Masking Technique: Masking Selected Sequence Variation by Incorporating Mismatches into Melting Analysis Probes

Rebecca L Margraf[1], Rong Mao[1,2], Carl T Wittwer[1,2].

[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT.
[2]Department of Pathology, University of Utah Medical School, Salt Lake City, UT.

Abstract

Background: Hybridization probe melting analysis can be complicated by the presence of sequence variation (non-pathogenic polymorphisms or other mutations) near the targeted mutation. We investigated the use of 'masking' probes to differentiate alleles with similar probe melting temperatures.

Materials and Methods: Selected sequence variation was masked by incorporating deletions, unmatched (non-complementary) nucleotides, or universal bases into hybridization probes. Such masking probes create a probe:target mismatch with all possible alleles at the selected polymorphic location. Any allele with additional variation at another site is identified by a lower probe melting temperature than alleles that vary only at the masked position. This technique was applied to *RET* proto-oncogene and HPA6 mutation detection using unlabeled hybridization probes, a saturating dsDNA dye, and high-resolution melting analysis.

Results: Masking probes clearly distinguished all targeted mutations from polymorphisms when at least one base pair separated the mutation from the masked variation. Polymorphisms immediately adjacent to mutations could usually be masked, except in certain cases, such as with single base deletion probes when both adjacent positions have the same polymorphic nucleotides. The masking probes can also localize mutations to specific codons or nucleotide positions.

Conclusion: Masking probes can be a useful tool to simplify hybridization probe melting analysis of complex regions and eliminate the need for sequencing.

Methods

Unlabeled Probe Technique

Closed-tube method that can target and genotype specific mutations within larger amplicon

- similar to Hybprobe technique except probes are unlabeled.

Protocol

1. Asymmetric PCR - to increase single-stranded product

Probe is 3' phosphorylated to eliminate extension

2. Hybridize with unlabeled probe followed by high resolution melting analysis

Probe-target duplex

Detection by dsDNA binding dye: LCgreen PLUS

Samples:

- De-identified genomic DNA samples were received from the Mayo Clinic (Table1).
- All variant samples have been genotyped as heterozygous unless otherwise stated.

Asymmetric PCR with unlabeled probes:

- Approximately 50 ng of DNA sample and 0.5 uM of unlabeled probe were added to a 10 uL PCR reaction using Roche LightCycler FastStart DNA Master Hybridization Probe kit and LCGreen™ Plus dye (Idaho Technology). Generally, the primers were used at 0.55 uM with a 1:10 ratio (Table 2).
- PCR on the Lightcycler®
  - 10 min UNG step at 50°C and 10 min PCR activation at 95°C.
  - 60 cycles of PCR: denaturation for 1 sec at 95°C, annealing for 1 sec at 62°C and elongation for 10 sec at 72°C.
- Duplexing procedure: After amplicon melt protocol ended at 95°C, samples were cooled to 40°C in the LightCycler, then placed at 4°C for >10 minutes.

High-resolution melting analysis for unlabeled probes:

Acquire temp 55 or 60°C, final temp 95°C. Raw data was converted to derivative plots.

Table 1: Sequence Variation in the *RET* Proto-Oncogene

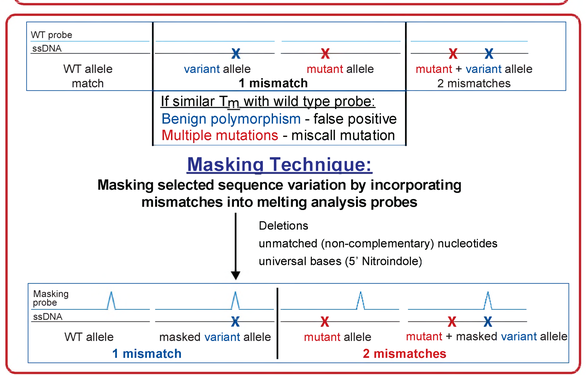
RET exon	Sequence variation position[a] Codon	Nucleotide	Amino acid change	Nucleotide change[b]
10	609	1826	C609Y	TGC >TAC
		1826	C609S	TGC >TCC
	611	1831	C611R	TGC >CGC
		1832	C611Y	TGC >TAC
		1832	C611F	TGC >TTC
	618	1852	C618R	TGC >CGC
		1852	C618G	TGC >GGC
		1853	C618Y	TGC >TAC
		1853	C618S	TGC >TCC
		1853	C618F	TGC >TTC
	620	1858	C620S	TGC >AGC
		1859	C620Y	TGC >TAC
		1859	C620S	TGC >TCC
		1859	C620F	TGC >TTC
		1860	C620W	TGC >TGG
11	631	1893	D631D	GAC >GAT[c]
	634	1900	C634S	TGC >AGC
		1900	C634R	TGC >CGC
		1900	C634G	TGC >GGC
		1901	C634Y	TGC >TAC
		1901	C634S	TGC >TCC
		1901	C634F	TGC >TTC
		1902	C634W	TGC >TGG
13	768	2304	E768D	GAG >GAC
	769	2307	L769L	CTT >CTG[c]

[a] Nucleotide position of sequence variation using *RET* cDNA sequence derived from *RET* mRNA GenBank NM_020630, where nucleotide position 181 is +1 ('A' of the ATG start codon).
[b] Sequence variation is heterozygous unless otherwise stated in the text. Wildtype codon sequence is listed > nucleotide of change is in bold.
[c] Polymorphism.

Table 2: Primers and Probes

RET exon	Primers[a]	Amplicon (base pair)	Codon of variation[b]	Probes (base pairs)[c]	Probe sequence[d]
10	GGGCAGCATTGTTGGGGGAG	146	609,611	WT 609/611 (30bp)	GGCTATGGCACCTGCAACTGCTTCCCTGAG
	TGGTGGTCCCGGCCGCCA		618,620	WT 618/620 (31bp)	GGAGAAGTGCTTCTGCGAGCCCGAAGACATC
11	TGCCAAGCCTCACACCAC	109	630, 631	WT exon 11 (27bp)	CGTGCGGCAGCTCGTCGCAGCAGTGG
	GACAGCAGCACCGAGAC		634	WT 634 (31bp)	TGCGATCACCGTGCGGCAGCTCGTCGCAC
13	ACTTGGGCAAGGCGATGCAG	274	768,769	WT exon 13 (30bp)	CCCGAGTGAGCTTCGAGACCTGCTGTCAGA
	GAACAGGGCTGTATGGAGC				
HPA6	TGGGATCCCAGTGTGAGTGCTCA	180	489	WT HPA6 (31bp)	CTGCAGACGGGCTGACCCTCTCGGGGGCTGC
	AGAAGTCGTCACACTCGCAGTAC			MUT-specific (31bp)	CTGCAGACGGGCTGACCCTCCTGGGGGCTGC

[a] Primers are listed 5' to 3', with the forward primer above the reverse primer.
[b] The underlined codons contain a polymorphism, while the other codons contain pathogenic mutations. The HPA6 mutation and polymorphism is in the same codon, 489.
[c] WT - wild type. MUT- mutation.
[d] Probe sequences are wild type and listed 5' to 3'. *RET* exon 10 and 13 are forward probes, while *RET* exon 11 and HPA6 are reverse probes. The possible mutation locations are highlighted in **bold** and the polymorphism locations are underlined. The masking probes have the same sequence as the wild type probes, except at the incorporated masking mismatch(es) displayed in each figure. The universal base and unmatched nucleotide masking probes were the same size (base pair) as the wild type probes, while the masking deletion probes were reduced in size by the number of deleted nucleotides from the wild type probe sequence.



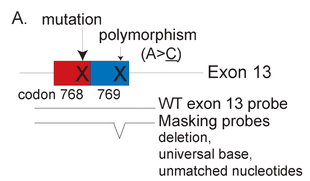
Results

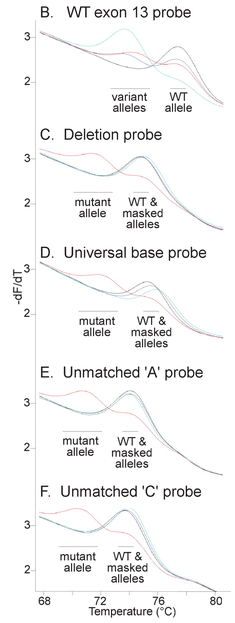
Masking Polymorphisms Near Targeted Mutations

Figure 1

Results:

- *RET* exon 13 has a common benign polymorphism (non-coding strand A>C) near a pathogenic mutation (Fig 1A).
- Both the polymorphism and the targeted mutation have one mismatch with the wild type probe, which resulted in similar probe melting temperatures (Fig 1B).
- Masking probes with a universal base, deletion, or an unmatched nucleotide at the non-targeted polymorphism location were evaluated (Fig 1C-F).
  * Unmatched nucleotides used for the masking probes did not complement the possible nucleotides at the polymorphism location (non-coding strand: A or C).
- Each masking probe reduced all possible alleles to one mismatch status with the probe, creating a nearly identical T_m for the masked polymorphism allele as for the wild type allele.
- The targeted mutant allele had an additional mismatch with the masking probes and was clearly distinguished by a 4°C lower T_m than the wild type or masked polymorphism alleles.



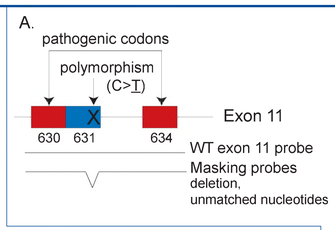


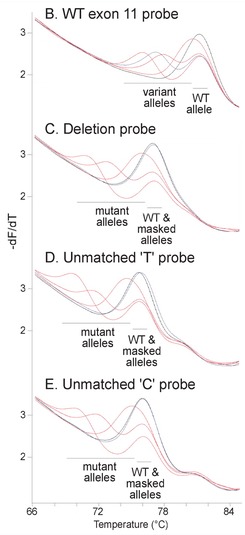
Legend: A: The diagram illustrates *RET* exon 13 with the pathogenic codon 768 in red and the polymorphism codon 769 in blue, while 'X' represents the variant position within the codons. The masking probes have wild type sequence with an incorporated mismatch of a universal base, unmatched nucleotide or deletion at the polymorphism location, represented by the 'V'. The graphs (B-F) are derivative plots of unlabeled probe high-resolution melting analysis data. For each graph: the black curve is homozygous wild type, the red curve is the heterozygous mutation at codon 768(GAG>GAC), the dark blue curve is heterozygous for the codon 769 polymorphism and the light blue curve is homozygous for the codon 769 polymorphism. Two melting temperature ranges are underlined on each panel, listing which alleles melted at each T_m range. B: The wild type probe (WT exon 13 probe). The codon 769 polymorphism was masked by four different masking probes: C: deletion probe, D: universal base probe (5-nitroindole), E: unmatched nucleotide 'A' probe, or F: unmatched nucleotide 'C' probe.

Figure 2

Results:

- *RET* exon 11 has two codons of possible pathogenic mutations at 630 and 634, and a polymorphism at codon 631 (Fig 2A).
- In order to analyze the two pathogenic codons but not the polymorphism, a masking deletion and two unmatched nucleotide probes were tested.
- With all three probes, the codon 631 polymorphism was masked from analysis with a nearly identical T_m as the wild type allele, allowing the lower T_m mutant alleles to be clearly detected (Fig 2C-E).





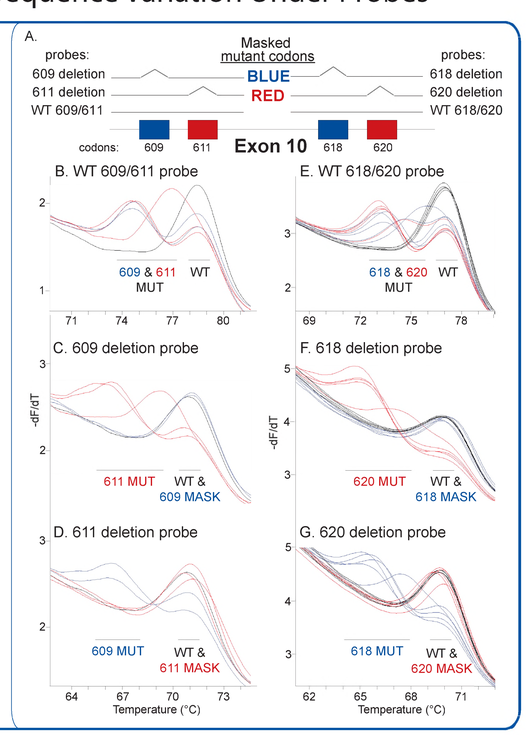
Legend: A: The diagram illustrates RET exon 11 with pathogenic codons 630 and 634 shown in red, with the codon 631 polymorphism is blue ('X' for codon position). The masking probes have wild type sequence that incorporates either a deletion or an unmatched nucleotide at the polymorphism location, represented by the 'V'. The graphs (B-E) are derivative plots of unlabeled probe high-resolution melting analysis data. For each graph: the black curve is homozygous wild type, the red curves are three unique heterozygous mutations within codon 634 (TGC>CGC, GGC, and TCC), while the blue curve is heterozygous for the codon 631(GAC>GAT) polymorphism. B: The wild type probe (WT exon 11 probe), over codons 630, 631 and 634. The codon 631 polymorphism was masked by three different masking probes: C: deletion probe, D: unmatched nucleotide 'T' or E: unmatched nucleotide 'C' probe.

Locating the Position of Sequence Variation Under Probes

Figure 3

Results:

- *RET* exon 10 mutations are mainly restricted to four pathogenic codon locations: 609, 611, 618, and 620.
- Although the wild type probes can detect sequence variation, they cannot identify which codon contains the mutation (or even if the detected sequence variation is within a pathogenic codon) due to similar T_ms (Fig 3B, E).
- To locate the mutation to a particular pathogenic codon, masking probes had a three base pair deletion over one codon (Fig 3A).
- In each case, mutations within the masked codon were as stable as the wild type allele, whereas alleles with the mutation outside of the masked codon had an additional mismatch with the probe and were clearly identified by lower T_ms (Fig 3C and D, F and G).



Legend: A: The diagram illustrates *RET* exon 10 where pathogenic mutations can be any nucleotide change within codons 609, 611, 618 and 620; all of wild type nucleotide sequence TGC. Each masking probe has a three base pair deletion of the wild type probe sequence over one pathogenic codon as illustrated. The codons predicted to be masked by each probe are listed by codon color (**BLUE** or **RED**) under 'masked mutant codons'. For the graphs (B-G): heterozygous mutations at codons 609 and 618 are the blue traces, heterozygous mutations at codons 611 and 620 are the red traces and the black traces are homozygous wild type samples. Codons 609/611 data are displayed in the left panels and codons 618/620 data are displayed in the right panels. Two melting temperature ranges are underlined for each graph with codon mutant alleles (MUT), wild type alleles (WT) and masked codon mutant alleles (MASK) noted in each panel. B: The wild type probe (WT 609/611 probe) over the codons 609 and 611. C: Masking 609 deletion probe. D: Masking 611 deletion probe. E: The wild type probe (WT 618/620 probe), over the codons 618 and 620. F: Masking 618 deletion probe. G: Masking 620 deletion probe. The five unique mutations at codons 609/611 and ten unique mutations at codons 618/620 are listed in Table 1.

Masking Sequence Variation Immediately Adjacent to the Targeted Mutation

Figure 4

Results:

- The positional effects of single base masking deletions in the probe relative to targeted mutations are shown in Figure 4.
- Five different single base deletion probes were designed across *RET* exon 11 codon 634 (Fig. 4A)
- Alleles with mutations in the second position of codon 634 (blue) had T_ms 3-4°C below the wild type allele with all probes, except when the probe deletion was over the mutation site masking all three mutations (Probe 4, Fig. 4 F).
- Similarly, a mutation at the third position of codon 634 (light blue, (TGC>TG**G**)) had a T_m 2-3°C below the wild type allele with all probes, except when the deletion was over the mutation site, masking only this mutation (Fig. 4G).
- Mutations at the first position of codon 634 (red) were also masked by probes with a deletion over the mutation site (Fig. 4E). However, when the deleted base position was immediately adjacent to these mutations (Fig. 4D and 4F), the T_ms of the mutations were very similar (within 0.8°C) to the wild type T_m.
  * Using an unmatched 'T' or 'G' nucleotide for masking instead of the deletion allowed clear distinction between the mutant and wild type alleles (data not shown).

Legend: A: The diagram illustrates *RET* exon 11 codon 634 (boxed) of the wild type sequence TGC. Each masking probe has a one base pair deletion of the wild type sequence, near or within codon 634 as illustrated in the diagram. The mutations listed at the three nucleotide positions of codon 634 and their melting curve traces are color coded. The three unique heterozygous mutations at the first position of codon 634 are red, the three unique heterozygous mutations at the second position of codon 634 are blue, while the heterozygous mutation at the third position of codon 634 are light blue. The black melting curve traces are homozygous wild type samples. B: Wild type probe (WT 634 probe). C-G: Masking deletion probes 1 through 5. Mutations that should be masked by a deletion probe are noted in the panels by the word 'Mask' in the mutation color (red, dark blue or light blue). The seven unique codon 634 mutations used in this assay are listed in Table 1.

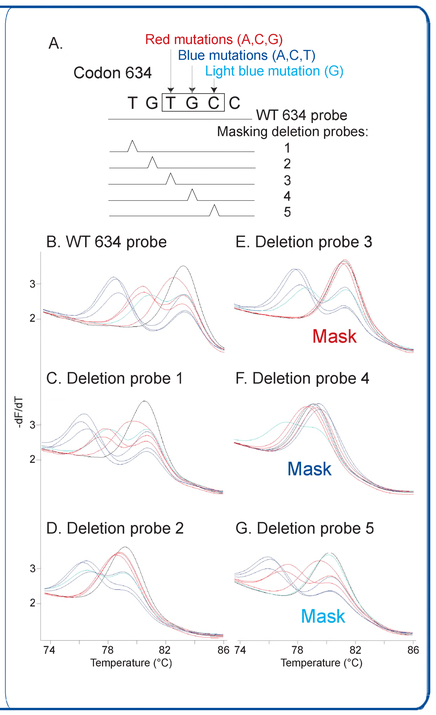
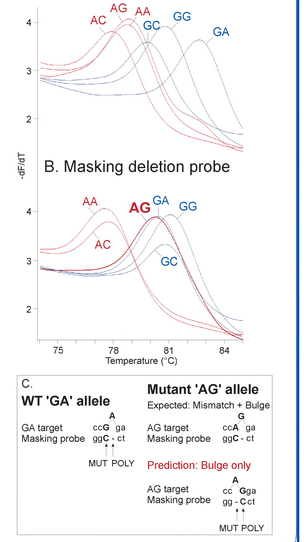


Figure 5

Results:

- The HPA6 c.1544G>**A** mutation is immediately adjacent to the polymorphism c.1545G>A or C.
- Use of the masking deletion probes increased the T_m separation between wild type (GG, GA, GC) and mutant alleles (**A**G, **A**A, **A**C), with an exception (Fig. 5).
- The wild type 'GA' and the mutant '**A**G' allelic nucleotide sequences resulted in very similar T_ms (Fig. 5B and E). Both these alleles are predicted to have only a single nucleotide bulge with the masking deletion probe (Fig. 5C).
  * Single base deletion probes create a single base bulge in the target DNA strand, usually at the position opposite the deleted base, but the bulge can be at alternate positions depending on the nearest neighbors. An immediately adjacent mutation would be expected to result in further destabilization (a mismatch next to a single base bulge). However, if the mismatched nucleotide in the probe can complement the otherwise bulged base in the target, then the position of the bulge "shifts", resulting in a single base bulge surrounded by matched pairs. In this case, both the wild type and mutant duplexes have a similar stability (both single base bulges).
- Such a situation cannot be avoided when the possible nucleotides for the mutation and the adjacent polymorphism are the same (e.g. both n.G>A).
  * Alternatively, use a masking probe with an unmatched nucleotide or universal base at the polymorphism location.



Legend: Homozygous engineered templates of six different combinations of the c.1544G>**A** mutation and adjacent polymorphism sequences (c.1545G>A or C) were tested: GG, GA, GC, **A**G, **A**A, and **A**C. The three mutant allele traces are in red (**A**G, **A**A, and **A**C), while the three wild type allele traces are in blue (GG, GA, and GC). A: The wild type probe (WT HPA6 probe). B: Masking deletion probe. The mutant '**A**G' allele with a T_m suggesting wild type is shown in bold type on the graph. C: The proposed duplexes of the two genotypes with very similar T_ms are displayed. The target sequences are shown above the complementary masking deletion probe sequence, with the mutation location (MUT) and polymorphism location (POLY) indicated. The dash ' - ' indicates the position of the probe deletion, located opposite the unpaired, bulged base. For the mutant '**A**G' allele with the masking deletion probe, the expected duplex with a mismatch and a single base bulge at the POLY position is displayed above the predicted duplex with only a single base bulge at the MUT position. It is predicted that for both the wildtype 'GA' allele and mutant '**A**G' allele, the single base bulge 'A' is surrounded by matched base pairs, resulting in similar T_ms.

Conclusion

Masking Technique:

- Clearly distinguished mutations from non-pathogenic polymorphisms.
  - Reduce false positives, negatives.
- Can mask polymorphism immediately adjacent to mutation.
- Locate mutations under probe (single nucleotide or codon).
  - Distinguished mutant alleles of the same T_m with wild type probe.
  - Reduced the number of confirmatory, mutation-specific probes.
- Detect and genotype mutations without sequencing.
  - Simplify probe melting analysis of complicated genes.

Thank you: Dr. Highsmith (Mayo Clinic)

Reference: Closed-tube genotyping with unlabeled oligonucleotide probes and a saturating DNA dye. Zhou L, Myers AN, Vandersteen JG, Wang L, Wittwer CT. Clin Chem. 2004 Aug;50(8):1328-35.

Highsmith, William E., Ph.D.
From: Highsmith, W. Edward Jr., Ph.D. [Highsmith.W@mayo.edu]
Sent: Monday, March 22, 2004 3:42 PM
To: IRB Minimal Risk Protocol; Highsmith, W. Edward Jr., Ph.D.; Biospecimens Committee
Cc: Highsmith, W. Edward Jr., Ph.D.
Subject: Request for Minimal Risk Protocol Approval

Minimal Risk Protocol Summary

This form will be submitted simultaneously to both the Institutional Review Board and the Biospecimens Subcommittee at Rochester or Scottsdale (if needed). In general, review and approval by both bodies is required prior to activation of the study.
Questions concerning the role of the Institutional Review Board should be directed to: Cindy L. Boyer, Research Services, 6-2808
Questions concerning the role of the Rochester Biospecimens Subcommittee should be directed to: Cheryl Nelson, Rochester Research Services, 4-5920
Questions concerning biospecimens in Scottsdale should be directed to: Linda Romme, Scottsdale Research Services, 2-4443.
Questions or comments regarding this form should be directed to the IRB Office.

LIVING OR DECEASED1 both
BIOSPECIMENS1 YES
DATATYPE1 deidentified
EXTERNAL COLLABORATORS1 YES
INTEND TO PUBLISH YES
PROPOSAL TITLE Provision of de-identified samples to ARUP laboratories for method validation
SITE ROC
PRINCIPAL INVESTIGATOR Highsmith, W. Edward Jr., Ph.D.
PI ID 14143372
CO INVESTIGATOR1 NotAnswered
CO INVESTIGATOR2 NotAnswered
CO INVESTIGATOR3 NotAnswered
CO INVESTIGATOR4 NotAnswered
CO INVESTIGATOR5 NotAnswered
STUDY COORDINATOR NotAnswered
SC ID NotAnswered

PROJECT PROPOSAL Melt-curve analysis is a newly developed technology

for the high-throughput, inexpensive detection of mutations in PCR amplified DNA. Dr. Rong Mao, a former fellow in the Mayo Molecular Genetics Laboratory (MGL), and colleagues at the University of Utah and ARUP Laboratories have developed a melt-curve analysis platform for the detection of mutations in the RET protooncogene using the HR1 High Resolution Melter from Idaho Technologies. Their work parallels work that is currently being done in the Mayo MGL using the same instrument. I propose to send Dr. Mao up to 60 de-identified samples that have been previously characterized with respect to RET gene mutations as part of clinical evaluations for the inherited cancer syndrome multiple endocrine neoplasia, type 2A. These samples either have been or will be evaluated on the HR1 platform in the Mayo MGL in an ongoing study exempted by the IRB April 1, 2003. We will collaborate on optimization and validation of an assay whcih could be faster and less expensive than currently existing methods.
FUNDING SOURCE n/a
FUND AMOUNT n/a

METHODS 1-2 examples of the approximately 40 disease causing RET mutations identifed by the Mayo MGL will be de-idetified and sent to Dr. Mao at ARUP Laboratories. No patient indentifiers will be included. The only information to accompany the specimen will be the identity of the RET mutation.
DATA OR SPECIMENS SOURCE Existing biospecimen
OTHER DATA OR SPECIMENS SOURCE NotAnswered
GCRC USEAGE No
COLLABORATOR NAMES Dr. Rong Mao
COLLABORATING INSTITUTIONS ARUP [ARUP is a commercial reference laboratory owned and operated by the University of Utah]
ACADEMIC INSTITUTION Yes
COMMERCIAL INSTITUTION Yes
BIOSPECIMENS OUTSIDE MAYO Yes
CONTACT INFORMATION Rong Mao, MD Associate Medical Director Molecular Genetics Section ARUP Laboratories Adjunct Assistant Professor of Pathology University of Utah School of Medicine Chipeta Way Salt Lake City, UT 84108 Tel: 801-583-2787 x 3165 Fax: 801-584-5207 e-mail: rong.mao@aruplab.com
EXTERNAL COLLABORATOR ROLE Evaluation of the HR1 method for mutation identification in the RET gene.
CLINICAL MATERIAL TO EXTERNAL COLLABORATORS De-identified DNA
BIOSPECIMEN TYPE DNA
BIOSPECIMEN SOURCE DNA
BIOSPECIMEN OTHER SOURCE NotAnswered
BIOSPECIMENS COLLECTED Existing
BIOSPECIMEN SAMPLE NUMBER 60
BIOSPECIMEN IDENTIFICATION Other
OTHER ID Mutation previously identified in clinical test
SPECIMENS STORAGE BUILDING Hilton

SPECIMENS STORAGE FLOOR 9
SPECIMENS STORAGE ROOM 9-16
SPECIMENS STORAGE OTHER NotAnswered
BIOSPECIMEN GERMLINE TESTING YES
RESULTS TO PATIENT OR RECORD NO
DE IDENTIFIED DATA No
SURVEY RESEARCH NO
ROCHESTER EPIDEMIOLOGY USED NO
NON MAYO PATIENT INFO NO
RESIDENTS OLMSTED COUNTY NO
PARTICIPANT CONTACT NO
HIPAA WAIVER CONFIDENTIAL DATA Yes
HIPAA WAIVER SUBJECT IDENTIFIERS DESTROYED Yes
HIPAA WAIVER SUBJECT IDENTIFIERS Yes
HIPAA WAIVER IDENTIFICATION Yes
WAIVER CONSENT MINIMAL RISK Yes
WAIVER CONSENT NO ADVERSE EFFECT SUBJECT Yes
WAIVER CONSENT REQUIRED TO DO RESEARCH Yes
WAIVER CONSENT SUBJECTS ADDITIONAL INFORMATION Yes
REQ EMAIL Highsmith.W@mayo.edu

Emailed to:
irbminimalriskprotocol@mayo.edu,Highsmith.W@mayo.edu,biospecimens@mayo.edu
PI email: Highsmith.W@mayo.edu

MAYO FOUNDATION

Please direct any questions regarding the completion of this form to the IRB Office at the phone extension or e-mail address to the left.

FROM: MAYO FOUNDATION INSTITUTIONAL REVIEW BOARDS
201 BUILDING, ROOM 4-60
PHONE 4-2329 • FAX 8-0051 • E-MAIL irbprogressreports@mayo.edu

DATE: **02/28/2005**

TO: **HIGHSMITH,W,E Jr., PhD**

RE: ANNUAL REVIEW OF IRB PROTOCOL **701-04** REVIEW TYPE: **Expedited** REVIEW COMMITTEE: **Expedited Review Committee**
"Provision of De-identified Samples to ARUP Laboratories for Method Validation"

Progress Report Instructions and Report Form

Please read these instructions completely and carefully

According to our records, the IRB has previously sent a progress report reminder notification. Federal regulation [45CFR46.1009(e)] requires the Institutional Review Boards (IRB) to review protocols at intervals appropriate to the degree of risk, but not less than once per year. At this time, the due date for the above named protocol's annual review is now 30 days away. **Approval of this protocol will expire on Mar-29-2005 unless the IRB approves a completed progress report prior to this date.** You are responsible for submitting a continuing or final progress report with all required materials in time for review by the IRB before this expiration date. *Failure to submit a complete progress report may cause your protocol to expire before it can be approved.* Please note that the deadline to make an Expedited Review Committee agenda, the deadline is noon central time, the Thursday prior to the meeting. Note that the deadline for an agenda may change due to holidays.

A complete progress report ***must*** include a **single-sided** copy of the most recently IRB-approved consent form(s) (if applicable). Double-sided copies will **not** be accepted. This document does not need to be included if the answer to 3a is "Yes" and the number entered for question 4 is "0" (zero).

DO NOT include registration numbers (clinic numbers) or any other patient identifiers in your progress report submission.

If all supporting documents to the progress report can be sent electronically, please e-mail the documents (along with this completed form) as separate attachments in the same e-mail, using "Progress Report" for the subject, to irbprogressreports@mayo.edu. Do not combine the progress report form with other materials into a singular attachment for e-mail. Submissions of this kind will not be accepted by the IRB.

If **any** of the supporting documents cannot be sent electronically, please print this completed progress report form, place it on top of the packet of the supporting documents, and send the entire packet to: IRB Progress Reports Secretary, 201 Building, Room 460.

Please do not submit more than one copy of your completed progress report to the IRB.
Keep a copy of your entire progress report for your records!

If the protocol involves the General Clinical Research Center (GCRC), you are responsible for sending a complete copy of the progress report and all supporting materials (except the protocol) to **Shari Brumm, GCRC, Domitilla 5-521**

Progress Report Form

Date: **02/28/2005**

Name of Principal Investigator: **HIGHSMITH,W,E Jr., PhD**

Review Type: **Expedited**

IRB #: **701-04** Review Committee: **Expedited Review Committee**

Title: **"Provision of De-identified Samples to ARUP Laboratories for Method Validation"**

Expires: **Mar-29-2005**

If the IRB consent type for this protocol is "waived," please complete the online progress report form at http://wolfpack2.mayo.edu/resis/irb/chart_review.cfm instead of using this form. The address above will need to be typed into your web browser's address bar.

Please complete this form by clicking on the appropriate check boxes and typing in the text fields.

PLEASE TYPE ALL NARRATIVE COMMENTS

Conflicts of Interest: The following reflects the current status for all study personnel:

☒ There are no new conflicts to disclose

☐ One or more study personnel now have a conflict of interest. (Please contact the Conflict of Interest (COI) Review Board to report and resolve this conflict before submitting to the IRB. A copy of the minute item response from the COI Review Board should be forwarded with this submission).

Please answer the following question BEFORE continuing with the rest of this form.

Does this IRB number refer to a grant application under which all active protocols are separately submitted to the IRB for review (i.e., no subjects are enrolled or no patient data collected under this IRB number)? .....Yes ☐ No ☒

→ **If "Yes", please list the IRB numbers (or titles if an IRB number has not yet been assigned) of protocols supported by this grant in the box below and then answer only questions 1 and 2.**

COMPLETION OF THIS SECTION IS REQUIRED FOR ALL STUDIES WHERE HIPAA AUTHORIZATION IS NOT BEING OBTAINED

Request for Waiver of HIPAA Authorization

A Request for Waiver of HIPAA Authorization is required in accordance with 45 CFR 164.512(i).

Please complete this section by checking all boxes that apply.

☒ All study data will be treated in a confidential manner and the same precautions used to protect patient clinical data will be employed.
NOTE: *If you are unable to check this box, please describe in the box below the precautions that will be taken to prevent inappropriate use of the data.*

☒ All subject identifiers will be destroyed upon completion of the research.
NOTE: *If you are unable to check this box, please explain in the box below why the retention of the identifiers is appropriate.*

☒ I certify that the subject identifiers will not be reused or disclosed to any other person or entity, except as required by law, for authorized conduct and oversight of the study, or for other IRB-approved research.

☒ The research could not be practicably carried out without access to and use of the subjects' identifying information.

I. Protocol Status

1. **Do you want to continue this protocol in an active status? (If any participants are still receiving study intervention or are being followed per protocol, the protocol must continue in an active status.) .....Yes ☐ No ☒**

2. This protocol is being conducted under this IRB number at (check all that apply) .....MCR ☐ MCJ ☐ MCS ☐
If this protocol is being conducted at more than one Mayo site under this IRB number, it is the responsibility of the protocol's principal investigator to submit a progress report that includes data from all participating Mayo sites.

II. Protocol Activity

3a. Is the research permanently closed to the enrollment of new people? .....Yes ☒ No ☐

3b. If "Yes", have all currently enrolled participants completed study interventions? .....Yes ☒ No ☐

4. How many participants have been enrolled at Mayo since IRB approval was last received? **50**
If this is the first progress report for this protocol, please enter the same number in questions 4 and 5. Do not leave either field blank.

5. How many participants have been enrolled at Mayo since the study was originally approved? **50**

6. How many participants (at Mayo) have been approved for enrollment by the IRB?.....**60**

7. If the IRB approved screening of additional participants in order to meet target accrual, please indicate the total number approved for screening (that is, the total number approved for enrollment plus additional screens).....**60**
If there is no approval of additional participants for the purposes of screening, please enter the response from question 6 in the box for question 7. Do not leave either field blank.

8. Are Mayo participants still being followed per protocol?.....Yes ☐ No ☒

9. Briefly summarize (in the box below, in 200 words or less) the protocol activity since IRB approval was last received. Include progress to date and future plans.
50 de-identified samples with previously characterized RET protooncogene mutations were sent to Dr. Mao at ARUP Laboratories for validation of a new test protocol. Results have been presented as a poster at a national meeting.

10a. Have any changes occurred to the Mayo personnel involved with this study that have not been submitted to the IRB via the Protocol Modification Request Form?.....Yes ☐ No ☒

10b. If "Yes", please list in the box below the full name and role (i.e., principal investigator, co-investigator, study coordinator, etc.) of all Mayo personnel being added or removed from the study. If any personnel are being replaced, please indicate if they will be remaining on the study under a different role.

Remember that personnel must successfully complete the Mayo Training Program for Protecting Human Subjects (http://researchweb.mayo.edu/mtp-phs/) prior to participating in a human research project.

11a. Have any changes in the specific aims, study procedures, or consent form occurred that have not been approved by the IRB? .....Yes ☐ No ☒

11b. If "Yes", please explain in the box below.

12a. Have any changes in the eligibility criteria occurred that have not been approved by the IRB?.....Yes ☐ No ☒

12b. If "Yes", please explain in the box below.

13. Since IRB approval was last received, has the study been audited or monitored by any outside sources (i.e., study sponsor, ECOG, NCCTG, NCI, etc.)? .....Yes ☐ No ☒
If "Yes", a copy of the sources' audit report, monitor report or summary must be included with this progress report.

14a. Has anything appeared in the pertinent medical literature that affects the conduct of this study, the anticipated benefits, or the potential risks?.....Yes ☐ No ☒

14b. If "Yes", please explain in the box below.

15. If any publications or presentations have resulted from the work related to this study, please list them in the box below.
Abstract - 1.Margarf RL, Mao R, Highsmith WE, Holtegaard LM, Wittwer CT, Mutation scanning of the RET protooncogene using unlabeled probes and high-resolution melting analysis. J Molec Diag 2004; 6(4):435.

III. Review of Risks to Research Participants

16a. Have any additional risks been identified since IRB approval was last received? ..... Yes ☐ No ☒

16b. If "Yes", and these risks have not been reported to the IRB, please summarize in the box below.

17a. Briefly describe (in the box below) the frequency and severity of all adverse events (including those already reported to the IRB) that have occurred since IRB approval was last received.

None

The investigator is reminded that all serious adverse events must be reported to the Serious Adverse Events/Deviations Board. Do not attach SAE/Deviation forms to this progress report.

17b. Also indicate (in the box below) whether the adverse events are similar in type, frequency and severity to what was expected before the study, and if not, how they differ from expectations.

17c. If this protocol is a multi-center study, please also describe (in the box below) whether Mayo's experience with adverse events in this study is comparable with that at other institutions.

18a. Was there any unusual increase in the frequency of serious but expected adverse events among Mayo participants? ..... Yes ☐ No ☐

18b. If "Yes", please describe in the box below.

IV. Informed Consent Evaluation – (Applies to both written and verbal consent)

19a. Have any problems occurred with regard to obtaining and documenting of the informed consent? ..... Yes ☐ No ☐

19b. If "Yes", please describe in the box below.

20. In the box below, briefly state each reason for the withdrawal of research participants (whether voluntary or not) from the study. For each reason given, please state the number of research participants withdrawn since IRB approval was last received.

21a. Have there been any unanticipated problems with the retention of participants? ..... Yes ☐ No ☐

21b. If "Yes", please describe in the box below.

22. Are the consent/assent form documents still acceptable (i.e., the information contained in the document is accurate and complete and there is no new information that may have been obtained since the last IRB approval which should be disclosed to participants)? .... Yes ☐ No ☐ Verbal Consent ☐

If "No", please e-mail (to irbprogressreports@mayo.edu) an electronic copy of all recommended changes to the consent/assent form(s).

Research: Pre-Submission Approval Form

Approval is required **before information is presented outside of ARUP and enters the public domain** to ensure that HIPAA and IRB protocols have been followed. Please ensure that this document is signed and appropriate documents are attached before submitting any information for publication/presentation outside of ARUP.

☑ Attach copy of (please indicate) manuscript, poster, abstract, or other presentation

Presentation/Poster presented at (specify meeting or conference): Association for molecular pathology meeting

ARUP Cited: Yes ☑ No ☐ If no, state reason: _____

☐ Global IRB #7275 applies, and PRCS-0020, *Internal Sample Request: De-Identification of Samples* has been followed **OR**

☑ Independent Institutional Review Board (IRB) approval, IRB# 701-04
Attach copy of approved IRB protocol.

OR

☐ IRB is not applicable. Please explain: _____

Scientist/Researcher: _____ Date: _____
Signature

Approval Signatures and Dates:

Medical Director: _____ Date: _____

R&D Group Manager or
ARUP Privacy Officer: _____ Date: _____

CONFIDENTIAL: This material is prepared pursuant to Utah Code Annotated, 26-25-1, et seq., for the purpose of evaluating health care rendered by hospitals or physicians and it NOT PART of the medical record.