         41       W3       IPLEX       SDHD_G12S_GT       ACGTTGGATG ACGTTGGATG       94       89.2       78.8       60.8       71.4 LD       R       6319.1 (CTCACCC       6506.3 (CTCACCTT       6509.3 (C       6416.2       TC         42       W3       IPLEX       RET_6309.G       ACGTTGGATG ACGTT	36	W3	iPLEX	RET C515S GCCT	ACGTTGGATG	ACGTTGGATGT	98	94.6	78.8	55.3	66.7	Da	R	5598.6	GACTGCA	СТ	5869.9	GACTGCAC	ЭС	5885.9	G.
38       W3       IPLEX       RET_E901K_GA       ACGTTGGATG ACGTTGGATG A       98       96       78.8       47.4       42.1       F       5824.8       TTGTCCCC       6072       TTGTCCCG A       6096       TT         39       W3       IPLEX       RET_V804LM_c.2413GT/A       ACGTTGGATG ACGTTGGATG ACGTTGGATG 4       93       63.3       78.8       59.8       70       d       F       5949.9       CAGGGGCCA       6221.1       CAGGGGCG       6221.1       CAGGGGAGC       6437.1       C,         40       W3       IPLEX       RET_C531R_TC       ACGTTGGATG ACGTTGGATG ACGTTGGATG       94       89.2       78.8       60.8       71.4       bh       R       6129       TGGGGAGC       6566.3       CCTCACCT(T       6500.3       CCTCACCTG       6566.3       CCTCACCT(T       6500.3       CCTCACCTG       6771.4       ttggTGGCC       6771.4       ttggTGGCCC       6771.4       ttggTGGCC       <	37	W3	iPLEX	KRAS Q61K CA	ACGTTGGATG	ACGTTGGATGT	96	99.9	78.8	48.8	42.1	s	R	5704.7	ATTGCAC	C	5991.9	ATTGCACT /	1	6031.8	Ā
39       W3       IPLEX       RET_V804LM_c.2413GT/A       ACGTTGGATG ACGTTGGATG       93       63.3       78.8       59.8       70       d       F       5949.9       CAGGCCC       A       6221.1       CAGGCCCCG       6400.2       TGGGGAG(C       6416.2       TC         40       W3       IPLEX       RET_C531R_TC       ACGTTGGATG ACGTTGGATG       96       94.6       78.8       67.7       80       Dg       R       6129       TGGGGAG(T       6400.2       TGGGGAG(C       6416.2       TC         41       W3       IPLEX       SDHD_612S_GT       ACGTTGGATG ACGTTGGATG(A       94       89.2       78.8       60.8       71.4       Dh       R       6319.1       CCTCACC       G       6566.3       CCTCACCT       6590.3       CCCCACT       6590.3       C       677.3       AGATATG/A       6839.3       A         42       W3       IPLEX       RET_F921K_GA       ACGTTGGATG ACGTTGGATG       97       100       78.8       56.8       70.6       R       6526.3       AGATATG/G       677.3       AGATATG/A       6839.4       A         44       W3       IPLEX       RET_E843D_GT       ACGTTGGATG ACGTTGGATG       98       94.6       78.8       52.7	38	VV3	iPLEX	RET E901K GA	ACGTTGGATG	ACGTTGGATGA	98	96	78.8	47.4	42.1		F	5824.8	TTGTCCCC	c	6072	TTGTCCCG	4	6096	τī
40         W3         IPLEX         RET_C531R_TC         ACGTTGGATG ACGTTGGATG         96         94.6         78.8         67.7         80         Dg         R         6129         TGGGGACT         6400.2         TGGGGAGC         6416.2         TC           41         W3         IPLEX         SDHD_G12S_GT         ACGTTGGATG ACGTTGGATG         94         89.2         78.8         60.8         71.4         Dh         R         6319.1         CTCACC G         6566.3         CCTCACCTT         6590.3         O           42         W3         IPLEX         RET_639G_CG         ACGTTGGATG ACGTTGGATGT         91         95.8         78.8         56.8         70.6         d         F         6542.2         ttcgGTGCC         6701.4         ttcgGTGCCG         6701.4         ttcgGTGCCG         6773.4         46         673.4         47.4         ttcg           44         W3         IPLEX         RET_E921K_GA         ACGTTGGATG ACGTTGGATG         98         94.6         78.8         52.7         45.5         h         F         6623.3         CCACCCAC         AC895.0         CCACCCAC         6894.5         CCACCCAC         7312.8         G         45         W3         IPLEX         RET_6843D_GT         ACGTTGGATG ACGT	39	W3	iPLEX	RET_V804L/M c.2413GT/A	ACGTTGGATG	ACGTTGGATGA	93	63.3	78.8	59.8	70	d	F	5949.9	CAGGCCC	A	6221.1	CAGGCCCG	3	6237.1	ĉ,
41       W3       IPLEX       SDHD_G12S_GT       ACGTTGGATG ACGTTGGATG/       94       89.2       78.8       60.8       71.4       Dh       R       6319.1       CCTCACC       G       6566.3       CCTCACCT(T)       6590.3       C         42       W3       IPLEX       RET_A639G_CG       ACGTTGGATG ACGTTGGATG       91       95.8       78.8       56.8       70.6       d       F       6454.2       ttogGTGCC       6701.4       ttogGTGCC       6701.4 <t< td=""><td>40</td><td>W3</td><td>iPLEX</td><td>RET C531R TC</td><td>ACGTTGGATG</td><td>ACGTTGGATGT</td><td>96</td><td>94.6</td><td>78.8</td><td>67.7</td><td>80</td><td>Dq</td><td>R</td><td>6129</td><td>TGGGGA</td><td>т</td><td>6400.2</td><td>TGGGGAG</td><td>:</td><td>6416.2</td><td>τī</td></t<>	40	W3	iPLEX	RET C531R TC	ACGTTGGATG	ACGTTGGATGT	96	94.6	78.8	67.7	80	Dq	R	6129	TGGGGA	т	6400.2	TGGGGAG	:	6416.2	τī
42         W3         IPLEX         RET_A639G_CG         ACGTTGGATG         ACGTTGGATG         91         95.8         78.8         56.8         70.6         d         F         6454.2         ttcgGTGCC         6701.4         ttcgGTGCC	41	W3	iPLEX	SDHD G125 GT	ACGTTGGATG	ACGTTGGATGA	94	89.2	78.8	60.8	71.4	Dh	R	6319.1	CCTCACC	G	6566.3	CCTCACCT	-	6590.3	ō
43       W3       IPLEX       RET_E921K_GA       ACGTTGGATG ACGTTGGATG       97       100       78.8       45.1       28.6       R       6526.3       AGATATG G       6773.5       AGATATG A       6883.4       A         44       W3       IPLEX       RET_Y791N_TA       ACGTTGGATG ACGTTGGATG       98       94.6       78.8       52.7       45.5       h       F       6623.3       CCACCCAI       6894.5       CCACCCACT       6950.4       CI         45       W3       IPLEX       RET_E843D_GT       ACGTTGGATG ACGTTGGATG       98       82.9       78.8       66.4       69.6       H       R       7041.6       GCCCATG G       7288.7       GCCACCCAI       6950.4       CI         46       W3       IPLEX       HRAS_G13R_GC       ACGTTGGATG ACGTTGGATG       81       91.7       78.8       66.2       65.4       Ds       R       7050.1       GTCCCGC       8137.3       GTCCCCGCA       8137.3       GTCCCCGCA       817.7       63.5       54.1       64.7       D       R       5051.3       GCCACTC G       5238.5       CCACCCCCC       5338.5       CC       4317.5       GCCAGGCT       5338.5       CC       5338.5       CC       5338.5       CC       5338.	42	W3	iPLEX	RET A639G CG	ACGTTGGATG	ACGTTGGATGO	91	95.8	78.8	56.8	70.6	d	F	6454.2	ttcgGTGC	ic i	6701.4	ttcgGTGCCC	3	6741.4 1	tte
44       W3       IPLEX       RET_Y791N_TA       ACGTTGGATG ACGTTGGATG       98       94.6       78.8       52.7       45.5       h       F       6623.3       CCACCCA       A       66894.5       CCACCCAC       T       6950.4       C         45       W3       IPLEX       RET_B843D_GT       ACGTTGGATG ACGTTGGATG       98       82.9       78.8       66.4       69.6       H       R       7041.6       GCCCATG G       7288.7       GCCACCCAT       6950.4       C         46       W3       IPLEX       HRAS_G61K_CA       ACGTTGGATG ACGTTGGATG       81       91.7       78.8       66.2       65.4       bs       R       7701.1       GTCCCGC       8137.3       GTCCCGCATA       8177.2       G         46       W3       IPLEX       HRAS_G13R_GC       ACGTTGGATG ACGTTGGATG       81       91.7       78.8       66.2       65.4       bs       R       7701.1       GTCCCGC       8137.3       GTCCCGCATA       8177.2       G         48       W4       IPLEX       HRAS_G13R_GC       ACGTTGGATG ACGTTGGATG       108       75.4       76.3       57.4       70.6       R       5107.3       GCCAGGCT       5378.5       GCCAGGCC       5378.5       GCCAGGC	43	W3	iPLEX	RET E921K GA	ACGTTGGATG	ACGTTGGATGT	97	100	78.8	45.1	28.6		R	6526.3	AGATATO	G	6773.5	AGATATGA	7	6853.4	Ā
45       W3       IPLEX       RET_E843D_GT       ACGTTGGATG ACGTTGGATG       98       82.9       78.8       66.4       69.6       H       R       7041.6       GCCCATG       G       7288.7       GCCCATGGT       7312.8       G         46       W3       IPLEX       HRAS_Q61K_CA       ACGTTGGATG ACGTTGGATGT       81       91.7       78.8       66.2       65.4       Ds       R       7850.1       GTCCCGC C       8137.3       GTCCCGCAA       8177.2       G         47       W4       IPLEX       HRAS_G13R_GC       ACGTTGGATG ACGTTGGATGT       99       97.3       76.3       54.1       64.7       D       R       5051.3       GCCAGGCT       5378.5       GCCAGGCC       5338.5       G         48       W4       IPLEX       RET_R820C_CT       ACGTTGGATG ACGTTGGATGT       108       75.4       76.3       57.4       70.6       R       5107.3       GCCAGGCT       5378.5       GCCAGGCT	44	VV3	iPLEX	RET Y791N TA	ACGTTGGATG	ACGTTGGATGT	98	94.6	78.8	52.7	45.5	h	F	6623.3	CCACCCA	A	6894.5	CCACCCACT	7	6950.4	õ
46         W3         IPLEX         HRAS_Q61K_CA         ACGTTGGATG ACGTTGGATG         81         91.7         78.8         66.2         65.4         Ds         R         7850.1         GTCCCGC         8137.3         GTCCCGCA         8177.2         G           47         W4         IPLEX         HRAS_G13R_GC         ACGTTGGATG ACGTTGGATG         99         97.3         76.3         54.1         64.7         D         R         5051.3         CGCACTC         G         5298.5         CGCACTCT         C         5338.5         C           48         W4         IPLEX         RET_R820C_CT         ACGTTGGATG ACGTTGGATGT         108         75.4         76.3         57.4         70.6         D         R         5107.3         GCCAGGCT         5378.5         GC	45	W3	iPLEX	RET E843D GT	ACGTTGGATG	ACGTTGGATGO	98	82.9	78.8	66.4	69.6	Н	R	7041.6	GCCCATG	G	7288.7	GCCCATGG1	-	7312.8	G
47         W4         IPLEX         HRAS_G13R_GC         ACGTTGGATG         99         97.3         76.3         54.1         64.7         D         R         5051.3         COCACTC         G         5298.5         COCACTCT         C         5338.5         C           48         W4         IPLEX         RET_R820C_CT         ACGTTGGATG         ACGTTGGATG         108         75.4         76.3         57.4         70.6         D         R         5107.3         GCCAGGC         5378.5         GCCAGGC         5398.5         GC           49         W4         IPLEX         RET_C609F/S/Y_G/T/C/A         ACGTTGGATG ACGTTGGATG1         82         95.2         76.3         54.2         58.8         D         F         5186.4         AGCTGGCG         5473.6         AGCTGGCT         5513.5         AG           50         W4         IPLEX         RET_A510V_CT         ACGTTGGATG         ACGTTGGATG         97         94.5         76.3         63.6         83.3         d         R         5437.5         GGCAGCT         5708.7         GGCAGCC         5724.7         G           51         W4         IPLEX         RET_L881V_CG         ACGTTGGATG ACGTTGGATG1         97         100         76.3 <t< td=""><td>46</td><td>W3</td><td>iPLEX</td><td>HRAS Q61K CA</td><td>ACGTTGGATG</td><td>ACGTTGGATGT</td><td>81</td><td>91.7</td><td>78.8</td><td>66.2</td><td>65.4</td><td>Ds</td><td>R</td><td>7850.1</td><td>GTCCCGC</td><td>c</td><td>8137.3</td><td>GTCCCGCA/</td><td>7</td><td>8177.2</td><td>G</td></t<>	46	W3	iPLEX	HRAS Q61K CA	ACGTTGGATG	ACGTTGGATGT	81	91.7	78.8	66.2	65.4	Ds	R	7850.1	GTCCCGC	c	8137.3	GTCCCGCA/	7	8177.2	G
48         W4         IPLEX         RET_R820C_CT         ACGTTGGATG ACGTTGGATGT         108         75.4         76.3         57.4         70.6         D         R         5107.3         GCCAGGCT         5378.5         GCCAGGCC         5394.5         G           49         W4         IPLEX         RET_C609F/S/Y_G/T/C/A         ACGTTGGATG ACGTTGGATGT         82         95.2         76.3         54.2         58.8         D         F         5186.4         AGCTGGCG         5473.6         AGCTGGCT         5513.5         AC           50         W4         IPLEX         RET_A510V_CT         ACGTTGGATG ACGTTGGATGT         97         94.5         76.3         63.6         83.3         d         R         5437.5         GGCAGCT         5708.7         GGGCAGCC         5714.7 </td <td>47</td> <td>VV4</td> <td>iPLEX</td> <td>HRAS G13R GC</td> <td>ACGTTGGATG</td> <td>ACGTTGGATGO</td> <td>99</td> <td>97.3</td> <td>76.3</td> <td>54.1</td> <td>64.7</td> <td>D</td> <td>R</td> <td>5051.3</td> <td>CGCACTC</td> <td>G</td> <td>5298.5</td> <td>CGCACTCT</td> <td>2</td> <td>5338.5</td> <td>õ</td>	47	VV4	iPLEX	HRAS G13R GC	ACGTTGGATG	ACGTTGGATGO	99	97.3	76.3	54.1	64.7	D	R	5051.3	CGCACTC	G	5298.5	CGCACTCT	2	5338.5	õ
49         W4         IPLEX         RET_C609F/S/Y_G/T/C/A         ACGTGGATG ACGTTGGATG         82         95.2         76.3         54.2         58.8         D         F         5186.4         AGGTGGC G         5473.6         AGGTGGC T         5513.5         A           50         W4         IPLEX         RET_A510V_CT         ACGTTGGATG ACGTTGGATG         97         94.5         76.3         63.6         83.3         d         R         5437.5         GGGCAGCT         5708.7         GGGCAGC(C         5724.7         G           51         W4         IPLEX         RET_L881V_CG         ACGTTGGATG ACGTTGGATGT         97         100         76.3         58.8         63.2         D         R         5644.7         TTCCGCCG         5891.9         TTCCGCCCC         5931.9         TCATTGCAG         6241.1         TCATTGCAT	48	VV4	iPLEX	RET R820C CT	ACGTTGGATG	ACGTTGGATGT	108	75.4	76.3	57.4	70.6	D	R	5107.3	GCCAGGG	т	5378.5	GCCAGGCC	:	5394.5	Ĝ
50         W4         IPLEX         RET_A510V_CT         ACGTTGGATG         97         94.5         76.3         63.6         83.3 d         R         5437.5         GGGCAGCT         5708.7         GGGCAGCC         5724.7         G           51         W4         IPLEX         RET_L881V_CG         ACGTTGGATG ACGTTGGATGT         97         100         76.3         58.8         63.2         D         R         5644.7         TTCCGCCCG         5891.9         TTCCGCCCC         5931.9         TT           52         W4         IPLEX         KRAS         GGLLR         AT/G         ACGTTGGATGT         96         99.9         76.3         51.2         45 s         R         5993.9         TCATTGC/G         6241.1         TCATTGC/A         6241.1         TCATTGC/A         6241.1         TCATTGC/A         6245.1         TC	49	VV4	iPLEX	RET_C609F/S/Y_G/T/C/A	ACGTTGGATG	ACGTTGGATGT	82	95.2	76.3	54.2	58.8	D	F	5186.4	AGCTGGG	G	5473.6	AGCTGGCT	ſ	5513.5	Â
51         W4         IPLEX         RET_L881V_CG         ACGTTGGATG         ACGTTGGATG         97         100         76.3         58.8         63.2         D         R         5644.7         TTCCGCCCG         5891.9         TTCCGCCCC         5931.9         TT           52         W4         IPLEX         KRAS         G61LR         AT/G         ACGTTGGATG         96         99.9         76.3         51.2         45 s         R         5993.9         TCATTGC/G         6241.1         TCATTGC/A         T         6265.1         TC	50	VV4	iPLEX	RET A510V CT	ACGTTGGATG	ACGTTGGATGO	97	94.5	76.3	63.6	83.3	d	R	5437.5	GGGCAG	т	5708.7	GGGCAGC	:	5724.7	G
52 W4 IPLEX KRAS G61LR AT/G ACCTTGGATGACGTTGGATGT 96 99.9 76.3 51.2 45 s R 5993.9 TCATTGC/G 6241.1 TCATTGC/AT 6265.1 TC	51	VV4	iPLEX	RET L881V CG	ACGTTGGATG	ACGTTGGATGT	97	100	76.3	58.8	63.2	D	R	5644.7	TTCCGCO	G	5891.9	TTCCGCCCC	2	5931.9	τī
	52	VV4	iPLEX	KRAS Q61L/R AT/G	ACGTTGGATG	ACGTTGGATGT	96	99.9	76.3	51.2	45	s	R	5993.9	TCATTGC.	G	6241.1	TCATTGCA	•	6265.1	τī

Table 1	
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Gene	Amount of mutations	Protein function/ Signaling pathway or Process					
RET	130	A member of the cadherin superfamily, encodes one					
		of the receptor tyrosine kinases/cell growth and					
		differentiation					
BRAF	1	Raf/mil family of serine-threonine protein kinase/					
		MAPK-signaling pathway					
VHL	3	A tumor-suppressing gene. The protein products of					
		VHL play a major role in the oxygen sensing					
		pathways.					
KRAS	3	A member of the small GTPase superfamily/ MAPK-					
		signaling pathway					
HRAS	5	A member of the small GTPase superfamily/ MAPK-					
		signaling pathway					
SDHD	2	Complex II of the respiratory chain, which is					
		specifically involved in the oxidation of succinate,					
		carries electrons from FADH to CoQ. The subunit D					
		protein is one of two integral membrane proteins					
		anchoring the complex to the matrix side of the					
		membrane.					
SDHB	1	Complex II of the respiratory chain, which is					
		specifically involved in the oxidation of succinate,					
		carries electrons from FADH to CoQ. The iron-sulfur					
		subunit is highly conserved and contains three					
		cysteine-rich clusters which may comprise the iron-					
		sulfur centers of the enzyme.					
CDKN1B	1	This gene encodes a cyclin-dependent kinase					
		inhibitor, which shares a limited similarity with CDK					
		inhibitor CDKN1A/p21. The encoded protein binds					
		to and prevents the activation of cyclin E-CDK2 or					
		cyclin D-CDK4 complexes, and thus controls the cell					
		cycle progression at G1.					

	Table 2	
Gene	Protein Mutation	Germline/Somatic
RET	RET_A510V_CT	g
	RET_A639G_CG	S
	RET_A640G_CG	g
	RET A641R GCCG	S

RET_A641S_GT	g/s
RET $\overline{A}883F$ $\overline{G}CTT$	g/s
RET A883T GA	g/s
RET_A919V_CT	s
RET C515S GCCT	g
RET C531R TC	g
RET C609F/S/Y G/T/C/A	g
RET_C609G/R/S_T/G/C/A	g/s
RET C611F GCTT	g
RET C611F GT	σ
RET_C611G/R/S_TG/C/A	σ
RFT_C611S_GC	5 o
RET_C611S_GCCT	5 0
RET_C611W_CG	S G
DET_C611V_CA	g
DET C611V CCAT	g
$\begin{array}{c} \text{KEI}\_\text{COILI}\_\text{OCAI}\\ \text{DET}\_C(1) \text{E}[S] \text{V}\_CT[C] \text{A} \end{array}$	g
$\begin{array}{c} \text{KEI}\_\text{COI8F/S/Y}\_\text{GI/C/A}\\ \text{DET}\_\text{COI8C/D/S}\_\text{TC/C/A} \end{array}$	<u>g/s</u>
$\begin{array}{c} \text{KEI}\_\text{C018U/R/S}\_\text{IU/C/A}\\ \text{DET}\_\text{C(10)V}\_\text{CC} \end{array}$	g/s
REI_C618W_CG	g
$\frac{\text{RE1}_\text{C620F/S/Y}_\text{G1/C/A}}{\text{RET}_\text{C620G/P/G}}$	g
REI_C620G/R/S_IG/C/A	g/s
REI_C620W_CG	g
RET_C630A_TGGC	S
RET_C630F/S/Y_G/T/C/A	g
RET_C630G/R_TG/C	g/s
RET_C634F/S/Y_G/T/C/A	g/s
RET_C634G/R/S_TG/C/A	g/s
RET_C634T_TGAC	S
RET_C634W_CG	S
RET_D489N_GA	S
RET_D631-R635dup_GAg	S
RET_D631A/G/V_AC/G/T	g
RET_D631D/E_CT/A 71	g
RET_D631N/Y_GA/T	g
RET_E511K_GA	g
RET_E623K_GA	g
RET_E632-L633del_GT	S
RET E632K GA	g
RET <sup>E768D</sup> GC	g/s
RET_E805K_GA	g
RET <sup>E818K</sup> GA	g
RET E843D GT	g
RET E884K GA	s
RET <sup>E901K</sup> GA	S
RET E921K GA	S
RET EL632-633DV GCCG	g

RET F619F CT	g
RET G321R GA	g
RET_G533C_GT	g/s
RET G550E GA	g/s
RET G691S GA	σ. σ
RET_G911D_GA	5
RET G011F GGTT	5
	3
DET 1952M CC	g
	g
REI_KOUSE_AU	g
KEI_K000E_AG	g
RET_K90/E_AG	g
RET_K907M_AT	g
RET_L790F_GT	g
RET_L881V_CG	g
RET_M700L_AT	g
RET_M848T_TC	g
RET_M918T_TC	g/s
RET_M918V_AG	g/s
RET_N777S_AG	g
RET P766S CT	S
RET P841L CT	S
RET P841P-GA	g
RET Q781R AG	g
RET R6000 GA	g
RET R635G CG	g
RET_R7700_GA	g
RET R820C CT	S
RET R833C CT	σ
RET R8440 GA	σ
BET R886W CT	σ
RET_ROOSK_GA	5
RET_ROTOR_GA	o o
DET S640L TCCT	g
DET S686N TCAA	g
DET SOLOL CT	g
	g
REI_589IA_IU	g
RE1_\$904C/F_CG/1	g/s
REI_S922F_CI	S
KE1_S922P_TC	S
RET_S922Y_CA	g
RET_T278N_CA	S
RET_T338I_CT	g
RET_T930M_CT	S
RET_V202M_GA	S
RET_V292M_GA	g

RET_V591I_GA	S
RET_V648I_GA	g/s
RET_V778I_GA	g
RET_V804L/M_c.2413GT/A	g
RET_Y791F_AT	g
RET_Y791N_TA	g
RET_Y806C_AG	g
SDHD_G12S_GT	g
SDHD_H50R_AG	g
SDHB_S163P_TC	g
HRAS_A11/G12dup_GC	S
HRAS_G12C_GT	S
HRAS_G13R_GC	S
HRAS_G13V_GT	S
HRAS_Q61K_CA	S
KRAS_Q61K_CA	S
KRAS_Q61L/R_AT/G	S
VHL_N78I	S
VHL_F76_del(TTC)	S
VHL_P59_del(C)	S
BRAF_V600E_	S
CDKN1B_V109G_TG	S
	RET_V591I_GA RET_V648I_GA RET_V778I_GA RET_V804L/M_c.2413GT/A RET_Y791F_AT RET_Y791F_AT RET_Y791N_TA RET_Y806C_AG SDHD_G12S_GT SDHD_H50R_AG SDHB_S163P_TC HRAS_A11/G12dup_GC HRAS_G13R_GC HRAS_G13R_GC HRAS_G13V_GT HRAS_Q61K_CA KRAS_Q61K_CA KRAS_Q61L/R_AT/G VHL_N78I VHL_F76_del(TTC) VHL_P59_del(C) BRAF_V600E CDKN1B_V109G_TG

# **References:**

Millis, M. (2011, Summer). Medium-Throughput SNP Genotyping Using Mass
 Spectrometry: Multiplex SNP Genotyping Using the iPLEX® Gold Assay. Springer
 Protocols, 700. Retrieved August 20, 2012, from

http://link.springer.com/protocol/10.1007%2F978-1-61737-954-3 5

This paper discusses and explains the basics of genotyping with the using MALDI-TOF Mass Spectrometry. This was the first paper that I have read right after being given the project. After fully interpreting this paper, I realized that I am going to be able to finish the project on my own. This paper served as a guide to me throughout the process of doing this project, as well as while writing the entire research report. In addition, this paper was used to make sure that I am not saying something that is factually incorrect.

 Gabriel, S., Ziaugra, L., & Tabbaa, D. (2009, January 1). UNIT 2.12 SNP Genotyping Using the Sequenom MassARRAY iPLEX Platform. Current Protocols in Human Genetics.

This paper describes in details the SNP genotyping method based on the Sequenom MassARRAY platform. It includes two step protocol (initial locus-specific PCR reaction, followed by single base extension using mass-modified dideoxynucleotide terminators) an assay structure and how using MALDI-TOF mass spectrometry identify the SNP allele. The paper is mentioned in the corresponded section of the Introduction.

3. Ricarte-Filho, J., Ryder, M., Ghossein, R., Fagin, J., Chitale, D., Rivera, M., et al. (2009, June 1). Mutational Profile of Advanced Primary and Metastatic Radioactive Iodine-Refractory Thyroid Cancers Reveals Distinct Pathogenetic Roles for BRAF, PIK3CA, and AKT1. CANCER RESEARCH. Retrieved July 1, 2012, from

http://cancerres.aacrjournals.org/content/69

The paper describes profiling of 111 mutations in RET, BRAF, NRAS, HRAS, KRAS, PIK3CA, AKT1 genes in clinical poorly differentiated, anaplastic and radioactive iodinerefractory differentiated thyroid cancers. The genotyping method is based on the Sequenom MassARRAY platform. It was shown that RAS mutations were prevalent in primary PDTC, whereas BRAF was more common in metastatic PDTC and ATC.

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PIK3CA or AKT1 mutations were rare. The paper is mentioned in the corresponded section of the Introduction.

4. MEN2 Database. (n.d.). AURP Scientific Resource for Research and Education.. Retrieved July 12, 2012, from <u>http://arup.utah.edu/database/MEN2/MEN2\_display.php</u> This database was only used for its mutations list during the collection phase of the project.

Human BLAT Search. (n.d.). UCSC Genome Browser. Retrieved August 17,
 2012, from <u>http://genome.ucsc.edu/cgi-bin/hgBlat</u>

The Human BLAT database was used for alignment of nucleic sequences. The sequences retrieved from COSMIC were inputted in the BLAT Database to be aligned with the rest of the nucleic sequence, as only a small part of it could be gathered from COSMIC.

Catalogue of Somatic Mutations in Cancer - COSMIC. (n.d.). Wellcome Trust
 Sanger Institute. Retrieved July 10, 2012, from

http://www.sanger.ac.uk/genetics/CGP/cosmic/

This database was used to retrieve the nucleic sequences that were used as the base to be inputted in the BLAT database. As it is impossible to use BLAT database with only knowing the position of the mutation, COSMIC was used to retrieve the minimal part of the sequence required to find the full nucleic sequence for any particular gene. 7. Genetics of Endocrine and Neuroendocrine Neoplasias (PDQ®). (n.d.). National Cancer Institute. Retrieved July 12, 2012, from

http://www.cancer.gov/cancertopics/pdq/genetics/medullarythyroid/HealthProfessional/Table4 This database was only used for its mutations list during the collection phase of the project.

OMIM Entry - # 171400 - MULTIPLE ENDOCRINE NEOPLASIA, TYPE IIA;
 MEN2A . (n.d.). OMIM - Online Mendelian Inheritance in Man . Retrieved July 19, 2012,
 from <a href="http://omim.org/entry/171400">http://omim.org/entry/171400</a>

This entry was used as the basis of understanding of the MEN 2A syndrome. It talks about the relationship between MTC and diseases such as pheochromocytoma, and about the basics of what MEN 2A syndrome actually is. In addition, it talks about what mutations are associated with the syndrome. It was specifically used for comparison with the MEN 2B and FMTC.

9. Jimenez, C., Hu, M. I., & Gagel, R. (2008, Spring). Management of Medullary Thyroid Carcinoma. Elsevier Saunders, ?, 15.

This MTC review was the first of many that I have read in the duration of this project. This review provided me with the basic information about MTC without which any attempt at actually finishing this project would have been obsolete. Many parts of the introduction are referred to this paper, as it was very influential. Similarly to many other sources, mutations were taken from this publication during the first phase of the project.  MacConaill, L Profiling Critical Cancer Gene Mutations in Clinical Tumor Samples. PLoS ONE (2009).

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0007887

This publication was one of many that were used only for its mutations. No part, except for the abstract, which contributed to the overall idea of the research report, has been read.

Ehsan Alvandi, Seyed Mohammad Akrami, Mohsen Chiani, Mehdi Hedayati,
 Babak Noori Nayer, Mohammad Reza Mohajeri Tehrani, et al. (2011, April 5). Molecular
 Analysis of the RET Proto-Oncogene Key Exons in Patients with Medullary Thyroid
 Carcinoma: A Comprehensive Study of the Iranian Population. Thyroid, 1. Retrieved
 September 1, 2012, from <a href="http://online.liebertpub.com/doi/abs/10.10">http://online.liebertpub.com/doi/abs/10.10</a>

This publication was one of many that were used only for its mutations, and or one small piece of information. No part, except for the abstract, which contributed to the overall idea of the research report, has been read.

Moura, M., Cavaco, B., Pinto, A., & Leite, V. (2011, February 16). High
 Prevalence of RAS Mutations in RET-Negative Sporadic Medullary Thyroid Carcinomas.
 JCEM ONLINE, 95, 6.

This paper has shown a study where 64% of the patients that had Sporadic MTC were found to have a BRAF mutation in position 600. This is very unusual as this mutation is considered to be PTC specific. In addition, this publication has given additional context to this research report. Similarly to many other sources, mutations were taken from this publication during the first phase of the project.

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OMIM Entry - # 162300 - MULTIPLE ENDOCRINE NEOPLASIA, TYPE IIB;
MEN2B . (n.d.). OMIM - Online Mendelian Inheritance in Man . Retrieved June 19,
2012, from <u>http://omim.org/entry/162300</u>

This entry was used as the basis of understanding of the MEN 2B syndrome. It talks about the relationship between MTC and diseases such as pheochromocytoma, and about the basics of what MEN 2B syndrome actually is. In addition, this entry talks about what mutations are associated with the syndrome. It was specifically used for comparison with the MEN 2A and FMTC.

hybridization, f. i., & (1989), I. e. (n.d.). OMIM Entry - + 164761 REARRANGED DURING TRANSFECTION PROTOONCOGENE; RET . OMIM Online Mendelian Inheritance in Man . Retrieved July 19, 2012, from

http://omim.org/entry/164761

This entry was used as the basis of understanding MTC, and why the mutations RET gene are found in so many cases of MTC. Unfortunately, as with many other publications, no definitive answer was given. This entry was also used as a guide, to make sure that what I say about mutations in RET gene is factually correct.

15. RT-PCR., & (2003), M. e. (n.d.). OMIM Entry - # 155240 - THYROID CARCINOMA, FAMILIAL MEDULLARY; MTC . OMIM - Online Mendelian Inheritance in Man. Retrieved July 19, 2012, from <u>http://omim.org/entry/155240</u> This entry was used as the basis of understanding of the FMTC syndrome. It talks about the relationship between MTC and diseases such as pheochromocytoma, and about the basics of what FMTC syndrome actually is. In addition, this entry talks about what mutations are associated with the syndrome. It was specifically used for comparison with the MEN 2B and MEN 2A.

16. Hazard, J., Hawk, W., & Crile, G. (1959, January 1). MEDULLARY (SOLID) CARCINOMA OF THE THYROID—A CLINICOPATHOLOGIC ENTITY. JCEM, 19. Retrieved June 26, 2012, from <u>http://jcem.endojournals.org/content/19/1/152</u> This publication was the first time MTC was classified. The paper itself was not read by this applicant, however, due to its historic relevance, it was referred to in the first paragraph of the introduction.

17. Cakir, M., & Grossman, A. (2009, May 25). Medullary Thyroid Cancer: Molecular Biology and Novel Molecular Therapies. Neuro Endocrinology, 25. This publication, alongside many MTC reviews, was used as the guideline for this research report. Many facts, such as information about Sporadic and Hereditary MTC were confirmed by this publication. Similarly to many other sources, mutations were taken from this publication during the first phase of the project.