CONFIDENTIAL

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Document Title PRESEPT Study: Prospective Evaluation of Septin 9 Performance for Colorectal Cancer Screening

Document Number: SPR0006

DMS File Number: Tbd

Revision Level	Effective Date	DCN	Description of Revision	Revision Author
Rev 1	11 April 2008	0659	Initial Release	Cathy Lofton-Day & Michael Wandell
Rev 2	11 August 2008	0820	Recommendations from CSSC, clarifications & editorial changes	Cathy Lofton-Day & Michael Wandell

Approval:

Department (name signatory)	Signature	Date (dd.mm.yy)
Study Director (Michael Wandell)		
Quality Assurance (Elli Neu)		
Project Management (Cathy Lofton-Day)		
Study Management (Neil Mucci)		
Biostatistics (Matthias Burger)		
Study Quality Assurance (Esmeralda Heiden)		

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Responsibility (name signatory)	Signature	Date (dd.mm.yy)
Study Director (Michael Wandell)		15 AUG 08
Quality Assurance (Elli Neu)		15.08.08
Project Management (Cathy Lofton-Day)		18-08-08
Study Management (Neil Mucci)		25.08.08
Biostatistics (Matthias Burger)	M. Burger	15/08/2008
Study Quality Assurance (Esmeralda Heiden)	E Heiden	15.08.2008

15.08.2008

Statement of Compliance

The PRESEPT study has been designed, and will be conducted, in accordance with the following regulations, standards and guidances to assure adequate protections for human subjects, adequate institutional and ethics review, and good clinical practices (GCPs). The Principal Investigators and Sponsor will assure adequate protections for human subjects and the scientific validity of the data that govern human investigations in the United States and the European Union.

- 45 CFR 46, U.S. Code of Federal Regulations applicable to clinical studies
- 21 CFR 50, Protection of Human Subjects
- 21 CFR 54, Financial Disclosure by Clinical Investigators
- 21 CFR 56, Institutional Review Boards
- 21 CFR 812 (Procedures for the conduct of clinical studies with medical devices)
- EN 13612, Performance Evaluation of In Vitro Diagnostic Medical Devices
- ICH GCP E6, Good Clinical Practice, Consolidated Guidance
- 21 CFR 820 Subpart C, Design Controls of the Quality System Regulation
- Guidance for Clinical Trial Sponsors: Establishment and Operation of Clinical Trial Data Monitoring Committees Federal Register (March 28, 2006; Volume 71, Number 59)
- Guidance for Industry: Computerized Systems Used in Clinical Investigations FDA, Part 11, Electronic Records; Electronic Signatures — Scope and Application, 2003
- Guidance for Industry: Guideline for the Monitoring of Clinical Investigators

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Principal Investigator:

Signed: Date:

Printed: *Title; Name*

LIST OF ABBREVIATIONS

CRC	Colorectal Cancer
CRF	Case Report Form
CSSC	Clinical Study Steering Committee
FDA	Food and Drug Administration
FOBT	Fecal Occult Blood Test
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IC	Informed Consent
ICH	International Conference on Harmonization
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
NAD	No Apparent Disease
NPV	Negative Predictive Value
OHSR	Office of Human Subjects Research
PI	Principal Investigator
PPV	Positive Predictive Value
QA	Quality Assurance
SOP	Standard Operating Procedure

Protocol Summary

Title: **PRESEPT Study: Prospective Evaluation of Septin 9 Performance for Colorectal Cancer Screening**

Population: 7500 prospectively enrolled Subjects fifty years old or older, at average or increased risk for colorectal cancer and guideline eligible for colorectal cancer screening.

Number of Sites: 15 – 20 study sites in the United States and Germany

Study Duration: 12 – 18 months, depending on accrual

Subject Duration: Less than one hour

Primary Objective

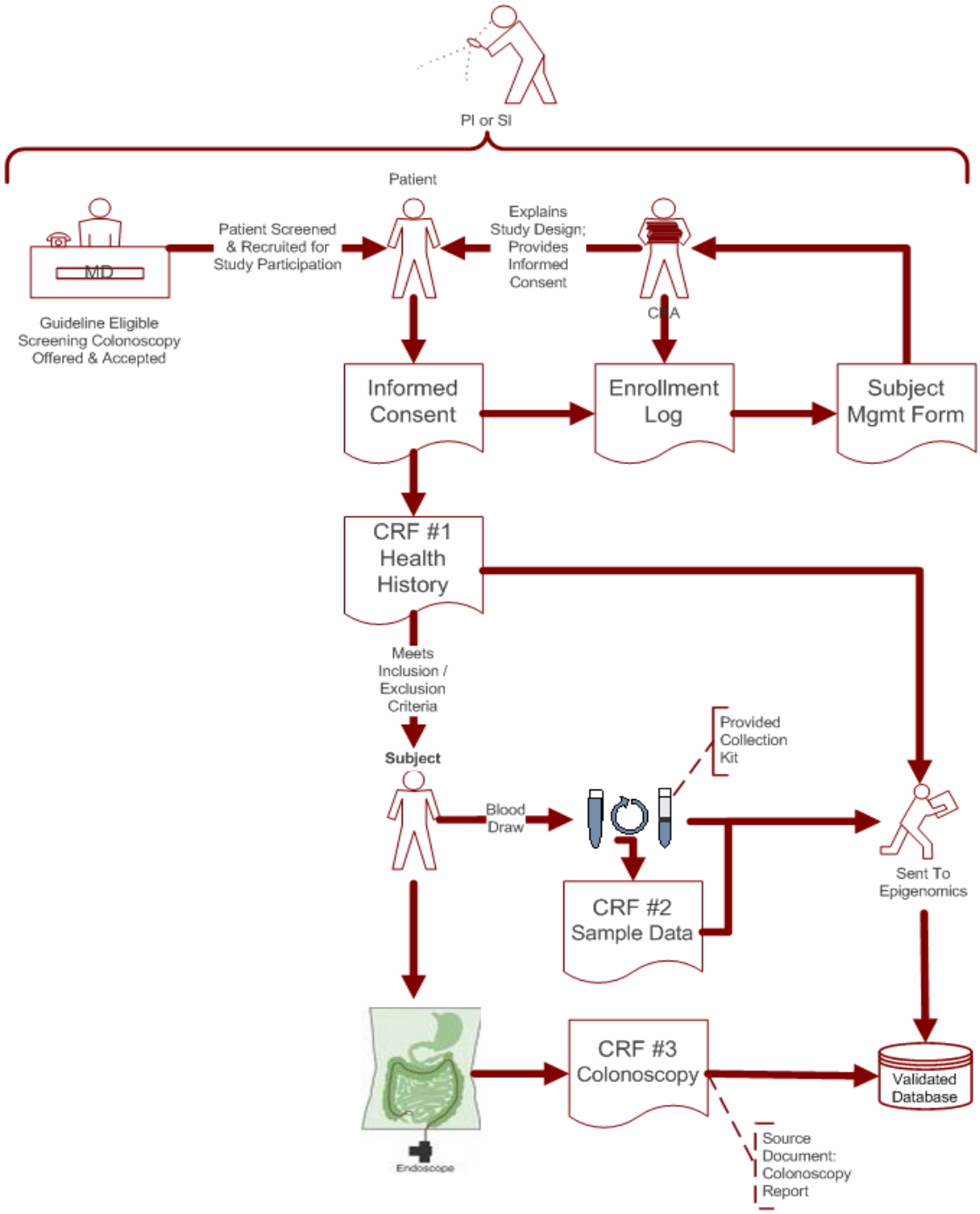
The primary objective of the investigation is to evaluate and describe the clinical performance of the Septin 9 Biomarker for detecting invasive colorectal adenocarcinoma in screening guideline-eligible individuals scheduled for screening colonoscopy.

Secondary Objective

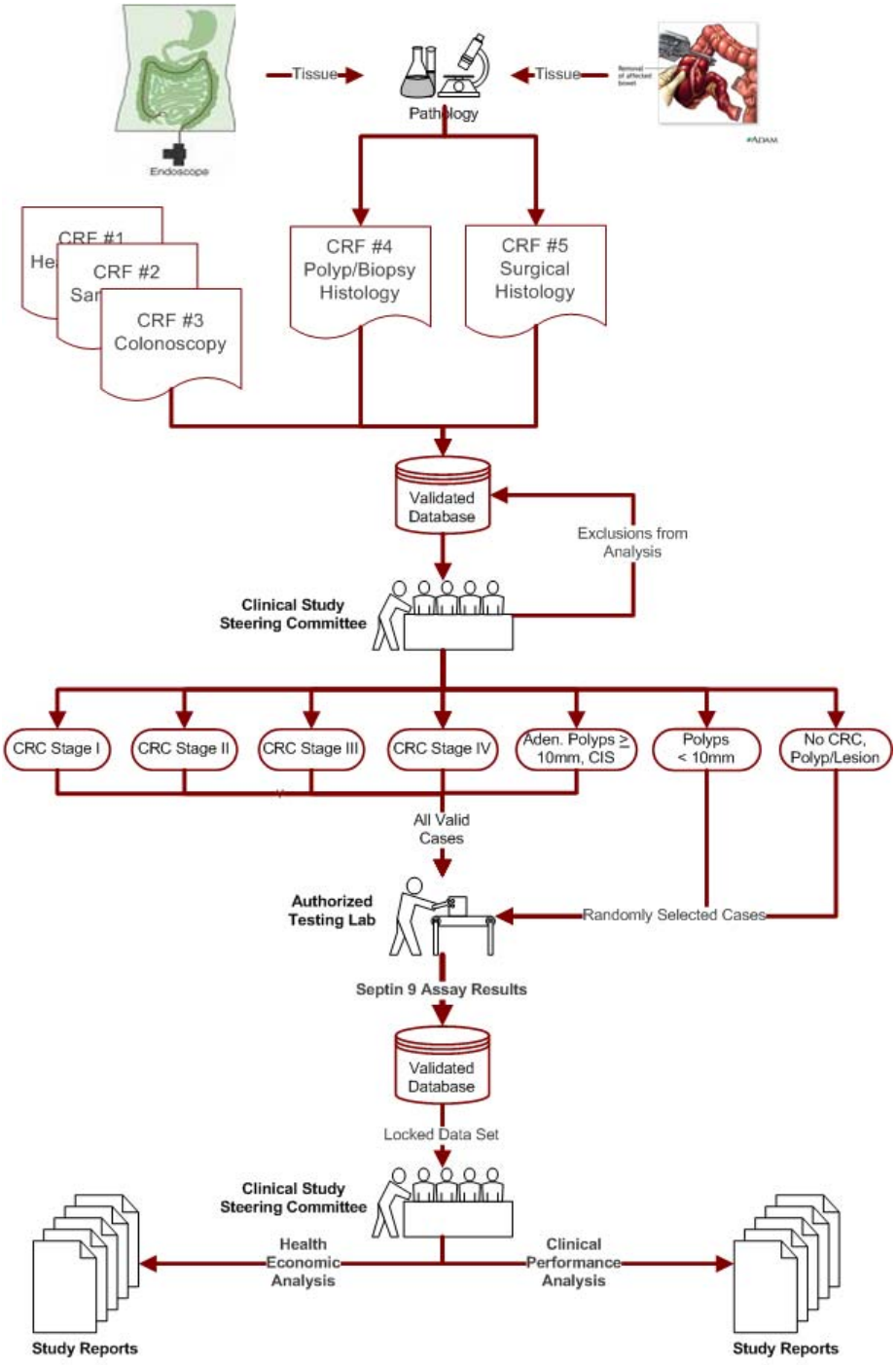
A second objective is to evaluate and describe the clinical performance of the Septin 9 Biomarker for colorectal adenomatous polyps 10mm or larger, flat lesions, or non-invasive adenocarcinoma.

Schematic representations of the study process follow.

Study Design Schematic I - Enrollment through Colonoscopy (All Subjects)



Study Design Schematic II - Tissue Analysis through Results Interpretation (Subjects requiring histopathological analysis)



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1 Study Responsibilities

1.1 Name and address of the Sponsor

Epigenomics Inc
1000 Seneca Street, Suite 300
Seattle, Washington 98101

1.2 Name and title of the person(s) authorized to sign protocol and protocol amendments for the Sponsor:

Study Director: Michael Wandell, PharmD, Senior Vice President, Regulatory & Quality

1.3 Name and title of the Sponsor's medical expert(s) for the investigation:

Esmeralda Heiden, MD, Pathologist & Medical Expert
Stephan Niemann, MD, Medical Director Diagnostics

1.4 Name and title of the Clinical Study Quality Assurance Personnel

Esmeralda Heiden, MD, Pathologist & Medical Expert

1.5 Name and title of the PRESEPT Study Lead Biostatistician

Matthias Burger, Biostatistician

1.6 Study Monitors (in addition to the above named Sponsor Representatives, the following personnel are authorized to act as Study Monitors):

In addition to the above named Sponsor Representatives, the following personnel are authorized to act as Study Monitors:

Jennifer Maas, Clinical Study Manager
Su Yamamura, Clinical Research Associate
Cathy Lofton-Day, PhD, Vice President, Molecular Biology, Diagnostics
Nura Sayed Suleiman, Clinical Research Coordinator
Marion Harting, Clinical Research Coordinator
Shannon Payne, Ph.D. Senior Scientist, Diagnostics
Nicole Rogers, Clinical Research Associate
Neil Mucci, Senior Clinical Research Manager
Ashley Mayo, Clinical Research Coordinator

2 Background Information and Scientific Rationale

2.1 Background Information

Colorectal cancer is the second leading cause of cancer related death in the U.S. with an estimated direct medical treatment cost of $8.3 billion in 2007. With a cure rate over 90% if diagnosed in early stages in the United States, there is now general agreement that average-risk adults aged 50 and older should be screened for colorectal cancer (CRC). If detected early, individual treatment costs for colon cancer are estimated at $30,000 per patient, whereas treatment for a patient who has developed late stage disease is estimated at $120,000.[1] However, less than 50% of the screening population has had a recent test.[2] Given today's treatment options for colorectal cancer, patient outcomes could potentially be greatly improved if more cancers were detected in early stages.

Epigenomics has identified methylated gene regions that are specific for colorectal cancer or pre-malignant tissue.[3] Aberrantly methylated genes represent attractive candidate markers for cancer screening, as cancer-specific methylation changes occur early in tumorigenesis, appear to be stable, yield a positive amplifiable signal, and can be assayed with high analytical sensitivity. Since methylation occurs early and in distinct genomic areas, it is possible to achieve high clinical sensitivity with a small number of methylated DNA markers. Studies have shown that aberrantly methylated DNA markers can be detected in tissue[4] and body fluids[5] and are highly correlated to colorectal cancer. Assays for the detection of methylated biomarker tumor DNA shed into the blood stream have been tested in clinical case control studies, but for acceptance into clinical routine, further evaluation and validation in screening guideline eligible individuals is required.

The purpose of this study is to collect blood specimens and clinical data from screening guideline eligible individuals designated by their physician to receive a screening colonoscopy, and to evaluate the performance of a colorectal cancer-specific DNA methylation biomarker for detection of colorectal cancer in this cohort. Based on the outcome of the colonoscopy, polypectomy, biopsy and surgical tissue histopathology, the clinical utility of Septin 9 as colorectal cancer screening test will be evaluated.

[1] www.eifoundation.org/national/nccra/report_card/docs/CRC_Cost_Fact_Sheet.doc
[2] Colorectal Cancer Facts and Figures (2005); American Cancer Society
[3] DNA-Methylation Biomarkers for Blood-Based Colorectal Cancer Screening.Clin. Chem., 2008; 54 (2): 414-423. Lofton-Day C, Model F, DeVos T, Tetzner R, Distler J, Schuster M, Song X, Lesche R, Liebenberg V, Ebert M, Molnar B, Grutzmann R, Pilarsky C, and Sledziewski A.
[4] M. Esteller. Relevance of DNA methylation in the management of cancer. Lancet Oncology, 1:4 (6), 351-358
[5] H-Z. Zou *et al.*, Detection of Aberrant p16 Methylation in the Serum of Colorectal Cancer Patients. Clin. Cancer Res., *8:* 188–191, 2002

2.2 Medical Rationale

From public health as well as health economics perspectives, the poor adoption of current screening options limits the effectiveness of CRC screening initiatives; as stated by Sidney Winawer, MD, "the best test is the one that gets done."[6] Current CRC screening guidelines include FOBT, sigmoidoscopy (alone or with FOBT), or colonoscopy. Non-invasive screening is conducted using FOBT, which while inexpensive, exhibits a low compliance rate (around 16% in the US) due to its use restrictions, perceived inconvenience and lack of consumer acceptance. The gold standard procedure for CRC detection is colonoscopy; it exhibits excellent performance characteristics, but has a limited utility as a first line screen due to its high cost, healthcare delivery resource limitations, and inadequate patient acceptance. It is believed a noninvasive, first-line screening assay capable of detecting individuals with colorectal disease, confirmed by colonoscopy, would have greater utility for population screening.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Because participation in this investigation is limited to Subjects already scheduled to undergo screening colonoscopy, the only potential risks to study Subjects are associated with a blood draw and possible loss of confidentiality of protected personal health information. Possible loss of confidentiality of protected personal health information is mitigated by study sites providing only pseudonymized data to the Sponsor (except during monitoring visits where source documents will be reviewed to verify the accuracy of CRFs). The risks for the Subject are the same as for any other standard clinical peripheral blood draw and include minor bleeding, bruising, fainting, local inflammatory reactions with swelling and very rarely, nerve damage.

2.3.2 Adverse and Serious Adverse Events

Due to the absence of contact of investigational Subjects with a device and the prohibition of the use of investigational tests for medical decision-making, it has been determined that no chance exists for the occurrence of Adverse or Serious Adverse Events. Therefore no provision has been made for the filing or handling of documentation associated with such occurrences.

2.3.3 Potential Benefits

Participating Subjects may receive a small payment (to be determined by each study PI) to compensate for the inconvenience associated with taking part in the study. Subjects may also benefit from the personal satisfaction derived

[6] 16 December 2003 Annals of Internal Medicine Volume 139 • Number 12

from helping medical science better understand colorectal cancer and contributing to the demonstration of a blood based molecular test to offer doctors and patients an alternative to other methods for colorectal cancer screening.

3 Objectives

3.1 Primary Objective: Evaluate Septin 9 Colorectal Cancer Detection

The primary objective of the investigation is to evaluate and describe the clinical performance characteristics (sensitivity, specificity, NPV, PPV) of the Septin 9 Biomarker for the detection of invasive colorectal adenocarcinoma in average to increased risk, screening guideline-eligible population.

3.2 Secondary Objective: Adenomatous Polyp or Nonpolypoid Lesion Detection

The secondary objective is to describe the performance characteristics (sensitivity, specificity, NPV, PPV) of the Septin 9 Biomarker for individuals having colorectal adenomatous polyps10mm or larger, flat lesions (nonpolypoid, flat/depressed colorectal neoplasm) or non-invasive adenocarcinoma.

3.3 Study End-Points

3.3.1 Primary Endpoint

Clinical/surgical diagnosis of invasivecolorectal adenocarcinoma detected by optical colonoscopy and confirmed by histology compared to the Septin 9 Biomarker classification.

3.3.2 Secondary Endpoint

Detection of adenomatous polyp(s) equal to or greater than 10 mm, flat lesion (s) or non-invasive adenocarcinoma by colonoscopy and confirmed by histology compared to the Septin 9 Biomarker classification will also be described.

4 Study Design

4.1 General Design

The study is designed as a prospective, open enrollment clinical investigation involving multiple clinical study sites in the United States and Germany. Subjects will be competitively enrolled at multiple sites until at least 50 invasive colorectal adenocarcinoma cases identified by screening colonoscopy and verified by clinical and histopathological examination have been enrolled. The primary objective of the investigation is to evaluate and describe the clinical performance of the Septin 9 Biomarker for detecting the 50 individuals with invasive colorectal adenocarcinoma identified in this population representative of the US screening guideline eligible population. Secondary objectives will be to evaluate and describe performance characteristics of the biomarker in individuals with adenomatous polyps 10 mm or larger, flat lesion (s) or non-invasive adenocarcinoma.

Collaborating sites will identify and contact patients scheduled for screening colonoscopy. These patients may be screened by the PI or designee to determine the patients' appropriateness for, and interest in, study participation. Study site personnel will meet with patients meeting eligibility guidelines and offer them participation. Patients interested in participation and who provide written informed consent will be enrolled as Subjects in the study.

Consented, enrolled Subjects will be interviewed by the PI, Sub-I or designee to obtain their personal health history using Attachment A. If, based on information provided in the health history interview, the Subject is determined not to meet inclusion or exclusion criteria, the Subject will be withdrawn from the study, excluded from analysis and the appropriate box checked on the Subject Management Form (Attachment M) which is held in the site's regulatory binder. Eligible consented Subjects meeting inclusion / exclusion criteria will have 40 ml of blood drawn prior to undergoing bowel cleansing in preparation for screening colonoscopy. Subject blood specimens will be processed according to Epigenomics' plasma preparation procedure (Blood Collection and Plasma Preparation SOP(LCSA006)(Attachment P)) and case report forms (CRFs)(Attachments A-E) (or their electronic equivalent) will be completed in compliance with Good Clinical Practice (GCP). Processed samples will be frozen and shipped to Epigenomics for storage. Electronic or paper copies of Attachments A-E will be provided to Epigenomics for entry into its secure database. Epigenomics will qualify, train and monitor sites for compliance with sample and data collection procedures, and will serve as the repository for samples and patient data.

Based on the colonoscopy results, polyp or biopsy histopathology, and/or surgical resection histopathology, all Subjects diagnosed with colorectal cancer or large adenomatous polyps or flat lesions, and subsets of Subjects with other polyps or no apparent disease (NAD, none of the foregoing) will be selected under the auspices of the Clinical Study Steering Committee (CSSC) (Section 6.1) for analysis using the Septin 9 methylation assay according to the defined statistical analysis plan (SAP, Section 10.8) . Plasma from

masked, selected cases will be analyzed using a research molecular diagnostics assay performed in one or more qualified molecular diagnostics laboratories collaborating with the Sponsor.

The assay results, clinical data and histopathological data will then be statistically analyzed by a subgroup of the investigators under the auspices of the CSSC, to determine the performance characteristics of the biomarker in this population. The Sponsor may also authorize use of these samples and data by partners seeking to validate in vitro diagnostic and clinical laboratory test services based on this technology for routine medical testing, which fully provide human subjects protections described herein. In addition to evaluating the clinical performance of the molecular diagnostic biomarker, the clinical performance data from the PRESEPT study will also be evaluated using a validated health economic analysis to estimate the potential health economic impact for the detection of colorectal cancer.

4.2 Study Duration

The projected study duration from “first Subject in” to “last Subject out” is 12 to 18 months. At study close-out, approximately 18 months from initiation, it is anticipated that 7,500 Subjects will be enrolled which will be sufficient to detect the required 50 cancers, a final analysis is planned. An interim analysis may take place at the end of 2008 at which time 5000 cases are expected to be enrolled.

4.3 Number of Subjects Required

The accrual target for colorectal cancer cases from the average and increased risk screening population is a minimum of 50. The CRC prevalence in the screening guideline-eligible population is difficult to determine but is estimated to be between 0.6-0.7% based on several studies.[7,8] Because prevalence of CRC and precancerous lesions (polyps) increases with age, an inappropriately low CRC prevalence will be avoided through a protocol design which reduces the probability of over-representation of younger, screening eligible age groups by assuring recruitment of a population representative of all screening eligible ages.

[7] Altenhofen, et al. Feasibility and first results: National programme of screening colonoscopies in Germany 2003-2005. Central Research Institute of Ambulatory Health Care in the Federal Republic of Germany, Berlin, June 6, 2007.

[8] Imperiale et al. Risk of advanced proximal neoplasms in asymptomatic adults according to distal colorectal findings. New Engl J Med 2000; 343 (3): 169-174.

Age Group	CRC Prevalence Observed (%)	Contribution Weight (%)	Study Target Enrollment	CRC Cases Expected
50-59	0.3	25	1875	5
60-69	0.6	45	3375	19
> 70	1.2	30	2250	28
Total	0.7	100	7500	52

Table 1. Anticipated age stratification of individuals enrolled in PRESEPT study and expected CRCs based on estimated prevalence.

Through the implementation of such a design it is projected that 12-15 study sites in the U.S. and 3-5 study sites in Germany, competitively enrolling approximately 7500 U.S. guideline eligible individuals aged 50 and older will yield 50 CRC cases. The Sponsor anticipates that identification of a minimum of 50 CRCs can be achieved by enrolling an approximately equal distribution of males and females, and balancing accrual across all sites to achieve a population approximating the percentages by age shown in Table 1 which were observed in a recently reported large population undergoing first-time screening colonoscopy.[7]

4.4 Duration of Subject Participation

Direct Subject participation is limited to the following activities: eligibility interview (strictly speaking not a study activity), providing informed consent, study enrollment, personal health history interview, and a single study blood draw.

It is estimated that the time spent by each Subject will be an hour or less (see Table 2). However, Subjects will be followed through colonoscopy, and surgery if required, via medical record review including colonoscopy reports, surgery reports and histopathology and clinical staging reports. The following table specifies the estimated time commitment for each Subject to participate in each study-related activity as defined by completion of required study documents (these activities are over and above the requirements of the Subjects' normal medical care).

More precisely, it is estimated that each Subject may expect to spend a minimum of 18 minutes and a maximum of 40 minutes for participation; in all other study activities the Subject is a passive participant who has consented access to their medical record for data capture.

[7] Altenhofen, et al. Feasibility and first results: National programme of screening colonoscopies in Germany 2003-2005. Central Research Institute of Ambulatory Health Care in the Federal Republic of Germany, Berlin, June 6, 2007.

	Informed Consent	Health History Att. A	Blood Draw Att. B	Colonoscopy Report Att. C	Biopsy / Polypectomy Histology Att. D	Surgical Histology & Staging Att. E
Direct Involvement	**X**	**X**	**X**	–	–	–
Subject Time	10-25 min	3-5 min	5-10 min	N/A	N/A	N/A

Table 2: Estimated Time of Study Subject Direct Involvement

5 Study Population

5.1 Selection of the Study Population

5.1.1 Subject Recruitment

- Any Subject scheduled for screening colonoscopy and that falls within an open accrual classification may be invited to participate in the study.
- Study sites are not required to enroll all suitable patients, if limited by resources.
- Collaborating sites may use alternative methods to identify and recruit potential Subjects for the study which will be submitted to the responsible IRB or IEC for approval.

5.1.2 Eligibility Criteria

The study will be conducted in Subjects scheduled for screening colonoscopy according to current U.S. CRC screening guidelines. In order to initially determine study eligibility, the Principal Investigator or designee may query the Subject and/or health system records to determine that the candidate Subject is:

- Scheduled for colonoscopy for colorectal cancer screening and not for diagnostic purposes;
- 50 years of age or older at the time of the scheduled colonoscopy (CRC screening guideline-eligible, according to current US guidelines[9]);

[9] Screening and surveillance for early detection of colorectal cancer and adenomatous polyps, 2008: A joint guideline from the American Cancer Society, the U.S. Mulit-Society Task Force on colorectal cancer and the American College of Radiology. CA Cancer J Clin. 2008,58: 1-31. Levin et al.

- Capable of providing informed consent for participation in the study, as determined by the patient's ability to read and understand the written informed consent without the need for a personal representative to assist in understanding the instructions;
- Potentially interested in participation and capable of doing so prior to starting bowel preparation.

5.1.3 Subject Informed Consent

Eligible patients will be asked to provide consent for study participation by agreeing to and appropriately signing a written informed consent approved by the authorized Institutional Review Board or an Independent Ethics Committee. All completed Informed Consent Forms are stored in the site's regulatory binder.

5.1.4 Subject Enrollment

Consented Subjects will be enrolled in the study and assigned a Subject identification code (Subject Code). Subject Codes are pre-printed on labels and included in a Sponsor-provided Enrollment Pack. The **Enrollment Pack** contains the Health History CRF (Attachment A) and Sample Processing CRF (Attachment B), a Subject Management Form (Attachment M) and the Subject Traveler (Attachment O) with Subject Code labels attached. For other CRFs (Attachments C-E)), additional Subject Code labels are provided. Study enrollment of a Subject and Subject Code assignment are documented on the Enrollment Log (Attachment F), and a Subject Management Form (Attachment M) is initiated.

5.1.5 Obtaining Subject Health History

The site PI or designee will interview enrolled Subjects prior to bowel preparation to obtain their relevant health history (Health History CRF: Attachment A) and determine their inclusion/exclusion status. Enrolled Subjects who do not meet inclusion and exclusion criteria according to their responses to the Health History CRF (Attachment A) questions must be withdrawn and will not have blood drawn; all Subjects who meet these requirements may progress to the next stage of the study, the blood draw.

- The Health History CRF (Attachment A) may be completed during the same interview as the consent process, if the Subject provides written informed consent prior to recording the CRF data.
- Completed CRFs (Attachments A-E) of all enrolled Subjects, including those not meeting inclusion / exclusion criteria, are stored in the regulatory binder.

5.1.6 Subject Blood Draw

If after obtaining the health history, a Subject is judged to meet the established inclusion/exclusion criteria, a blood sample will be obtained and plasma isolated according to Epigenomics' (Blood Collection and Plasma Preparation SOP(LCSA006) (Attachment P). A **Sample Processing Kit** will be available for each eligible Subject. The Sample Processing Kit contains Sample ID barcoded sample aliquot cryovials and additional Sample ID labels to be used to label the Sample Processing CRF (Attachment B).

After assigning an eligible Subject the Sample Processing Kit, the site PI or designee will ensure all labels present in the kit match and are legible. Labels not required for the Sample Processing CRF (Attachment B) will be attached to the Subject Management Form for later use and filed in the regulatory binder.

5.2 Inclusion Criteria

- Informed Consent provided
- Capable of providing adequate health history
- Age 50 or older at time of colonoscopy (colorectal screening guideline eligible)
- Accessible for blood draw prior to start of bowel preparation for colonoscopy
- First large bowel endoscopy in lifetime

5.3 Exclusion Criteria

- Anorectal bleeding or hematochezia within last 6 months for which patient sought medical attention
- Known iron deficiency anemia in the last 6 months for which patient sought medical attention
- Previous history of colorectal polyps or CRC
- High risk for colorectal cancer (2 or more 1^0 relatives with CRC; 1 or more 1^0 relative(s) < 50 years with CRC; known HNPCC or FAP)

6 Study Procedures/Evaluations

6.1 Clinical Study Steering Committee (CSSC)

6.1.1 Overview

A Clinical Study Steering Committee will be appointed by the Sponsor to oversee the study design for Septin 9 evaluation, conduct reviews and to resolve issues that may arise during the study. The CSSC will be maintained at seven members; the Sponsor will have the responsibility to add members to fill any resignations.

6.1.2 Composition

The CSSC will include a medical expert in colorectal cancer population screening studies unrelated to any study site, a biostatistical expert in cancer study design unrelated to the study analysis, two to three site principal investigators, and two Sponsor representatives (e.g., the study director and the project manager).

6.1.3 Clinical Study Steering Committee Responsibilities

Prior to initiation of the study, CSSC will agree to a charter defining its responsibilities, meeting times and agendas and methods for issue resolution. The following are responsibilities assigned to the CSSC by the Sponsor:

- Review and approve study design and protocol (following IRB/IEC approval)
- Review and approve major study amendments
- Select Subjects for inclusion in interim analysis and final analysis of the Septin 9 Biomarker
- Assure the study is conducted according to GCP quality and ethical standards
- Assure the study data are complete
- Coordinate study interim and final results analysis, publication(s) and presentation(s)
- Assess/adjudicate study endpoints to guarantee that data meet protocol-specified criteria, such as correct staging of colorectal cancer
- Establish, implement and distribute to investigators a publication policy prior to Interim Analysis

6.1.4 Clinical Study Steering Committee Members

The following individuals will be authorized by the Sponsor to act in accordance with statute, regulations, and guidances to fulfill the objectives defined above.

- David Ransohoff, MD, Professor of Medicine, Cancer Epidemiology, Cancer Prevention and Control, University of North Carolina School of Medicine, Chair
- Dale Snover, MD, Adjunct Professor, Department of Laboratory Medicine and Pathology, University of Minnesota Medical Schoo
- Brent Blumenstein, Ph.D., Principal, Trial Architecture Consulting
- Michael Wandell, Pharm.D., Study Director,Epigenomics
- Cathy Lofton-Day, Ph.D., Project Manager, Epigenomics
- Neal Osborn, MD, Co-Director of Clinical Research, Atlanta Gastroenterology
- Timothy Church, PhD, Professor, School of Public Health, University of Minnesota
- Prof. Dr. Thomas Rösch, Charité - Universitätsmedizin Berlin

6.2 Protocol Amendments

Changes in the protocol, as authorized by CSSC, such as changes in the Subjects' eligibility, inclusion, exclusion criteria or major changes to the sample collection or collection of additional data will be reported to the IRB/IEC if required. The approved protocol plus attachments and all amendments will be maintained in each site's regulatory binder.

6.3 Study Document Management

6.3.1 Site PI Responsibilities

The site PI assumes overall responsibility for the ethical and clinical conduct of the study within the institution, as such is accountable for the management of study documents. The Sponsor recognizes the challenges associated with these activities within a large organization and will support each PI in the training and monitoring of study staff to assure correct performance of these activities. The activities which the PI is responsible for include, but are not limited to:

- The accuracy, legibility, and completeness of the data reported to the Sponsor in Attachments A-E, study management documents and in all required reports.
- The secure storage and maintenance of signed informed consent(s), CRFs (Attachments A-E and M), copies of medical records and other

source documents kept at the site in compliance with applicable regulations, local statute and institutional policies.

- Study documents will be completed and retained in compliance with applicable requirements of 21 CFR Part 812, EN 13612 and ICH Good Clinical Practices that are summarized herein.
- Responsibilities of Sub-Investigators, research nurse, study coordinators, research assistants, laboratory personnel and support staff will be defined in the Delegation of Responsibility (Attachment I) and verified in the Training Log (Attachment N) for each site.

6.3.2 Source Documents

All Subject information entered into CRFs (Attachments A-E and M) will be obtained from, or constitute, valid source documents. Specifically, source documents that may be used for obtaining the required information for the study include:

- Information collected by an authorized member of the study staff directly from a Subject, or in reference to a Subject's sample may be entered directly into the appropriate CRF (Attachment A-E and M) which constitutes the source document.
- Electronic or paper medical records (printed electronic records must be labeled with study number, signed and dated).
- Recorded data from automated instruments and printouts of the interpretation of these results from validated software.
- Copies or transcriptions certified after verification as being accurate and complete.
- Microfiches, photographic negatives, microfilm or magnetic media accurately representing medical records, clinical records (colonoscopy) or pathology reports.

6.4 CRFs and Accessibility of Key Source Documents

6.4.1 Case Report Forms (CRFs) (Attachments A-E)

Case Report Forms (CRFs) (Attachments A-E) are the data collection instrument for the study and may be recorded in paper or electronic form. CRFs are to be completed by the site PI, or his/her designee, in accordance with GCP compliant study record completion and storage procedures (Attachment H: Instructions for Case Report Form (CRF) Completion, Storage and Maintenance). During the course of the study and ongoing care of each study Subject, data is recorded in an Enrollment Log (Attachment F), Subject

Management Form (Attachment M) and 3 to 5 different CRFs (Attachments A-E) , depending whether or not colon biopsy or surgery was performed. CRFs include:

- Health History CRF (Attachment A)
- Sample Processing CRF (Attachment B)
- Colonoscopy Report CRF (Attachment C)
- Incisional Biospy/Polypectomy Histology CRF (Attachment D)
- Segmental Colon Resection: Tissue Histology and Staging CRF (Attachment E)

6.4.2 Colorectal Adenocarcinoma Source Documents

Certified copies of source documents obtained from the medical records of the ~50 Subjects with histologically verified colorectal adenocarcinoma including adenocarcinoma in situ (CIS, Stage 0) will be obtained and stored in the Regulatory Binder with the Subjects' CRFs.

The purpose of the certified copies is to facilitate the monitoring with readily accessible data on the staging of Subjects in which colorectal adenocarcinoma was diagnosed. The site PI or Sub-Investigator will assure that he/she automatically receives certified copies of these documents during the course of the diagnostic work-up and therapy of the cases. Should it be required to contact other medical disciplines to obtain copies of these documents, it will be the site PI's or Sub-Investigator's responsibility to establish these contacts and to obtain these copies in a timely manner. The following documents represent the source documents required for all colorectal adenocarcinoma cases:

- Histopathological report(s) on tissue removed during colonoscopy (source document for Incisional Biospy/Polypectomy Histology CRF) (Attachment D)
- Histopathological report(s) on tissue removed during surgery (source document for Segmental Colon Resection: Tissue Histology and Staging CRF) (Attachment E)
- Pathological slides for requested cases.
- Medical report(s) demonstrating the staging of the patient according to the TNM classification
- If applicable, histopathological report(s) on metastatic tissue removed during surgery (identical to source document for Segmental Colon Resection: Tissue Histology and Staging CRF)(Attachment E).

- If applicable, medical reports of investigations conducted during the diagnostic staging work-up or during surgery indicating or verifying the presence of metastases. In case of multiple metastases at one location or metastases at more than one location, documentation of one or several reports unambiguously verifying the presence of metastasis at least at one location should be achieved.
- Source documents may include, but are not limited to: Computed tomographic (CT) scans of chest, abdomen, pelvis; positron emission tomography (PET) scan; magnetic resonance imaging (MRI) scan; needle biopsy; abdominal ultrasonography; and/or, chest X-ray.

6.5 CRF Corrections

For detailed information regarding CRF Corrections see Attachment H, Instructions for Case Report Forms Completion, Storage and Maintenance, Section 2.1.

6.5.1 Discovered by Site, Prior to Submission

If a CRF (Attachment A-E) or Subject Management Form (Attachment M) requires correction prior to submission to Epigenomics the responsible person will strike through the original entry without impairing it legibility and enter the correct response in a legible manner next to it. This amended copy of the Attachment will be submitted and the site PI will assure the change is appropriately made and documented.

6.5.2 Discovered by Site, After Submission

If, after submission of a CRF to Epigenomics, the site PI, or designee, determines that a change, addition or deletion is needed to increase the accuracy of the document, the responsible person will strike through the original entry without impairing its legibility and enter the correct response in a legible manner next to it. This amended copy of the CRF will be re-submitted to the Sponsor and the site PI will assure the change is appropriately made and documented.

6.5.3 Discovered by the Sponsor, Prior to Submission.

If a CRF is found during monitoring that requires correction prior to submission to Epigenomics, the Study Monitor will bring it to the attention of the site PI or designee. If it is agreed a change, addition or deletion is needed to increase the accuracy of the document, the responsible person will strike through the original entry without impairing its legibility and enter the correct response in a legible manner next to it. This amended copy of the CRF will be submitted and the site PI will assure the change is appropriately made and documented.

6.5.4 Discovered by the Sponsor, After Submission

If during data entry, data validity checking or data clean-up, a CRF is found that requires correction after receipt and review at Epigenomics, the Study Director or designee will contact the site PI or designee (if so authorized by the Delegation of Responsibility) to assure the accuracy of the information provided. If it is agreed a change should be made to increase the accuracy of the document, the Principal Investigator or Sub-Investigator will submit an amended copy of the CRF and the Study Director will assure the change is appropriately made and documented.

6.6 Study Documents

6.6.1 Regulatory Binder

The Sponsor will provide each site a regulatory binder in which to store the approved protocol and amendments, blank IRB/IEC-approved informed consent forms, copies of all filled-out CRFs (Attachments A-E), Investigators' CVs, IRB/IEC approval letter, Sponsor correspondence, Delegation of Responsibility Log with signature list and contact information, Sponsor Monitoring Reports, and Investigator's Brochure.

6.6.2 Subject Enrollment Log (Attachment F)

The Subject Enrollment Log is a sequential listing of all enrolled Subjects that links each Subject's unique study identifier (Subject Code) with his or her identity. The purpose of the log is both to provide a master list of all Subjects enrolled at the site and to provide a link between the assigned Subject Code and the confidential Subject identifying information (including name and medical record number). The log is filed in the regulatory binder at the site, and is not provided to the Sponsor. This document provides traceability back to the original Subject and their Informed Consent while safeguarding the Subject's protected health information. Each consented Subject will be assigned a unique Subject Code before any specimens or data are collected. This link will be documented on the Enrollment Log with the following information:

- Name
- Medical record reference number
- Date of enrollment
- Subject Code

6.6.3 Health History CRF (Attachment A)

Must be completed for all enrolled Subjects.

Health history information obtained by the site PI or designee through an interview with the study Subject will be entered into the CRF. The data captured in the Health History CRF is used to verify that the Subject falls into the population at average or increased risk for colorectal cancer and guideline eligible for colorectal cancer screening. Subjects meeting inclusion/exclusion criteria according to the personal health history questions as defined in the Health History CRF may proceed to the blood draw and may be included in analysis. Subjects who do not meet inclusion / exclusion criteria are not appropriate for analysis and should be withdrawn according to defined procedure. This CRF will be the source document for demographic information and medical history including data such as:

- Age at blood collection
- Gender
- Ethnicity
- Colorectal cancer screening history
- Family history of CRC
- Symptoms present at the time of colonoscopy

6.6.4 Sample Processing CRF (Attachment B)

Must be completed for all enrolled Subjects having blood drawn.

The Sample Processing CRF will also be its own source document as the blood specimen collection, processing, storage and shipment are being done exclusively for the study and the data is not part of the Subject's medical record.

Information related to blood sample collection and plasma processing will be recorded on the Sample Processing CRF.

- Time and date of blood draw
- Time and date of plasma processing start
- Total plasma volume and number of plasma aliquots
- Confirmation of buffy coat sample
- Time and date of aliquot freezing
- Compliance of individual processing steps with Plasma SOP (LCSA006).

6.6.5 Colonoscopy CRF (Attachment C)

Must be completed for all enrolled Subjects having colonoscopy. Note: For Subjects with repeat colonoscopy, a separate Colonoscopy CRF (Attachment C) will be completed for each and the duplication noted..

The Subject's colonoscopy report will be the source document for the Colonoscopy CRF and will include the following.

- Date of colonoscopy
- Macroscopic findings and observations from the colonoscopy
- Completeness of colonoscopy
- Polyp related findings: number, size, shape, location
- Interventions performed

6.6.6 Incisional Biospy/Polypectomy Histology CRF, (Attachment D)

Completed only for those Subjects having tissue removed endoscopically at colonoscopy.

The histopathological report on the tissue removed during colonoscopy will be the source document for the Incisional Biospy/Polypectomy Histology CRF and will include the following. Note: for Subjects with multiple histology or pathology reports for the same procedure, a separate Incisional Biospy/Polypectomy Histology CRF will be completed for each and the duplication noted.

- Histology of polyps/lesions (3 largest)
- Size of polyps/lesions (3 largest)
- Location of polyps/lesions (3 largest)
- Histologic type of carcinoma

6.6.7 Segmental Colon Resection: Tissue Histology and Staging CRF (Attachment E)

Completed Only for Subjects Having Colorectal Cancer or Polyp Resection Surgery

The histopathological report on the tissue removed at surgery, either derived from an electronic or a paper medical record, will be the source document for the Segmental Colon Resection: Tissue Histology and Staging CRF and will include the following. Note: for Subjects having multiple histology or pathology reports for the same procedure, a separate Segmental Colon Resection: Tissue

Histology and Staging CRF will be completed for each and the duplication noted.

- Colorectal cancer diagnosis information
- Tumor site
- Tumor size
- Histologic type of tumor
- Histologic grade of tumor
- Staging (pTNM)
- Surgical information
- Date of surgery

6.6.8 Subject Management Form (Attachment M)

Must be completed for all enrolled Subjects.

The Subject Management Form is intended as a tool to track the status of acquisition of Subject data and sample information and record transfer of data and sample information to Epigenomics. This form is also used to record withdrawal of a Subject from the study and transfer this information to Epigenomics.

7 Study Sample Evaluations

7.1 Specimen Collection, Preparation, Handling and Shipping

7.1.1 Instructions for Specimen Preparation, Handling, and Storage

For all Subjects, blood draw and plasma separation will be performed following Epigenomics' Collection and Processing of Plasma SOP (LCSA006).

7.1.2 Specimen Shipment

Shipment of frozen plasma specimens will be performed following the instructions as detailed in PRESEPT Clinical Investigation, Attachment G: Specimen Storage and Shipment Instructions.

7.2 Sample or Sample Aliquot Exclusion Criteria

Samples or sample aliquots will be evaluated upon arrival at Epigenomics. Those found to exhibit any of the following may be excluded from the study analysis:

- Gross hemolysis (bright orange or red color)
- Protocol non-compliant collection, processing, storage or shipping
- Plasma samples with inadequate volume for Septin 9 analysis

7.3 Treatments Not Authorized or Permitted

Treatments not authorized or permitted before and/or during the specimen collection:

- Bowel preparation before colonoscopy
- Intravenous fluid administration

7.4 Laboratory Evaluations

The Septin 9 Biomarker has been designed to determine the methylation status of a region of the Septin 9 gene using free-floating DNA extracted from blood plasma. The biomarker detects the presence of methylated Septin 9 DNA in the Subject's plasma sample and the data is reported as a binary variable (methylated Septin 9 DNA present vs. absent).

To perform the assay, DNA is extracted from 5 ml of Subject plasma and is then chemically converted to retain DNA methylation information using sodium bisulfite. The bisulfite treated DNA is analyzed by real-time PCR using an assay specific for Septin 9. The Septin 9 Assay is run in triplicate for each Subject sample and a sample is determined to be positive for colorectal cancer if Septin 9 methylated DNA is detected in at least one of the three replicates.

Plasma specimens from a subset of Subjects will be selected and analyzed for the presence of methylated Septin 9 by an authorized laboratory. A subset of masked coded specimens will be selected for testing under the auspices of the CSSC as described in Section 6.1.

7.5 Future Use of Stored Specimens and Clinical Data

7.5.1 The PRESEPT Study Evaluation

The study described herein will conduct the initial testing of specimens at an authorized laboratory to evaluate the performance characteristics compared to the clinical status of the Subjects in order to determine the performance of Septin 9 methylation for detection of colorectal cancer.

7.5.2 IVD Assay Validation

It is envisioned that future testing of the specimens and use of the clinical data will support an in vitro diagnostic device application to FDA for a Premarket Approval Application and CE marking a medical device currently in development.

7.5.3 Future Validation of CRC and Polyp IVD Assay

It is also planned that these specimens will be used for future validation of in vitro diagnostic tests and laboratory developed test services for improved detection of colorectal cancer and precancerous lesions.

8 Clinical Monitoring

8.1 Training

The site PI, Sub-Investigators and other authorized study site personnel will receive training on the protocol from Epigenomics before the start of the specimen and data collection, and as required for new or additional personnel. Site personnel involved in the specimen and data collection will receive required training either from the site PI or from Epigenomics. Onsite training may include monitoring the first several Subject enrollments to provide assurance that study protocol and human study protection regulations are being followed.

8.2 Study Monitoring Plan

The study will be monitored according to the plan described below. The site PI will allocate adequate time for such monitoring activities. The site PI will also ensure that the monitor or other compliance/quality assurance reviewer is given access to all study and related documents, study related facilities and staff as noted in the clinical trials agreement associated with this protocol (e.g. diagnostic laboratories, pathologists) and has adequate space to conduct the monitoring visit.

Monitoring visits will be scheduled with the site PI and other investigation-related personnel for the site such that there is at least one visit during the specimen and data collection and one after data and specimen collection has finished (this might be done together with the Site Close Out Visit). Other monitoring visits will be scheduled for mutually convenient times to coincide with achievement of specific accrual goals for each site.

8.3 Monitoring Schedule

8.3.1 Initial Routine Monitoring Visit

On or around the estimated time for accrual of 10% of each sites proportion of the total 7500 projected enrollment.

8.3.2 Routine Monitoring Visit

On or around the estimated time for accrual of 66% of each site's proportion of the total 7500 projected enrollment (just prior to dataset close out for Interim Analysis of the first 5000 enrollments).

8.4 Monitoring Visit Goals

- Assure compliance with all aspects of this protocol.
- Ensure that the data and specimen collection instructions are properly followed.
- Check that all required documentation is present and complete in the site's regulatory binder.
- Discuss with the site PI or investigation-related personnel, any problems which were not foreseen at the Site Initiation Visit. Whenever possible, solutions or a plan for how the problem will be solved should be agreed upon during the visit.
- Ensure data quality, such as accuracy, correctness, legibility and completeness.
- Ensure that bias is minimized during the screening and enrollment process
- Certified copies of selected completed cases or access to an electronic medical record system (all necessary CRF elements complete and accurate) will be provided to Epigenomics by mutual agreement.

8.5 Monitoring Visit Activities

All Epigenomics' or other designated clinical monitors and data auditors must have access to all relevant source documents as required by the protocol's monitoring plan and/or Epigenomics' auditing plan and/or regulatory requirements from authorized regulatory agencies.

In order to fulfill the aforementioned goals each monitoring visit may include the following, depending on the time-point of the visit and the needs of the particular site and/or PI:

- Meeting with the site PI, or designee, to monitor the screening, enrollment and clinical data collection procedures and documentation process

- Pathology review of diagnostic slides or images from the selected cases included in the Investigation by Epigenomics' appointed pathologist for the study or a third party, independent pathologist in case of disagreement between the primary pathologist and Epigenomics' appointed pathologist. The cases to be reviewed will be defined prior to the monitoring visit between the parties.
- Observing specimen collection, processing, and storage for compliance with protocol and related procedures.
- Checking the site's regulatory binder for completeness.
- Checking consent forms for enrolled Subjects for completeness.
- Checking storage facilities for the site's regulatory binder, other investigation-related documentation, specimen collection supplies, and specimens for adequacy.
- Checking with the site PI, or designee, the process of Subject enrollment.
- Checking CRF (Attachments A-E) data against the source documentation. In general, during the visit the monitor will select the clinical records that he/she wants to check and the PI, or designee, will prepare the records, whenever possible during the same visit or for a subsequent visit.

9 Quality Control and Quality Assurance

9.1 Monitoring, Audit and Inspection

The investigator(s)/institution(s) will permit study-related monitoring, audits and inspections by the Independent Ethics Committee (IEC) and/or Institutional Review Board (IRB), the Sponsor or designee, government regulatory bodies and institution/university compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator(s)/institution(s) will ensure the capability for inspections of applicable study-related facilities (e.g. diagnostic laboratories, pathologists, etc).

10 Statistical Considerations

10.1 Introduction

The goal of the statistical analysis is to estimate the clinical performance of the Septin 9 Biomarker in an average to increased risk colorectal screening guideline eligible population. An interim analysis will be performed upon enrollment of 5000 Subjects to determine Septin 9 performance on an initial selection of Subject specimens (see section 10.3.1). Enrollment will continue until 50 Subjects with CRC, Stage I-IV, are accrued, at which time a final analysis of the data will be conducted according to a pre-defined statistical analysis plan (SAP).

10.2 Sample Size Considerations

Given an anticipated prevalence for CRC detection of approximately 0.7% in the proposed study population, we would expect the following detection rate depending on the number of individuals enrolled.

~33 CRC cases in **5000** enrolled patients

~40 CRC cases in **6000** enrolled patients

~46 CRC cases in **7000** enrolled patients

~53 CRC cases in **8000** enrolled patients

~59 CRC cases in **9000** enrolled patients

Since the actual number of CRC disease cases detected will be subject to stochastic effects, there is a risk associated with not reaching the minimum number of CRC case requested. Using a negative binomial distribution assumption, the probability of collecting 50 CRC cases within N individuals enrolled is shown in Figure 1.

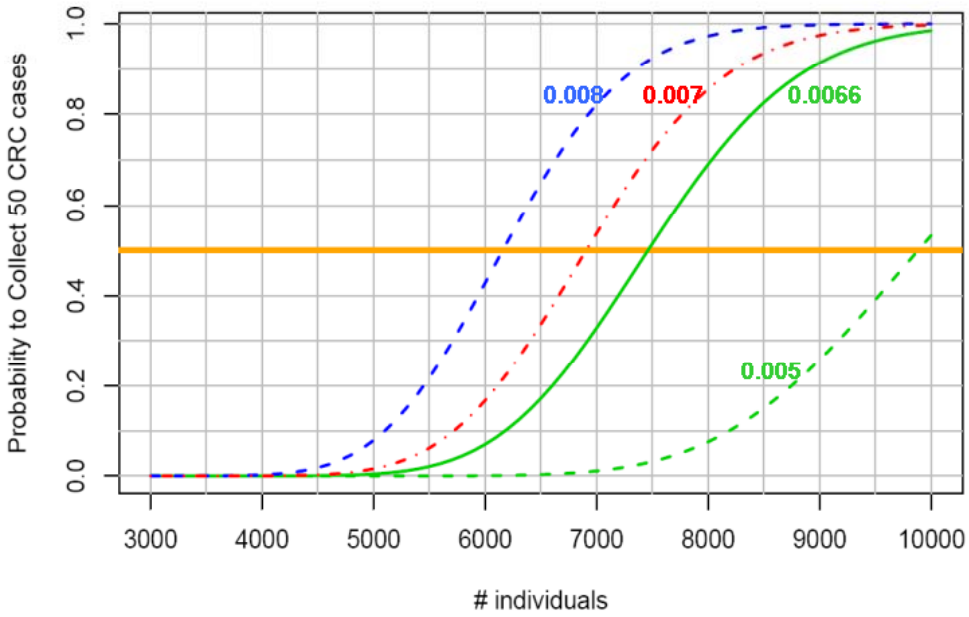

Figure 1: Probability to collect truly 50 CRC cases as a function of the number of enrolled patients for a set of prevalence rates. Green (solid): the best estimate scenario, green (dashed) low prevalence scenario, blue high prevalence scenario, red stratification scenario.

10.3 Results Analysis

10.3.1 Interim Analysis (n = 5000 Enrolled)

Upon enrollment of the first 5000 valid cases, the Clinical Study Steering Committee will select a subset of the enrolled Subjects (~1000) for Septin 9 methylation testing. Aliquots of plasma from selected cases will be shipped to the authorized testing laboratory for analysis with the Septin 9 Assay. This subset of cases will be the basis for the interim analysis and will represent:

- All samples from cases of colorectal cancer (Stages I – IV, excluding Stage 0) at interim (~30).
- All cases with one or more large adenomatous (≥ 10 mm) polyps/lesions (including Stage 0).
- A random selection of approximately 250 Subjects with one or more small polyps (all ≤9 mm) and that do not fall into the two previous categories.

- A random selection of approximately 500 Subjects that do not fall into the three previous categories.
- All analytical tests will be conducted in masked fashion by an authorized clinical laboratory with data submitted directly to the Clinical Study Steering Committee that will direct the analysis of the data.
- The data will be analyzed according to a pre-established statistical analysis plan.

10.3.2 Final Analysis (n = 50 CRC Cases Detected)

After a total of 50 CRC cases (total including Interim Analysis) have been accrued, the Clinical Study Steering Committee will select for clinical testing a subset of the valid, completed cases enrolled after the interim testing (~1000). These Subjects will represent:

- All remaining samples from cases of colorectal cancer (Stages I – IV, excluding Stage 0) enrolled and/or completed since the interim analysis (~20).
- All samples from cases with large adenomatous ($\geq$ 10 mm) polyps/lesions (including Stage 0) enrolled since the interim analysis.
- A random selection of approximately 250 Subjects with one or more small polyps ($\leq$9 mm) and that do not fall into the two previous categories enrolled since the interim analysis.
- A random selection of approximately 500 Subjects that do not fall into the three previous categories enrolled since the interim analysis.
- All analytical tests will be conducted in masked fashion by an authorized clinical laboratory with data provided to the Clinical Research Steering Committee, which will determine the most appropriate forum for data analysis.

10.4 Study Subject Withdrawal and Exclusion from Analysis

An enrolled Subject originally included in the clinical investigation will be withdrawn from participation and excluded from analysis in a timely manner according to the following criteria.

- Subject's physician determines withdrawal from the study is in the best interests of the health and safety of the Subject.
- Study Subject requests withdrawal through revocation of Informed Consent.

In these cases, study documentation will be completed justifying exclusion from analysis, all CRFs (Attachments A-E) will be removed from the regulatory binder and all physical specimens will be retrieved, destroyed and excluded from testing; if testing has occurred, the results will be excluded from analysis. However, such information and data may be retained as part of the study records for regulatory purposes or to ensure the scientific integrity of the study.

10.5 Potential Subject Exclusion from Analysis

A Subject originally included in the clinical investigation may be excluded from analysis if any of the following criteria are present:

- Significant protocol violation
- Subjects with colonoscopy procedure reports designated as being “incomplete” or “inadequate” for any reason (inadequate bowel prep, patient intolerance, obstruction, etc.) will be excluded from selection for biomarker analysis even if the procedure report is available, unless a colorectal cancer was found and confirmed by pathology.
- Subject specimens or data lost or damaged.
- Insufficient amount of plasma per Subject remains for marker testing due to invalidation of plasma aliquots from improper plasma preparation, shipment or storage (see section 7.0).
- Complete Subject data set including date to complete all applicable CRF’s is unavailable within 30 days of close of study enrollment.

10.6 Documentation by Study Site of Subject Withdrawal or Exclusion

Subject withdrawal or exclusion must be documented, including the reason for withdrawal or exclusion, on the Subject Management Form (Attachment M).

10.7 Clinical Study Steering Committee Oversight

The entire data set will be analyzed under the direction of the CSSC according to a pre-established statistical analysis plan and the final results will be submitted for publication.

10.8 Analysis Plan

10.8.1 Goal of Analysis

Estimate the clinical performance of the Septin 9 Biomarker in an average to increased risk population in terms of:

- Sensitivity
- Specificity
- Positive Predictive Value
- Negative Predictive Value

10.8.2 Analysis Sets

Interim data analysis will be conducted on the single quality-approved data set containing the data available at the specified time point. Selection of specimens for this analysis is described in section 10.3.1.

The Final Analysis will be conducted on the single quality-approved full data set. Selection of specimens for this analysis is described in section 10.2.2.

10.8.3 Statistical Hypothesis Testing

The main results of the experiment will be reported in a descriptive manner. However, statistical test methodology will be employed to evaluate the marker performance at an interim time and at the end of the study.

The study will investigate the following set of hypotheses:

H0: sensitivity $\leq$ 0.4 OR specificity $\leq$ 0.80

HA: sensitivity > 0.4 AND specificity > 0.80

These values will be evaluated at an experiment-wise Type I error controlled at 10%. Allocation of the error for each of the two analyses (interim and final) will be determined by the number of CRC cases observed at the interim. The number of CRC cases as a proportion of 50 will be the observed information. Power will be calculated accordingly. The aim is to maintain power at approximately 80%.

10.8.4 Parameter Estimates

Estimations of sensitivity and specificity will be based on the qualitative outcome of the Septin 9 methylation test. Sensitivity is defined as the test

positive fraction in disease case, while specificity is defined as (1- false positive fraction) in non-diseased individuals.

Overall sensitivity (se) and specificity (sp) estimates will be converted into positive and negative predicted values using the formulae below and the population prevalence (p) estimate found in Table 1.

$$PPV = \frac{se * \rho}{se * \rho + (1 - sp) * (1 - \rho)} \qquad NPV = \frac{sp * (1 - \rho)}{sp * (1 - \rho) + (1 - se) * \rho}$$

Example: Assume the prevalence of colonoscopy-confirmed CRC in the population addressed is 0.7%, the weighted average sensitivity is approximately 60% and specificity is approximately 90%. Then, the predictive value of positive test result would be 4.06% and the predictive value of negative test would be 99.69%. In other words, the odds of disease for those who test positive is about 6 times the prevalence rate. For those who test negative for Septin 9 methylation, the probability of disease is less than half the prevalence rate.

10.8.5 Performance Expectations

Previous case-control studies on CRC patients and healthy controls provide estimates of their clinical performance values of the Septin 9 Biomarker. Based on these studies the following results are expected in the final analysis.

- Observed sensitivity of the Septin 9 Biomarker equal to or greater than 60%
- Observed specificity of the Septin 9 Biomarker equal to or greater than 90%

10.8.6 Sample Size, Power Consideration, Precision of Estimates

Sample size is based on the minimum number of CRC cases given a study size of up to 7500 and prevalence estimates of between 0.6-0.7%. The degree of statistical information (i.e. precision of estimates) provided by this experiment will be quantified in terms of expected length of confidence intervals for estimated variables.

In summary, SEPT 9 results from plasma samples analyzed at both interim and final will be combined for the final report. The sample set includes the following:

- All samples from cases of colorectal cancer (Stages I – IV, excluding Stage 0).
- All cases with one or more large adenomatous polyps/lesions ($\geq$ 10 mm) (including Stage 0).

- A random selection of approximately 500 Subjects with one or more small polyps (all ≤9 mm) and that do not fall into the two previous categories.
- A random selection of approximately 1000 Subjects that do not fall into the three previous categories.

10.8.7 Sensitivity

Given 50 CRC cases, the sensitivity estimate will have a 95% confidence interval of length smaller than 0.29 (i.e. maximal length for an estimate of 50%).

Given 30 CRC cases (expected number of cancers at interim analysis), the sensitivity estimate will have a 95% confidence interval of length smaller than 0.375 (i.e. maximal length for an estimate of 50%).

10.8.8 Specificity

The analysis of cases with one or more adenomatous polyps/lesions 10mm or larger is expected to exhibit a false positive fraction similar to 0.20. For example, given 100 cases, estimates in the range (0.15-0.25) have attached 95% confidence intervals of length less than 0.18.

Given 1000 control cases, specificity estimates in the high range (0.90-0.95) will be substantiated with 95% confidence intervals of length smaller than 0.037.

10.8.9 Procedure for Accounting for Missing, Unused and Spurious data

Data augmentation techniques will not be used. Subjects with missing Septin 9 test results will be excluded from analysis. Subjects with incomplete data on critical clinical covariates (colonoscopy results, age, gender, tumor histology, polyp size) will be excluded from selection for Septin 9/biomarker analysis.

10.8.10 Procedure for Reporting Deviation(s) from the Original Statistical Plan

If necessary, we will describe and justify any deviations from the original statistical plan in the final report.

11 Ethics/Protection of Human Subjects / Independent Ethics Committee

11.1 Institutional Review Board

This study is to be conducted according to US and international standards of GCP, applicable government regulations and Institutional research policies and procedures (FDA Title 21 part 50 and International Conference on Harmonization guidelines).

This protocol and any amendments will be submitted to a properly constituted Independent Ethics Committee (IEC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IEC/IRB concerning the conduct of the study will be made in writing to the investigator(s)/institution(s) and a copy of this decision will be provided to the Sponsor before commencement of this study. The investigator(s)/institution(s) should provide a list of IEC/IRB members and their affiliations to the Sponsor.

11.2 Informed Consent Process

11.2.1 Informed Consent Template (attached)

11.3 Actions to Protect Subject Confidentiality

Individual Subjects' information collected for the clinical investigation will be kept confidential at the participating clinical laboratories and/or by the site PI (contractually bound to confidentiality with Epigenomics) in charge of performing the investigational testing. Data collected on the CRFs (Attachments A-E and M) will be entered into a validated 21 CFR part 11 compliant database at Epigenomics.

Information may be disclosed to the FDA and any other regulatory authorities as required to obtain IVD approval or clearance according to national and international regulations.

11.4 Ethical Considerations

Since the study is a prospective study in which blood will be collected prior to colonoscopy, and no changes in clinical practice will occur as a result of this test we consider that there is no significant risk for the Subjects. In this respect, the results from the investigational assay

cannot be used to influence any decisions regarding the medical care of the individual Subjects.

In order to protect Subjects' privacy none of the following personal identifiers will be included in the Attachments A-E and M (CRFs and Subject Management Form) or the Fax Log (Attachment Q) (adapted from HIPAA identifiers list):

- Names (including initials)
- Date of birth
- Geographic subdivisions (smaller than the state)
- Phone, fax numbers
- Subject's addresses
- Social Security, medical record numbers
- Any other unique number, characteristic or code

All Subjects will be assigned a unique identifier (Subject Code provided by Epigenomics) and the Enrollment Log (Attachment F) linking the identifiers and the clinical record/ number/name will be kept in the site's regulatory binder for the clinical investigation. This log will allow proper monitoring of the clinical records and/or pathology reports by the appointed monitors in order to assure completeness, accuracy and correctness of the data collected at each site. This unique identifier will also be assigned to the collected specimens and will be used for all subsequent steps in the clinical investigations.

12 Data Handling and Record Keeping

12.1 Data Management Responsibilities

12.1.1 Delegation of Responsibility

The site PI may delegate any study-related tasks to sub-investigators or other site personnel and will document such delegation by completing and signing the Delegation of Responsibility Log (Attachment I). The site PI, or designee, is responsible for updating and signing the Delegation of Responsibility Log as changes occur until the end of the clinical investigation.

The site PI, or designee, will store the signed, completed Delegation of Responsibility Log in the site's regulatory binder.

12.1.2 Contact Information Form

The contact information list for the Investigation contains all the names and contact details of the site PI and other site personnel involved in the investigation (Attachment J: Contact List for Clinical Investigation (Investigational Site)).

The site PI or designee is responsible for filling in and updating the Contact List whenever new information is obtained and for storing it in the regulatory binder. Any updates on the list must be promptly made available to Epigenomics.

12.2 Data Capture Methods

Data collection at the sites will be performed with paper CRFs (Attachments A-E and M) or validated, 21 CFR Part 11 compliant electronic data capture (EDC) software programs.

At Epigenomics, each paper or electronic version of faxed Attachment A-E and M will be entered by trained data entry personnel into a validated database by double data entry. For EDC data entry, validated data transfer software will be used.

The database has limited access to authorized individuals only and provides an audit trail for the generation, modification and/or deletion of records.

The database is validated according to the FDA Guidance “General Principles of Software Validation” and is compliant with 21 CFR Part 11.

12.3 Study Report

Epigenomics is responsible for the generation of the Study Report. The Study Report is the final documentation of the results and interpretation of the clinical investigation.

12.4 Record Retention

The site PI(s)/institution(s) is/are responsible for retaining all study documents, including the underlying source documents, according to regulatory authorities’ requirements and guidelines. ICH GCP (ICH 4.9) requires the retention of “essential documents” for at least two years after the approval of a marketing application or until there is no pending or contemplated applications or development is formally discontinued. Similarly, FDA GCP requirements for studies done to support device approval is at least 2 years after FDA approval, no more requests for approval are contemplated, or after work on a new drug or device has been discontinued.The site PI or Epigenomics may withdraw from the responsibility to maintain records for the period required above and transfer custody of the records to any other person who will accept responsibility for them under 21 CFR Part

812.140, including the requirement stated in section 6.3.1 in this protocol according to 21 CFR Part 812.145.

These documents should be retained for a longer period if required by an agreement with Epigenomics. In such an instance, it is the responsibility of Epigenomics to inform the site PI as to when these documents are no longer required.

12.5 CRF, Essential Record Storage and Archiving

Essential records should be maintained in a legible condition. Adequate and suitable space should be provided for the secure storage of all essential records upon the completion of the investigation. The facilities must be secure, with appropriate environmental controls and adequate protection from fire, flood and unauthorized access.

Access to archives must be restricted to authorized personnel. Any change in ownership and location of the documentation must be documented in order to allow tracking of the stored records.

An archive index/log will be maintained to record all essential documents that have been entered into the archive, and to track and retrieve documents on loan from the archive.

The site PI will make Epigenomics aware of the storage arrangements for the documents to be stored at investigator sites. If the site PI becomes unable to store their essential documents, the Sponsor should be notified in writing so that alternative storage arrangements can be agreed.

If the institution/site is no longer able to maintain custody of their essential documents, he/she must notify Epigenomics in writing and the investigator needs to ensure that appropriate arrangements can be made.

12.6 Protocol Deviations

Documentation is required for all deviations from the protocol related to sample and/or data collection and handling or deviations from the eligibility, inclusion, or exclusion criteria for Subjects and/or samples.

All deviations must be reported promptly to Epigenomics. Deviations likely to affect the safety or health of the Subject should be reported to the IRB/IEC following the specific IRB/IEC requirements and procedures. Deviations based on logistical changes or administrative aspects do not require reporting to the IRB/IEC.

If the site discovers a protocol deviation has occurred, the following will be done:

- The deviation will be logged in Attachment K: Study Protocol Deviation Log, by the site PI, or designee. The Deviation Log will be stored in the regulatory binder.
- The deviation will be described in detail in Attachment L: Study Protocol Deviation Report Template and stored together with the Subjects' CRFs (Attachments A-E)

13 Financing and Insurance

Financing, investigator(s)/institution(s) payment(s) and insurance aspects of the investigation are addressed in the Investigator's contract.

14 Publication Plan

Epigenomics intends that the results of this clinical investigation will be published under the direction of Clinical Study Steering Committee who will establish a publication policy prior to data analysis. Consistent with regulatory disclosure requirements, neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by Epigenomics for the purposes of performing the study, will be published or distributed to any third party without Epigenomics' prior consent.

All results of the investigation will remain the property of Epigenomics.

15 Attachments

15.1 Prototype Informed Consent Form

15.2 Case Report Forms

15.2.1 Attachment A: Health History Case Report Form

15.2.2 Attachment B: Sample Processing Case Report Form

15.2.3 Attachment C: Colonoscopy Case Report Form

15.2.4 Attachment D: Incisional Biospy/Polypectomy Histology Case Report Form

15.2.5 Attachment E: Segmental Colon Resection: Tissue Histology and Staging Case Report Form

15.3 Study Management Documents

15.3.1 Attachment F: Subject Enrollment Log

15.3.2 Attachment G: Specimen Storage and Shipment Instructions

15.3.3 Attachment H: Instructions for Case Report Form (CRF) Completion, Storage and Maintenance

15.3.4 Attachment I: Delegation of Responsibility

15.3.5 Attachment J: Contact List for Clinical Investigation (Investigational Site)

15.3.6 Attachment K: Study Protocol Deviation Log

15.3.7 Attachment L: Study Protocol Deviation Report Template

15.3.8 Attachment M: Subject Management Form

15.3.9 Attachment N: Training Log

15.3.10 Attachment O: Subject Traveler

15.3.11 Attachment P: Blood Collection and Plasma Preparation SOP

15.3.12 Attachment Q: Fax Log

15.3.13 Attachment R: Fax and Sample Shipment Schedule Instructions

16 Literature References for Epigenomic's Methylation Biomarkers

DNA-Methylation Biomarkers for Blood-Based Colorectal Cancer Screening.Clin. Chem., 2008; 54 (2): 414-423. Lofton-Day C, Model F, DeVos T, Tetzner R, Distler J, Schuster M, Song X, Lesche R, Liebenberg V, Ebert M, Molnar B, Grutzmann R, Pilarsky C, and Sledziewski A.

Comparative DNA methylation analysis in normal and tumour tissues and in cancer cell lines using differential methylation hybridisation. Int J Biochem Cell Biol. 2007;39(7-8):1539-50. Lewin J, Plum A, Hildmann T, Rujan T, Eckhardt F, Liebenberg V, Lofton-Day C, Wasserkort R.

Identification and validation of colorectal neoplasia-specific methylation markers for accurate classification of disease. Mol Cancer Res. 2007 Feb; 5(2):153-63. Model, F. et al.

August 2007: CNAPS-V Conference, Moscow, Russia. Presentation: "Septin 9 Methylation as a Plasma Biomarker for Colorectal Cancer"

May 2007: Digestive Disease Week 2007, Washington DC, U.S.A. Presentation "Detection of colorectal adenoma and cancer by blood-based analysis of DNA methylation" by Prof. Dr. Matthias Ebert

April 2007: AACR Annual Meeting 2007, Los Angeles, U.S.A. Poster "Clinical case-control study in plasma shows that the DNA methylation biomarker, Septin 9, detects 70% of Stage I-III colorectal cancer patients "

March 2007: CHI Conference "Epigenomics", San Diego, USA. Presentation: "Blood Based Screening for Colorectal Cancer and Preneoplastic Disease"

RESEARCH SUBJECT INFORMATION AND CONSENT FORM

TITLE:	PreSEPT Study: Prospective Evaluation of SEPT9 Performance for Colorectal Cancer Screening
PROTOCOL NO.:	SPR0006 WIRB® Protocol #20080713
SPONSOR:	Epigenomics, Inc. Seattle, Washington United States
INVESTIGATOR:	Name Address City, State Zip Country
SITE(S):	Name Address City, State Zip Country
STUDY-RELATED PHONE NUMBER(S):	Name Telephone Number

What am I being asked to do?

You are being asked to take part in a clinical research study of a new type of test, which may be used to screen persons such as you for colorectal cancer (cancer of the lower bowel) with a blood test. Your study doctor or a clinical research associate under his/her direction will explain the clinical study to you. Clinical research studies are voluntary and include only people who choose to take part. This consent form may contain words that you do not understand. Please ask the study doctor or the study staff to explain any words or information that you do not clearly understand. Please take your time to make your decision about taking part. You may take home an unsigned copy of this consent form to think about or discuss with friends and family before making your decision. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.

Why am I being asked take part?

You are being asked to take part in this study because you have been scheduled for a colonoscopy (a procedure where a tube is inserted through your rectum to look for evidence of cancer in your colon [large bowel]) to screen for colorectal cancer. Participation in this study will involve providing blood.

What is the purpose of this clinical research study?

The purpose of this study is to evaluate the ability of an investigational blood test to detect colorectal cancer compared to colonoscopy. An investigational blood test is one that is not approved by the U.S. Food and Drug Administration (FDA). By taking a blood sample of persons receiving colonoscopy and then testing those blood samples from those who are later found to have or not have colorectal cancer, we can find out how well one or more blood test "markers" works as an alternative to colonoscopy. These markers detect small changes in one or more genes in your DNA (deoxyribonucleic acid - the chemical that makes up genes). Your blood specimen will be tested using one or more tests to detect changes in one or more genes in your DNA. Changes in these genes may indicate the presence of colorectal cancer in some individuals. A genetic analysis (test) for other changes to your DNA associated with diseases unrelated to cancer will not be done. It is expected at least one of these tests will be submitted to regulatory authorities for approval to be marketed. Your blood sample and related data/information will be used to support the research, development, and approval of these tests by Epigenomics and/or its collaborators.

Who will provide funding?

A commercial company, Epigenomics, Inc., which has offices in the United States and Germany, is paying for and sponsoring the study.

How many subjects will take part in the research study?

About 7,500 subjects from the United States and Germany are expected to take part in this study. These subjects will meet current medical guidelines (recommendations) for colorectal cancer screening. That is, fifty (50) years of age or older and without signs or symptoms that suggest colorectal cancer.

If I take part, what will I be expected to do?

If you agree to take part in this research study, the following will be expected of you:

1. You will read and sign this consent form.
2. You will answer approximately twelve confidential questions regarding the health of you and your close relatives (Health History Interview) asked by your study doctor or a study team member.
3. You will allow your study doctor or another health professional to draw a little less than three tablespoons of blood, collected in up to four tubes from a vein in your arm, for later testing. Your blood will be stored for later research and testing with one or more tests done by the study sponsor and/or its collaborators or researchers.

When you have finished providing a health history and providing blood, your part of the study is done. Your time to participate is estimated to be less than 30 minutes. You will sign a separate consent form for the colonoscopy, which is part of your standard care. We are also asking permission to get information about any samples that are taken when you have your colonoscopy or surgery, and/or share portions of the tissue removed during colonoscopy or surgery for further analysis, if needed whether the samples contain cancer or not. Your study doctor or a study member also may contact you by letter or telephone within the next two years to ask if you would be willing to contribute more blood samples or to ask you additional questions regarding the status of your health related to this study. You are not agreeing to provide additional samples or to answer any additional questions.

How long will you keep my blood sample? Where will my blood sample be stored?

Your blood sample may be kept for a long time, possibly up to 20 years. Your blood sample will be stored by Epigenomics. However, Epigenomics may transfer your blood sample or a portion of it to its collaborators and/or researchers (including Abbott Molecular Inc. and Quest Diagnostics) for use in this study and future cancer research.

Will my blood sample be used for other research?

In addition to the use in this study, your blood sample may be used by Epigenomics and/or its collaborators for the research, development, and regulatory approval of other medical tests or treatments related to cancer that may have commercial value. Research using your sample may result in commercial products or valuable discoveries, some of which may be patented. There are no plans for you to receive any money from any commercial products or discoveries resulting from the research. You consent to have your blood specimen saved for future cancer research, even though the purpose of the future research is not known.

What are the risks I can expect from taking part?

Your blood sample will be taken by needle puncture of a vein. This method involves inserting a needle into a vein in the arm and withdrawing a sample of blood. It is routinely used to obtain blood for physical examinations. Needle puncture of a vein is accompanied by minor discomfort at the site of the needle entry and may result in slight bruising, feeling of faintness, local swelling and in rare cases infection or even nerve damage.

There may be risks or side effects which are unknown at this time.

What are the benefits I can expect from taking part?

You may take satisfaction from helping medical science better understand colorectal cancer and contributing to the demonstration of a blood based molecular test to offer doctors and patients an alternative to other methods for colorectal cancer screening.

There is no guarantee that you will receive any medical benefits from being in this study.

You will not be provided with any results or information regarding the testing of your blood sample. The results will not be used in any medical decisions for you. Your study doctor will not know what the results of your blood test are. For additional information regarding any potential risks or benefits of taking part in this study, ask your study doctor or study team member.

Are there costs for participating?
You will not be charged for any study-related procedures. Your colonoscopy will be billed in the usual way.

Is there payment for participation?
You will be paid $25.00.

What choices do I have if I do not take part in this study?
Your alternative is to not be in this study.

Whether you do or do not take part in this study, your actions will have no bearing on your health care or the way your doctors or health care providers treat you, or any rights or benefits which you are otherwise entitled.

Will my medical information be kept confidential?
Your study doctor and your study team will do their best to make sure that the personal health information in your medical record and obtained from this study will be kept confidential. Personal health information includes information in your medical records or obtained from this study that can identify you. This will be done by removing your name and other identifying information from the documents used in the study and replacing them with code numbers. All the personal information regarding your health history and whether you have or do not have colorectal cancer will be maintained on a confidential basis, and your name will not be shared with anyone outside your study team. However, we cannot guarantee absolute confidentiality. Your personal health information may be given out if required by applicable law or regulations.

How will my medical information be used and shared?
The study team will provide Epigenomics with information from your Health History Information, including your age, findings from your colonoscopy and other information collected from the study. The study team will provide this information to Epigenomics in case report forms or other documents. The information provided to Epigenomics will not include your name, address, telephone number, or other information that can identify you.

Authorization to use and disclose information for research purposes

What information may be used and given to others?

The study doctor will get your personal and medical information. For example:

- Past and present medical records
- Research records
- Health history interview
- Case report forms or other documents
- Records about phone calls made as part of this research
- Records about your study visits.
- Information gathered for this research about:
  - Physical exams
  - Laboratory and other test results
- Records about the study device.

Who may use and give out information about you?

The study doctor, the study team and other health professionals at the health care institution, and the study monitor.

Who might get this information?

- The sponsor of this research. "Sponsor" means any persons or companies that are:
  - working for or with the sponsor, or
  - owned by the sponsor.

Your information <u>may</u> be given to:

- Representatives of the U.S. Food and Drug Administration (FDA) or other regulatory agencies,
- Researchers and collaborators in the U.S. or other countries, including Abbott Molecular Inc. and Quest Diagnostics,
- Department of Health and Human Services (DHHS) agencies,
- Governmental agencies in other countries,
- Western Institutional Review Board® (WIRB®).

Why will this information be used and/or given to others?

- for research, quality assurance, and data analysis;
- to perform this study and future research and development related to cancer;
- use it to publish studies or for presentations at scientific meetings; and
- use it to improve the design of future studies, and/or to file applications with governmental agencies in the U.S. and other countries to obtain approval for screening or diagnostic tests or other medical products.

If the results of this study are made public, information that identifies you will not be used.

What if I decide not to give permission to use and give out my health information?
Then you will not be able to be in this research study.

May I review or copy my information?
Yes, but only after the research is over.

May I withdraw or revoke (cancel) my permission?
Yes, but this permission will not stop automatically.

You may withdraw or take away your permission to use and disclose your health information at any time. You do this by sending written notice to the study doctor. If you withdraw your permission, you will not be able to stay in this study.

When you withdraw your permission, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others.

Is my health information protected after it has been given to others?
There is a risk that your information will be given to others without your permission.

What happens if I am injured because I took part in this study?
It is important that you tell your study doctor, _____, if you feel that you have been injured because of taking part in this study. You can tell the study doctor in person or call him/her at _____.

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study has no plans to pay for medical treatment.

Neither your study doctor nor Epigenomics have any plans to compensate you for any injury or additional expenses you may have because of this study. Your health insurance company may or may not pay for treatment of injuries as a result of your participation in this study. In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this consent form.

What are my rights if I take part in this study?
Your participation in this study is voluntary. You may decide not to participate or you may leave the study at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

How can I withdraw from the research study?

Tell the study doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop. If you withdraw from the study, any portion of your blood sample that has not been used will be destroyed and no new information will be collected from you. Any information collected from you before you withdraw from the study and any data generated from the use of your sample will be excluded from the study. However, such information and data may be retained as part of the study records for regulatory purposes or to ensure the scientific integrity of the study.

The study doctor or the sponsor may stop you from taking part in this study at any time without your consent for any of the following reasons:

- if it is in your best interest;
- if you do not follow the study rules;
- if the study is stopped;
- you do not later consent to any future changes that may be made in the study plan;
- or for any other reason.

What if there are new findings?

We will tell you about new information or changes in the study that may affect your health or might change your decision to be in this study. You may be asked to sign a revised consent form if this occurs.

Who can answer my questions about the study?

If you have any questions about your participation in this study, if at any time you feel you have had a research-related injury, or if you have any questions, concerns, or complaints about the research, contact your study doctor, _____, at _____.

If you have questions about your rights as a research subject or if you have questions, concerns, or complaints about the research, you may contact:

Western Institutional Review Board® (WIRB®)
3535 Seventh Avenue, SW
Olympia, Washington 98502
Telephone: 1-800-562-4789 or 360-252-2500
E-mail: Help@wirb.com.

WIRB is a group of people who perform independent review of research.

WIRB will not be able to answer some study-specific questions, such as questions about appointment times. However, you may contact WIRB if the research staff cannot be reached or if you wish to talk to someone other than the research staff.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

If you agree to be in this study, you will receive a signed and dated copy of this consent form.

If you want more information about this study, ask your study doctor.

How do I agree to participate in the study?

If you voluntarily choose to participate, you can provide your consent for the study personnel, the sponsor, and its collaborators to include you by agreeing to, and signing, the following statement.

I have read the information in this consent form. I have had my questions answered. I agree to take part in this study.

I authorize the use and disclosure of my health information to the parties listed in the authorization section of this consent for the purposes described above.

By signing this consent form, I have not given up any of my legal rights.

Subject's Name (printed): _____

Subject's Signature: _____

Date: _____

Person Conducting Informed
Consent Discussion's Signature: _____

Date: _____

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Attachment A: HEALTH HISTORY CRF

1.0 Subject Code……

2.0 Epidemiological information

Age: _____ years

Gender: ☐ Male ☐ Female

Ethnicity (check one):

American Indian or Alaska Native…….…….☐
Asian ……. ☐
Black or African-American…….. ☐
Native Hawaiian or Other Pacific Islander…… ☐
Caucasian…….. ☐
Hispanic……. ☐
Other ……. ☐

3.0 Inclusion Criteria

Has informed consent been given? ☐ Yes ☐ No

Is the subject screening guideline-eligible for colonoscopy? ☐ Yes ☐ No

Will subject's blood be drawn prior to bowel preparation? ☐ Yes ☐ No

Is this the patient's first large bowel endoscopy in lifetime? ☐ Yes ☐ No

4.0 Exclusion Criteria

Does the patient report a family history of CRC? ☐ Yes ☐ No
(2 or more 1^0 relatives with CRC; 1 or more 1^0 relative(s) < 50 years with CRC; known HNPCC or FAP)?

Does the subject report having had rectal bleeding in last 6 months for which he/she sought medical care? ☐ Yes ☐ No

Does the subject report having had anemia in last 6 months for which he/she sought or received medical care? ☐ Yes ☐ No

Does the patient have a previous history of colorectal polyps or CRC? ☐ Yes ☐ No

Subjects 50 years of age or older, responding 'yes' to all inclusion and 'no' to all exclusion criteria may proceed; Subjects not meeting these requirements will be withdrawn from study and excluded from further analysis.

I confirm that all entries are complete and accurate:

_____ _____ _____
Date Signature Printed Name

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Attachment B: SAMPLE PROCESSING CRF

1.0 Subject Code…… ……..

2.0 Blood Draw Date/Time:

____ /___ /_____ __ __ : __ __
DD / MM / YYYY HH:MM (24hr)

3.0 Process Overview

Allow whole blood to sit at room temperature for at least 30 minutes prior to centrifugation

Freeze plasma within 4 hours of blood draw

Record dates in DD/MM/YYYY format - Example: 23/09/2008

Record times in HH:MM 24hour format – Example: 1:00pm = 13:00

4.0 Processing Steps

Whole blood remained at room temperature for at least 30 minutes… ☐ Yes ☐ No

Plasma processing start time __ __:__ __
HH:MM (24hr)

1st centrifugation at 1350 ±150 g for 12 min…… ☐ Yes ☐ No

2nd centrifugation at 1350 ±150 g for 12 min……☐ Yes ☐ No

Buffy coat collection from one Vacutainer tube …… ……☐ Yes ☐ No

5.0 Specimen ID (label)……

Place Specimen ID Label Here

6.0 Total Plasma Volume:……._____ ml

Number of plasma aliquots: _____

7.0 Aliquot Freezing Date/Time: ___ /___ /_____ __ __ : __ __
DD / MM / YYYY HH:MM (24hr)

Plasma frozen within 4 hours of blood draw ☐ Yes ☐ No

Plasma stored temporarily at -20ºC, for 24 hours maximum ☐ Yes ☐ No

I confirm that all entries are complete and accurate:

_____ _____ _____
Date Signature Printed Name

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Attachment C: COLONOSCOPY CRF

Place Epigenomics Subject Code Label Here

1.0 Subject Code:……..

2.0 General:

Date of colonoscopy (DD/MM/YYYY) ……..…… ____/____/____
Initial colonoscopy:…….. ☐ Yes ☐ No
Repeat colonoscopy: ……..…….. ☐ Yes ☐ No
Was bowel preparation noted to be inadequate on report?……. ☐ Yes ☐ No
Length of time in colon……..……_____ (mins) ☐ unknown
Colonoscopy reported incomplete (e. g. Cecum not visualized)?…… ☐ Yes ☐ No

3.0 Interventions Performed:

Polypectomy?…… ☐ Yes ☐ No
Biopsy?…….. ☐ Yes ☐ No

4.0 Further Actions Planned:

Repeat colonoscopy?…… ……. ☐ Yes ☐ No
Surgical intervention? ……. ☐ Yes ☐ No

5.0 Macroscopic Findings:

No Polyp, Lesion* or Carcinoma ☐
Polyp(s)/Lesion(s)…… ☐
Suspected Carcinoma…… ☐

6.0 Number of Polyp(s)/Lesion(s):

One or two…… ☐
Three to ten…… ☐
More than ten……..…… ☐

7.0 Type of 3 Largest Polyp(s)/Lesion(s)* (largest "1", second largest "2", etc.):

	1	2	3
Sessile ……..……	☐	☐	☐
Pedunculated……..……	☐	☐	☐
Flat Lesion……..	☐	☐	☐
Unknown/Undefined ..……..	☐	☐	☐

8.0 Location of 3 Largest Polyp(s)/Lesion(s)*:

	1	2	3
Proximal colon (Cecum, Right (ascending) colon, Hepatic flexure, Transverse colon)	☐	☐	☐
Distal colon: (Splenic flexure, Left (descending), Sigmoid, Rectosigmoid)…….	☐	☐	☐
Rectum……	☐	☐	☐
Unknown……..……..	☐	☐	☐

9.0 Size of 3 Largest Polyp(s)/Lesion(s):

	1	2	3
1 to 9 mm ……..	☐	☐	☐
10 mm or larger ……..……	☐	☐	☐

10.0 Tissue Available for histology: if yes check box…… ☐ ☐ ☐

* Defined as nonpolypoid flat/depressed colorectal neoplasm

I confirm that all entries are complete and accurate:

_____ _____ _____
Date Signature Printed Name

Attachment D: INCISIONAL BIOPSY/POLYPECTOMY HISTOLOGY CRF

1.0 Subject Code ……

Place Epigenomics Subject Code Label Here

2.0 Location of 3 Largest Polyp(s)/Lesion(s) (If possible relate "A" to 1, "B" to 2, and "C" to 3 corresponding to those identified on Attachment C: Colonoscopy CRF)

	A	**B**	**C**
Proximal colon…….. (Cecum, Right (ascending) colon, Hepatic flexure, Transverse colon)	☐	☐	☐
Distal colon…… (Splenic flexure, Left (descending) colon, Sigmoid, Rectosigmoid)	☐	☐	☐
Rectum……	☐	☐	☐
Unknown……..	☐	☐	☐

3.0 Size of Polyp(s)/Lesion(s)

Greatest dimension:	___ mm	___ mm	___ mm
Not available……	☐	☐	☐

4.0 Histological findings.

4.1 Type of Polyp/Lesion ***Check 1 response per column***	**A**	**B**	**C**
Inflammatory……	☐	☐	☐
Hyperplastic (metaplastic)……	☐	☐	☐
Tubular adenoma (adenomatous polyp)……	☐	☐	☐
Villous adenoma……	☐	☐	☐
Tubulovillous adenoma (villoglandular polyp)……	☐	☐	☐
Serrated adenoma (any subtype)……	☐	☐	☐
Adenocarcinoma……	☐	☐	☐
Other……	☐	☐	☐
4.2 Level of Dysplasia ***Check 1 response per column***	**A**	**B**	**C**
No dysplasia ……	☐	☐	☐
Low grade (mild or moderate dysplasia)……	☐	☐	☐
High grade (severe dysplasia, carcinoma in situ)	☐	☐	☐
Invasive Adenocarcinoma……	☐	☐	☐
Not Specified or N/A……	☐	☐	☐

5.0 Date of Pathology Report: ____/____/_____
DD / MM / YYYY

I confirm that all entries are complete and accurate.

_____	_____	_____
Date	**Signature**	**Printed name**

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Attachment E: SEGMENTAL COLON RESECTION: TISSUE HISTOLOGY AND STAGING CRF

Place Epigenomics Subject Code Label Here:

1.0 Subject Code……..

2.0 Date of Surgery: ____/____/_____
DD/ MM / YYYY

3.0 Tumor Site (Check all that apply)

Cecum……	☐	Left (descending) colon……	☐
Right (ascending) colon……..	☐	Sigmoid colon……	☐
Hepatic flexure……..	☐	Rectosigmoid……..	☐
Transverse colon……..	☐	Rectum……..	☐
Splenic flexure……	☐	Colon, not otherwise specified…….	☐
		Cannot be determined……..	☐

4.0 Tumor Size:

Greatest dimension: ____ cm, or……..……Cannot be determined ☐

5.0 Histologic Type (Check any that apply)

Adenocarcinoma NOS……	☐	Other carcinoma or malignant tumor…..	☐
Other adenocarcinoma type……..	☐	Not determined……..	☐

6.0 Histologic Grade (Check one)

Low-grade (well to moderately differentiated)	☐		
High-grade (poorly to undifferentiated)……	☐	Cannot be assessed……	☐

7.0 Pathologic Staging (pTNM)

7.1 Primary Tumor (pT) (Check one)

pTX: Cannot be assessed……	☐	**pT3**: Tumor invades through the muscularis propria into the subserosa or nonperitonealized pericolic or perirectal soft tissues……	☐
pT0: No evidence of primary tumor…	☐	**pT3a/b**: Tumor invades through muscularis propria into subserosa or nonperitonealized pericolic or perirectal soft tissues, invades < 5mm beyond muscularis propria border……	☐
pTis: Carcinoma in situ, intra-epithelial (no invasion) or w/ lamina propria invasion……	☐	**pT3c/d**: Tumor invades through muscularis propria into subserosa or nonperitonealized pericolic or perirectal soft tissues, invades >5 mm beyond muscularis propria border……	☐
pT1: Tumor invades submucosa……	☐	**pT4a**: Tumor directly invades other organs ……	☐
pT2: Tumor invades muscularis propria	☐	**pT4b**: Tumor penetrates the visceral peritoneum…	☐

7.2 Regional Lymph Nodes (pN) (Check one)

pN0: No regional node metastasis…….	☐	**pN2**: Metastasis in >4 regional lymph nodes……	☐
pN1: Metastasis in 1-3 regional nodes	☐	**pNX**: Cannot be assessed……	☐

7.3 Distant Metastasis (M) (Check one)

M0: No metastasis……	☐		
M1: Distant metastasis……	☐	**MX**: Cannot be assessed……	☐

I confirm that all entries have been reviewed for completeness, accuracy and legibility:

Date	Signature	Printed Name

Epigenomics Inc.
1000 Seneca Street, Suite 300
Seattle, WA 98101, USA

Epigenomics AG
Kleine Präsidentenstr. 1
10178 Berlin, Germany

Attachment F: Subject Enrollment Log

Epigenomics Subject Code Label	Subject Name	Clinical Record Number	Informed Consent signed (Yes/No)	Enrolled by: (Name/Initials)	Enrollment Date MM/DD/YYYY	Subject meets inclusion/ exclusion criteria (Yes/No)

Attachment G: Specimen Shipment Instructions

- Notify Epigenomics via phone or e-mail BEFORE shipping, for agreement on a shipping date. Provide courier name and tracking number for the shipment. We will be able to follow up if the expected shipment does not arrive.
- Avoid shipping for delivery on Fridays or public holidays unless agreed in advance. If there were any delay in delivery, the samples would thaw over the weekend or holiday, as our facility is not open to receive those deliveries.
- Store frozen samples in your –80^{O}C freezer for at least several weeks, or until you have accumulated enough for a shipment.
- Temperature monitoring devices may be provided to monitor temperature during shipment. Please see product instructions for handling details and include the activated devices in each shipment.
- Use insulated shipping boxes, with an inner foam box and outer cardboard box. Some sites prefer to use their own boxes in order to avoid storing large numbers of boxes, which is fine. If you need shipping boxes, please order them and add to your invoice for reimbursement by Epigenomics. An example of an appropriate box would be: ThermoSafe item #355, which is available from VWR as item #15714-500 or Fisher Scientific as item #03-530-17.
- Package the specimens according to Standard UN 3373 (IATA Shipping Instructions 650) for 'Biological Substance, Category B', i.e. two water tight and pressure safe layers with absorbent material in between are required. Do not allow samples to thaw while preparing package for shipment!
- Use dry ice pellets, not blocks. Blocks may crush the samples.
- Include sufficient dry ice for planned shipping time, and include enough dry ice to protect the samples in the event of a one-day delay in transit. Thawed samples cannot be used for our research. Please **do not** try to save shipping costs by putting less dry ice in the package. Epigenomics will pay all shipping costs.
- Fill any empty space in the box with wadded up newspaper or foam peanuts. Air space in the box will cause the dry ice to sublimate ("melt") faster.
- Include in the package a paper packing list of contents of the shipment. E-mail a copy of the packing list to your Epigenomics contact person.
- Ask your Epigenomics contact to which Epigenomics location you should send your shipment:

Epigenomics Location	USA	Germany
Shipping address	Attn: Study Management Epigenomics, Inc. 1202 Terry Ave., Suite 300 Seattle, WA 98101 USA +1-206-883-2909 or -2929	Attn: Study Management Epigenomics AG Kleine Präsidentenstr. 1 D-10178 Berlin Germany +49-30-24345-0

- Follow the shipping instructions below for your type of shipment:

Shipment Type	**Domestic Shipment <u>within</u> the U.S.**	**Domestic Shipment <u>within</u> the EU**	**Intercontinental Shipment and/or Requiring Customs Clearance**
Use this Courier *(Unless alternate courier is pre-approved by Epigenomics)*	Use FedEx **only**	Contact Epigenomics to arrange a courier pickup at your location.	Use WorldCourier -If WorldCourier is not available, use other courier approved in advance by Epigenomics (preferably one that is capable of refilling dry ice if necessary) (**Never** <u>use FedEx for int'l shipments.</u>)
Shipment costs	Use Epigenomics FedEx account #2321-5928-6	Billed directly to Epigenomics by courier	Epigenomics will reimburse, as agreed in the contract
Shipping Instructions	Ship Priority Overnight for delivery the next day	Contact Epigenomics to arrange a courier pickup at your location.	Specify that courier should refill the dry ice if necessary. Remember to complete Customs forms as required.
Dry Ice	Use **minimum** 15 pounds (7kg) for each box. Use more for larger boxes, proportional to the size of the box.	Use **minimum** 20 pounds (10kg) for each box. Use more for larger boxes, proportional to the size of the box.	Use **minimum** 25 pounds (12kg) for each box. Use more for larger boxes, proportional to the size of the box.

Questions? Need to notify us of a shipment?
Either call or e-mail your Epigenomics contact person.

If that person cannot be reached or you don't know whom to contact,

E-mail us:
samples@epigenomics.com

Or call:
Our U.S. location +1-206-883-2900 (9 a.m. to 5 p.m. PST)
Our German location +49-30-24345-0 (9 a.m. to 5 p.m. (4:30 Fridays) CET) and ask for Study Management

Attachment H: Instructions for Case Report Form (CRF) Completion, Storage and Maintenance

1.0 Responsibilities

1.1 Site Principal Investigator (PI)

1.1.1 The PI is responsible for the data reported to Epigenomics in CRFs and in required reports.

1.1.2 The PI is responsible for the accountability and timely transfer of CRF data to Epigenomics, and for the secure storage of CRFs at the investigational site.

1.1.3 The PI or designee is responsible for the storage of all completed CRFs in the site's Regulatory Binder. All study documentation will be stored in a secure, limited access area in accordance with the Study Protocol.

1.1.4 The PI may delegate in writing CRF completion and maintenance to appropriate investigational site personnel. Even when delegated, the PI maintains the ultimate responsibility. Whenever a task is delegated, the PI must document the task and the name of the designee on the Delegation of Responsibility Log (Attachment I).

1.2 Investigational Site Personnel

1.2.1 Per PI written authorization on the Delegation of Responsibility Log (see Attachment I), investigational site personnel may be responsible for any or all of the aforementioned tasks.

2.0 Instructions for completion, correction, storage, and maintenance of CRFs

2.1 CRF Completion

2.1.1 Authorized investigational site personnel will transcribe study data from source documents to CRFs (paper or electronic). All study data must be accurate, correct, complete, and legible.

2.1.2 All CRF data will be obtained from source documents and must be consistent with the source data or the discrepancies explained. (Exceptions: CRF #1 Health History and CRF #2 Sample Processing will serve as source documents for the study.)

2.1.3 The PI or designee will update or complete CRFs promptly after any new subject data (e.g. colonoscopy report, pathology report) becomes available.

2.1.4 The PI or designee signs each completed CRF, certifying that the data is complete and accurate.

2.1.5 Once complete, each CRF should be promptly provided to Epigenomics. Any one of three options selected to suit the needs of the collaborating site may be used:

- Paper CRFs (originals or certified copies) may be transmitted to Epigenomics;
- Paper CRFs may be transmitted directly to Epigenomics' database via a validated facsimile (fax) transmission system; or
- Data from authorized electronic CRFs using validated electronic data capture software may be transmitted by direct data uploading into Epigenomics' database following a defined procedure.

2.2 CRF Correction

The PI or designee may make corrections to the CRF as authorized by the PI. The following guidelines must be followed for any corrections to data entries:

2.2.1 Any change or correction to a CRF should be dated, initialed, and explained (*if necessary*) by the person responsible for making the change.

2.2.2 The change should not obscure the original entry (i.e. an audit trail is maintained); this applies to both written and electronic changes or corrections. No correction fluid may be used. The original entry should be crossed out with a single line. This allows the original entry to remain legible*. Example: "~~cold~~" sinus infection. The wrong data entry "cold" is crossed out and the correct response, "sinus infection" is written beside the entry.* Clearly initial and date beside the lined out item.

2.2.3 If a monitor at a site visit requests a correction to CRF data, a note to file will be generated by the monitor or PI or designee, and signed by both in order to document why data entries were changed.

2.2.4 Records will be retained in the site's Regulatory Binder regarding changes and corrections made to clinical data relevant for the clinical investigation.

2.2.5 Site personnel must not make changes to CRFs after completed CRFs have been transmitted to Epigenomics, unless specifically instructed to do so by Epigenomics.

Epigenomics may request data clarifications in writing after submission of the CRFs as part of Epigenomics' data management edits procedures and data analysis. The PI or designee will process the data queries and clarifications promptly and will report to Epigenomics in writing. Copies of all data edits will be retained in the site's regulatory binder and are part of the official audit trail.

2.3 CRF Storage and Maintenance

2.3.1 After transmission to Epigenomics, paper CRFs should be stored in a secure, limited access area at the investigational site.

2.3.2 Original CRFs will be maintained at the investigative site or appropriate designated storage facility.

2.3.3 The PI will be required to retain the CRFs along with all study documentation for a period defined in the protocol.

Attachment I: Delegation of Responsibility

STUDY PROTOCOL number: ___SPR0006____

Principal Investigator: .....

Site:..... *Address:*.....

Name	Initials	Signature	Role	Authorized Duties	Start Date	PI Verification / Signature
			Principal Investigator	ALL		

Authorized Duties:

1. Screening
2. Informed Consent
3. CRF Completion
4. Specimen Collection
5. Specimen Processing
6. Query Resolution
7. Other (specify)

If individual leaves or changes duties, strike through row and date. Re-enter information with new duties.

I delegate the above specified duties as assigned and will directly supervise the above named personnel in the performance of protocol requirements.

_____ _____

PI Signature Date (dd.mm.yy)

Attachment J: Contact List for Clinical Investigation

Study Protocol Number: _____

Personnel	Name & Title	Address	Telephone Numbers	Email
Principal Investigator				
Clinical Research Associate or Study Nurse				
Responsible Laboratory Person				
Sub-Investigator 1				
Sub-Investigator 2				
Pathologist				
Sponsor Study Director				
Sponsor Monitor				
Sponsor Sample Management				

Attachment J: Contact List for Clinical Investigation

Study Protocol Number: _____

Personnel	Name & Title	Address	Telephone Numbers	Email

Attachment K: Study Protocol Deviation Log

Study Protocol Number: _____

Study site: _____

Subject Code	Date of Deviation (dd.mm.yy)	Deviation	Date Reported to IRB/IEC (dd.mm.yy)	Date reported to Sponsor (dd.mm.yy)	Log Author Name

Attachment L: Study Protocol Deviation Report Template

Study Protocol No.	Study Name	Report Author	
Name of Site	Date of Report (dd.mm.yy)	Report Sent To	Copies Sent To

Subject Code:_____

Date of deviation:_____
(dd.mm.yy)

Deviation summary:

Reason for the deviation:

Impact of deviation:

Proposed corrective action, as applicable:

(If a corrective action is necessary its effectiveness needs to be demonstrated. Corrective action can be a study protocol admendment)

Report Author:

---------- ---------- ----------
Printed name signature date (dd.mm.yy)

Epigenomics Inc.
1000 Seneca Street, Suite 300
Seattle, WA 98101, USA
FAX# +1 206 883-2981

Epigenomics AG
Kleine Präsidentenstr. 1
10178 Berlin, Germany
FAX# +49 30 24345 56004

Attachment M: SUBJECT MANAGEMENT FORM

Subject Code:	Place Epigenomics Subject Code Label Here:
Date Consent Form Signed:	_ _ / _ _ / _ _ _ _ DD / MM / YYYY
Date Subject Enrolled:	_ _ / _ _ / _ _ _ _ DD / MM / YYYY
Date Colonoscopy Scheduled:	_ _ / _ _ / _ _ _ _ DD / MM / YYYY

CRF A – Health History			
Complete?	☐	Faxed to Epigenomics?	☐
Filed in Regulatory Binder?	☐		

Date Blood Draw Scheduled:	_ _ / _ _ / _ _ _ _ DD / MM / YYYY

CRF B – Sample Processing			
Complete?	☐	Sample CRF Faxed to Epigenomics?	☐
Filed in Regulatory Binder?	☐	Subject Code Linked to Specimen ID?	☐
Date Sample Shipped to Epigenomics:		_ _ / _ _ / _ _ _ _ DD / MM / YYYY	

CRF C – Colonoscopy			
Colonoscopy Completed?	☐	Faxed to Epigenomics?	☐
Filed in Regulatory Binder?	☐	Tissue to pathology?	☐

Date Surgery Scheduled:	_ _ / _ _ / _ _ _ _ DD / MM / YYYY

CRF D – Incisional Biopsy/Polypectomy Histology		**Not applicable**	☐
Complete?	☐	Faxed to Epigenomics?	☐
Filed in Regulatory Binder?	☐	CRC diagnosis?	☐

CRF E – Tissue Histology and Staging		**Not applicable**	☐
Complete?	☐	Faxed to Epigenomics?	☐
Filed in Regulatory Binder?	☐		

Subject Status – Upon completion of this lower section, please fax to Epigenomics		
Withdrawn?	☐	By Subject's Physician? ☐ By Subject? ☐
Potential Protocol Violation? (See Protocol, Section 11)	☐	Blood drawn after Bowel Prep? ☐ Blood draw Insufficient or Not Done? ☐ Colonoscopy Incomplete? ☐ Colonoscopy Not Performed within Time Required? ☐ Other Protocol Violation? ☐
Fulfills exclusion/Does not fulfill inclusion	☐	Case Complete? ☐

Attachment N: Training Log

Topic:.....

Attach handouts used if appropriate.

Date of Training:..... Length of Training Session:.....

Trainer: .....

Qualifications of Trainer:.....

.....

Persons Trained:

Printed Name	Signature	Date

Attachment O: SUBJECT TRAVELER

1.0 Subject Code……

Subject Name: _____

Attachment P: Blood Collection and Plasma Preparation SOP

1. PURPOSE

This document describes how to collect whole blood samples and prepare plasma and peripheral blood leukocytes (PBL) from these samples for the analysis of DNA.

2. DEFINITIONS

2.1 Subject Code

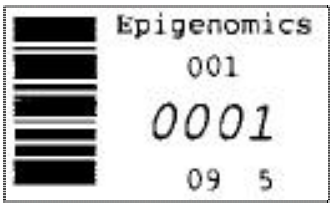
Human readable code, consisting of:

- Unique 3-letter code per Collection site/Provider, e.g. RCR
- Unique sequential number per subject, e.g. 0001, 0002

Example: First two subjects from the site "RCR" would be RCR0001 and RCR0002.

2.2 Sample ID

A human readable code as shown below, consisting of three lines:



3-digit code is the same for all subjects in the study

Large font 4-digit code is the same on all the cryovials for one subject

2-digit code distinguishes all the cryovials for one subject from each other
1-digit QC "checksum" code helps detect barcode scanning errors

Note: The Sample ID will be linked to the Subject Code by placing the Sample ID label from the Sample Processing Kit on the Sample Processing CRF.

3. MATERIALS AND EQUIPMENT

3.1 Provided by Site

Site(s) will require and must provide the following:

- Gloves, disinfectant and swabs
- Box, absorbent material (e.g. paper) and dry ice for shipment of samples
- Any other supplies not provided by Epigenomics (e.g. packaging material)
- Centrifuge, calibrated annually, capable of 1350 (±150) *g*
- –70°C or colder freezer (or approved dry ice storage container)

3.2 Provided by Epigenomics

3.2.1 Enrollment Pack

- Subject Management Form (with Subject Code labels for CRFs)
- Health History CRF
- Subject Traveler (with Subject Code labels for blood collection and plasma processing)

- Sample Processing CRF

3.2.2 Sample Processing Kit

Below is a description of such a kit

1. 2ml pre-labeled cryovials for plasma (10 pcs.)
2. 1ml pre-labeled cryovial for buffy coat with red cap insert (1 pc)
3. Sample ID label for placement on Sample Processing CRF

3.2.3 Bulk supplies

- 10ml EDTA Vacutainer tubes (BD lavender top, plastic)
- Vacutainer needles and/or butterfly needles, 20G or 21G
- Vacutainer needle holders
- 15ml polypropylene centrifuge tubes
- 50ml polypropylene pooling tubes
- Plastic disposable bulb pipettes
- Small boxes for storage and transport of filled cryovials

4. PROCEDURE

4.1 Blood Draw

For each subject providing a blood sample:

4.1.1 Write the Subject's name on the Sample Traveler.

4.1.2 Optional step: If blood will be drawn in a different location than where subject was enrolled, send the Sample Traveler and attached Sample Processing CRF (stapled together) with the Subject to the blood draw station. Then continue with Step 4.1.3.

4.1.3 Verbally confirm Subject's identity and prepare blood draw.

4.1.4 Label four EDTA Vacutainer tubes with Subject Code labels (attached to the Sample Traveler) and write the patient's name (or other identifier) on the four tubes.

4.1.5 Use Vacutainer system to draw blood from subject. Fill four EDTA Vacutainer tubes (total 40 ml of whole blood). For specific blood draw instructions, see Vacutainer package insert.

4.1.6 Immediately after filling each tube, invert the tube 10 times gently. Inversion can be performed while subsequent tubes fill.

4.1.7 Record date and time of blood draw on the Sample Processing CRF.

4.1.8 Allow blood tubes to stand at room temperature for a minimum of 30 minutes prior to centrifugation and check corresponding box on Sample Processing CRF.

4.1.9 Adhere Subject Code labels (from Traveler) to one 50ml pooling tube and two 15ml centrifuge tubes.

Note: Specimens must be processed and plasma frozen within 4 hours of blood draw.

4.2 Plasma processing

4.2.1 Record plasma processing start time (start of first centrifugation) on Sample Processing CRF.

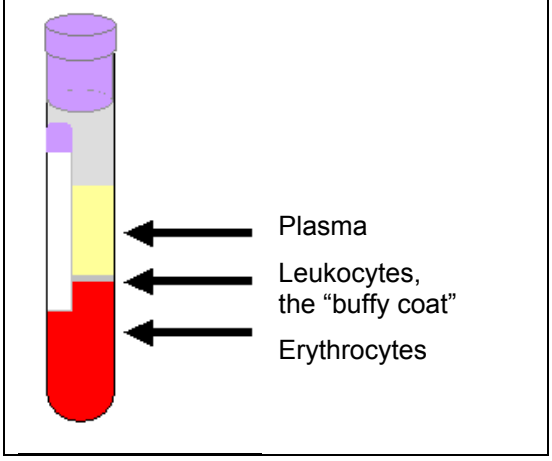
4.2.2 If centrifuge has an external brake, ensure that brake switch is off in order to prevent disruption of the cell layer.

4.2.3 Centrifuge blood in Vacutainer tubes at room temperature for 12 minutes at 1350 (±150) g. If centrifuge uses RPM (revolutions per minute), see centrifuge product brochure for conversion.

4.2.4 Remove tubes from centrifuge. If any of the Vacutainer tubes demonstrates gross hemolysis (bright red plasma), then this tube should be discarded. Continue processing of the other, non-hemolyzed, tubes. Check box on Sample Processing CRF indicating that (1st) centrifugation step has been completed.

4.2.5 Using a disposable bulb pipette, transfer plasma from collection tubes to the 15ml centrifuge tubes.

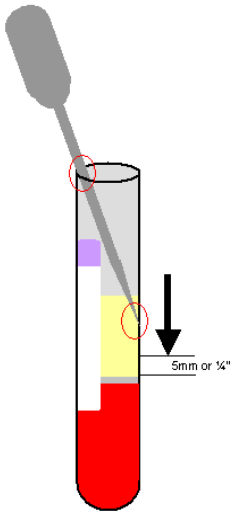
Vacutainer® after centrifugation



Note:

Centrifugation separates plasma from leukocytes and erythrocytes as shown at left.

Leaving sufficient residual volume in the tubes after the centrifugation and not disturbing the buffy coat when pipetting is the most critical step in the sample preparation process. DO NOT compromise quality by reducing the residual volume when pipetting.



Pipetting Instructions:

- After centrifugation, buffy coat is visible as very small whitish band above the erythrocytes.

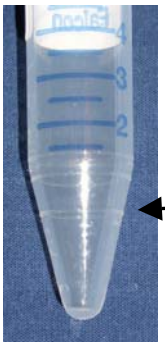
-Be careful not to disturb the buffy coat (cellular) layer in the original Vacutainer tubes.

-To minimize risk of aspirating cells from the buffy coat, hold Vacutainer upright and tilt the pipette to touch the Vacutainer in two positions (see red circles in the drawing on the left) and slowly move pipette down while aspirating.

-Always place the pipette at the top of the plasma layer and STOP aspirating ≥5mm or ≥ 1/4" above the buffy coat in order to avoid contaminating the plasma with cells (see arrow). If cells are aspirated, dispose of pipette and Vacutainer.

4.2.6 After transferring plasma to the 15ml centrifuge tubes as described, set aside at least one Vacutainer (from each subject) for later collection of buffy coat. Discard remaining Vacutainer tubes in biohazard trash.

4.2.7 Centrifuge the plasma in 15ml centrifuge tubes at room temperature for 12 minutes at 1350 (±150) *g* as described in 4.2.3. Check box on Sample Processing CRF indicating that (2nd) centrifugation step has been completed.



Minimal amount of residual volume (≥0.5ml) in centrifuge tube, see arrow pointing to first transparent mark from bottom.

4.2.8 With an unused bulb pipette, transfer plasma from the 15ml centrifuge tubes to the 50ml pooling tube.

Note: Leave a residual amount of ≥0.5ml of plasma (10-12mm or 1/2" height or lowest ring) in bottom of centrifuge tubes to avoid contamination with pelleted cells).

4.2.9 Gently mix plasma in pooling tube and record total plasma volume on Sample Processing CRF.

Processing blood as directed should result in ~4ml of plasma per Vacutainer tube, with a total volume of 15-20ml of plasma.

4.2.10 Obtain an Epigenomics Sample Processing Kit. Adhere the Sample ID label contained in kit to the designated position on the Sample Processing CRF (thereby linking the Sample ID with the Subject Code on the Sample Processing CRF). While working with the Subject's cryovials and the Sample Processing Kit, constantly verify that the same Sample ID is linked to the Subject.

4.2.11 Transfer the buffy coat from the Vacutainer tube(s) retained in 4.2.6 into the small (1ml) cryovial (with the red cap insert) using a fresh bulb pipette. Again, confirm the correct linkage of Subject Code and Sample ID by checking the labels on the tubes with those placed on the Sample Processing CRF. Check box on Sample Processing CRF indicating buffy coat

sample has been collected.

4.2.12 Using another new bulb pipette, transfer plasma from the pooling tube into the corresponding 2ml cryovials pre-labeled with the Sample ID. Fill each tube to the 2ml mark (the faint ring below the threading). In order to allow for expansion during freezer, do not fill above the 2ml mark!

4.2.13 Ensure that Sample ID on cryovials corresponds to Sample ID placed on the subject's Sample Processing CRF.

4.2.14 Record the number of filled cryovials on Sample Processing CRF.

4.2.15 Place all plasma and buffy coat aliquots (up to 11 cryovials from one subject) into the specimen storage box provided. Do not separate tubes from one subject into different boxes.

4.2.16 Freeze plasma in cryovials upright in –70°C or colder freezer or buried in dry ice (within 4 hours of blood draw). (Note: While freezing at –70°C or colder is preferred, initial storage at –20°C or colder for up to 24 hours, followed by transfer to a –70°C or colder freezer or dry ice container, is acceptable. Once at –70°C, the samples must be maintained continuously at that temperature. Document temporary initial storage at –20°C by marking the appropriate field on the Sample Processing CRF.)

4.2.17 Record date and time plasma and buffy coat are placed into freezer or dry ice on the Sample Processing CRF.

4.2.18 Store samples in –70°C or colder freezer (or in approved dry ice storage container) until shipment.

4.2.19 Discard all blood collection and processing tubes and pipettes as biohazard waste.

4.2.20 Complete documentation.

4.2.20.1 Ensure that all steps of this protocol have been carefully followed and Sample Processing CRF has been filled out completely.

4.2.20.2 Sign and date the Sample Processing CRF.

4.2.20.3 Fax completed Sample Processing CRF to Epigenomics.

4.2.20.4 File Subject Traveler in Regulatory Binder.

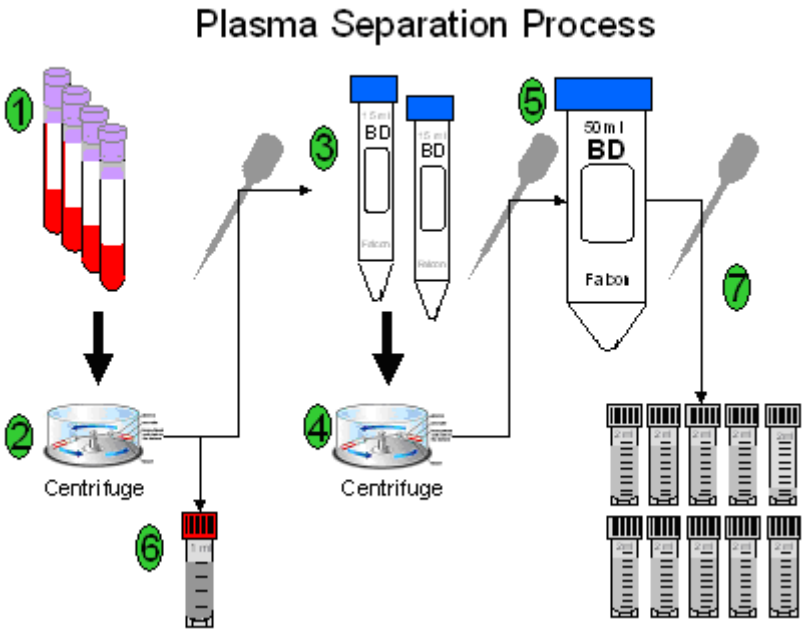
4.2.20.5 Return original Sample Processing CRF or a certified copy to the PI or designee for filing in the Regulatory Binder.

5. HEALTH AND SAFETY CONSIDERATIONS

5.1 Universal Precautions

In accordance with the Site's policies and guidelines, use personal protective equipment to prevent exposure to bloodborne pathogens or other potentially infectious materials, and dispose of all clinical waste appropriately.

Appendix A – **Plasma Preparation Summary**



		Plasma must be prepared and frozen within 4 hours of blood draw		
Process Step	**Image**	**Process Action**	**Specification**	**Remarks**
Blood Collection	1	Blood draw	Draw blood before bowel cleaning procedure using EDTA Vacutainer tubes*	
		Blood tube handling	Invert each Vacutainer 10 times gently immediately after blood draw	
			Keep sample at room temperature for 30min prior to centrifugation	
Plasma Preparation	2	1st Centrifugation	Centrifuge Vacutainers at room temp., 12 min, 1350 (±150) *g*, brake off	
	3	1st Transfer	Transfer to plasma to 15ml centrifuge tubes*	Pipette plasma carefully, leave residual volume
	4	2nd Centrifugation	Centrifuge 15ml centrifuge tube(s) at room temp., 12 min, 1350 (±150) *g*, brake off	
	5	2nd Transfer	Pool all plasma in 50 ml pooling tube*	Use new pipette, pipette plasma carefully, leave residual volume
		Assign Sample ID	Place Specimen ID label from Sample Processing Kit onto Sample Processing CRF	
	6	Buffy Coat	Transfer buffy coat from at least one Vacutainer into 1ml (red capped) cryovial	Red cell contamination is expected and acceptable
	7	3rd Transfer	Aliquot plasma into up to ten 2ml cryovials	Use new pipette, fill to 2ml mark
Freezing			Freeze in -70°C or colder freezer, or in dry ice	Initial storage at –20°C for up to 24 hours is allowed; then transfer to –70°C or colder freezer or dry ice.
Storage			Store at -70°C or colder until shipment	
Shipment			Use ample dry ice	See Specimen Shipping Instructions for details
* **Label all tubes with Subject Code labels (attached to Sample Traveler)**				

Appendix B – Specimen Storage Instructions

GENERAL

- Freeze plasma and buffy coat specimens *upright* in a cryovial storage box. (Note: While freezing at –70°C or colder is preferred, initial storage at –20°C or colder for up to 24 hours, followed by transfer to a –70°C or colder freezer or dry ice container, is acceptable.)
- Once frozen, maintain samples continuously at –70°C or colder.

When outside the freezer, such as when transferring to a different freezer in another location or preparing for shipment, boxes containing cryovials should be buried in dry ice.

- Freezer or dry ice specimen storage container temperature must be checked and documented at least once each workday.
    - Please make Epigenomics aware of any existing automatic or manual temperature monitoring procedures (and associated documentation) already in place at your site.
    - Alternatively, use this simple temperature monitoring process:
        - Place a thermometer in the dry ice to measure temperature of a dry ice container. For freezers, use display temperature *(only if calibrated annually)* or store a thermometer in a container of glycerol within the freezer; remove to read temperature.
            - The thermometer should meet NIST standards of accuracy (example: VWR item #61054-546).
        - Check the temperature once at the beginning of each workday and record the date, temperature, and staff initials on a paper temperature log.
            - Deviations from acceptable storage temperatures must be recorded and explained on the log; for example, "door left open to prepare shipment."
- If samples thaw or are exposed to temperatures warmer than -70°C, document this on a Protocol Deviation Report.
    - Momentary increases in freezer temperature due to opening and closing the freezer door are expected and allowed, and not considered to be protocol deviations. Avoid prolonged warming of the freezer to temperatures above -70°C by minimizing time with the freezer door open.
    - Do not ship to Epigenomics any specimens that have been thawed.
- The freezer or dry ice storage box containing the specimens should either be locked or in a secure area accessible only to authorized site staff.
- Batch ship frozen samples according to Epigenomics' Specimen Shipment Instructions.

STORAGE OF PLASMA SPECIMENS IN –70°C OR COLDER FREEZER

- Storage in a -70°C or colder freezer is preferred (but an alternative is outlined below).
- Epigenomics specimens should be stored in a designated area(s) of the freezer.
- A backup storage plan should be in place in the event of freezer failure.

TEMPORARY STORAGE OF PLASMA SPECIMENS IN APPROVED DRY ICE STORAGE BOX

The following short-term storage option is acceptable, if previously agreed upon by Epigenomics, for sites without a –70°C or colder freezer, provided that an appropriate temperature-monitoring program is in place.

- Specimens shall be placed upright in specimen storage boxes and buried in dry ice pellets inside an approved insulated dry ice storage container.
- Dry ice should be replenished to maintain adequate storage temperature.
- It is the site's responsibility to locate a local vendor and arrange delivery of dry ice pellets.
- It is the site's responsibility to take the appropriate safety precautions when storing and handling the dry ice (such as personal protective equipment and adequate ventilation).
- Thawed specimens cannot be used for our study. Plan to use 10 to 20 pounds of dry ice for every 24-hour period depending upon the size of the container.

Attachment Q: Fax Log

Site:_____

PI:_____

CRF

Abbreviations:
A=Health History CRF
B=Sample Processing CRF
C=Colonoscopy CRF
D=Incisional Biopsy CRF
E=Tissue Histology and Staging

M=Subject Management Form

Date DD/MM/YY	**Sender Name**	**Subject Code**	**Form (use abbreviations)**	**Page/Total**

Attachment R: Fax and Sample Shipment Schedule Instructions

There are 3 sections in the Regulatory Binder for organizing CRFs and Subject Management Forms:

1) "Active" forms
    a. All unfilled or partially filled CRFs
    b. All unfilled or partially filled Subject Management Forms
2) Forms "ready to fax"
    a. All completed (but not yet faxed) CRFs
    b. All completed (but not yet faxed) Subject Management Forms including forms for subjects withdrawn from study
3) "Completed and faxed" forms
    a. All completed and faxed CRFs
    b. All completed and faxed Subject Management Forms
    c. Faxed Subject Management Forms from subjects that have withdrawn from study

Procedure:

All completed CRFs and Subject Management forms should be faxed on a weekly basis on a specified day whenever possible. Take completed forms from "ready to fax" section, fax to Epigenomics, list faxed documents on the Fax Log, and then place in "Completed and Faxed" section.

CRFs that are not yet completed and Subject Management forms that are not yet completed should be filed in the "Active" forms section. Once completed, place them in the "Ready to fax" section.

Samples can be shipped according to one of three different schedules depending on frozen storage capacity:

1) Weekly shipment
2) Every other week shipment
3) Monthly shipment

All sample shipments should be sent on Mondays or Tuesday's at the latest.
A fax and shipment calendar is provided as a reminder of the fax and sample shipment schedule. All samples with corresponding completed CRFs and Subject Management forms received by the payment cut-off date noted on the calendar will be processed for payment according to the rules for completed cases. Payment will be forwarded on a quarterly basis according to the schedule on the calendar provided.