

Genetic Diagnostic Laboratory & Referral Service

UNIVERSITY OF PENNSYLVANIA MEDICAL CENTER

Tel: (215) 573-9161 • Fax: (215) 573-5940

Retinoblastoma (RB1)

Background: Retinoblastoma (RB) is a malignant tumor of the developing retina that occurs in children, usually before the age of five years. Retinoblastoma may be unilateral or bilateral. Approximately 60% of patients have unilateral RB with a mean age at diagnosis of 24 months; approximately 40% have bilateral RB with a mean age at diagnosis at 15 months. Germline mutations in the RB1 gene (chromosomal location 13q14) predispose individuals to the disease who are then at increased risk of developing other RB-related (non-ocular) tumors.

Assay: Full sequencing of RB1 gene in DNA isolated from blood or tumor
Deletion/Duplication analysis of RB1 gene in DNA isolated from blood or tumor

Utility: Diagnostic confirmation, carrier detection and prenatal diagnosis

Sensitivity: The probability that an RB1 gene mutation will be detected in an index case depends upon whether the tumor is unilateral or bilateral, unifocal or multi-focal; whether we test tumor or blood and whether the family history is positive or negative. If the tumor is/was bilateral the sensitivity is 89%. If the tumor is/was unilateral and there is no family history, tumor tissue should be tested first. If tumor is not available the sensitivity of testing DNA from a blood sample is ~10%. Sensitivity of testing if tumor is provided is ~77%. Mutations in non-coding sequences, deletions or duplications will not be detected by sequencing but may be detected by the deletion/duplication assay.

Turn around: Full sequencing of RB1 gene in DNA isolated from blood or flash frozen tumor: 8-12 weeks
Full sequencing of RB1 gene in DNA isolated from tumor in paraffin block: 12-15 weeks
Familial mutation analysis of RB1 gene in DNA isolated from blood: 2-3 weeks
Prenatal diagnosis of RB1 point mutation in DNA isolated from cultured CVS or amniocytes: 2-3 weeks
Deletion/Duplication assay in blood or tumor: 4-6 weeks
Deletion /Duplication assay in cultured amniocytes or CVS: 2-3 weeks

Fees: \$1460 full gene sequencing of RB1 in DNA isolated from blood or flash frozen tumor,
\$1580 full gene sequencing of RB1 in DNA isolated from tumor in paraffin block,
\$500 screening for deletion/duplication in RB1 gene in DNA isolated from blood or flash frozen tumor
\$340 familial mutation analysis of RB1 gene in DNA isolated from blood
\$340 prenatal diagnosis of RB1 point mutation in cells cultured from CVS or amniocytes
\$340 prenatal diagnosis of RB1 deletion or duplication in cells cultured from CVS or amniocytes

Samples of maternal and paternal bloods must be submitted with all prenatal testing

CPT codes: Full sequencing from blood or frozen tumor: 83891, 83894x21, 83898x24, 83904x21, 83909x2, 83912
Full sequencing from tumor in paraffin block: 83891x3, 83894x21, 83898x24, 83904x21, 83909x2, 83912
Screening for deletion/duplication in blood: 83891, 83900, 83901x4, 83909, 83912x3
Familial mutation from blood: 83891, 83898x6, 83904x6, 83912
Prenatal diagnosis for point mutation in CVS or amniocytes: 83891x2, 83898x4, 83904x5, 83912
Prenatal diagnosis for deletion/duplication in CVS or amniocytes: 83891x2, 83898x4, 83904x5, 83912

In cases of patients with unilateral RB tumor and no family history, we recommend testing DNA isolated from the tumor before testing for mutation in RB1 gene in DNA isolated from blood. Please advise the lab if tumor is available but not submitted with blood sample, so that we hold the blood until we have received and tested the tumor. If we are not notified in advance that tumor will be coming, we will sequence the blood sample and if tumor is subsequently sent to be tested, there will be another sequencing charge.

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Mutation Analysis for Retinoblastoma INSTRUCTIONS FOR SAMPLE SUBMISSION

▶ Each sample must be accompanied by:

1. A requisition for DNA analysis completed by the physician, nurse or genetic counselor requesting screening. **Please note: an ICD-9 code must be provided.**
 2. The patient's pedigree.
 3. An informed consent signed by the patient/parent/guardian and the professional obtaining the consent. Please have patient initial at top of each page and send **all** pages of the consent.
 4. A verification of blood tubes form signed by the patient, parent or guardian.
 5. A completed registration form with check, money order, credit card authorization or information and authorization for billing the referring institution.
-

IN THE EVENT THAT ALL PROPERLY COMPLETED FORMS DO NOT ACCOMPANY THE SPECIMEN, YOU WILL BE NOTIFIED AND SPECIMEN WILL BE HELD UNTIL PAPERWORK IS COMPLETE.

▶ **Preparing Sample:**

- Obtain 2 EDTA tubes of blood (lavender top).
- Label each tube with the patient's name and date sample was obtained
- Have patient/parent/guardian examine the tubes and sign the verification form.
- If you are sending fresh tumor please flash freeze and send frozen on dry ice.
- If you are sending paraffin embedded tumor, please be certain it is labeled with the patient's name

▶ **Shipping Sample: Ship blood at room temperature Federal Express or other overnight courier, which guarantees AM delivery to arrive Monday-Friday.** There is no one in the laboratory evenings and weekends to receive samples. As above, send fresh tumor on dry ice.

▶ **Shipping Address:**

Dr. Arupa Ganguly
Genetic Diagnostic Laboratory
University of Pennsylvania School of Medicine
Room 415 Anatomy-Chemistry Building
3620 Hamilton Walk
Philadelphia, PA 19104

REQUEST FOR RETINOBLASTOMA TESTING

PATIENT FIRST NAME _____ PATIENT LAST NAME _____

DOB _____ SEX _____ RACE _____ ETHNICITY _____

STREET _____

CITY _____ STATE _____ ZIP _____ COUNTRY _____

HOME PHONE _____

DID THE PATIENT HAVE _____ UNILATERAL OR _____ BILATERAL RETINOBLASTOMA?
ARE THERE ANY OTHER INDIVIDUALS IN THE FAMILY WITH A HISTORY OF RETINOBLASTOMA? _____

PLEASE ATTACH A 3 GENERATION PEDIGREE WITH ALL CANCERS INDICATED EVEN IF THERE IS NO FAMILY HISTORY OF RETINOBLASTOMA.

REFERRING PHYSICIAN _____ PHONE _____ FAX _____

GENETIC COUNSELOR _____ PHONE _____ FAX _____

INSTITUTION _____

STREET ADDRESS _____

CITY _____ STATE _____ ZIP _____ COUNTRY _____

ICD9 code _____ (must be provided by patient's care provider)

samples of maternal and paternal bloods must be submitted with all prenatal samples

TEST/TESTS REQUESTED

- Sequencing of the coding regions of RB1 in DNA isolated from flash frozen tumor
- Sequencing of the coding regions of RB1 in DNA isolated from blood
- Sequencing of the coding regions of RB1 in DNA isolated from paraffin embedded tumor
- Screening for deletion/duplication of RB1 gene in DNA isolated from frozen tumor by MLPA & multiplex PCR
- Screening for deletion/duplication of RB1 gene in DNA isolated from blood by MLPA & multiplex PCR
- RB1 familial mutation in DNA isolated from blood (attach report of proband)
- Prenatal diagnosis for point mutation of RB1 in DNA isolated from cultured amniocytes or chorionic villi (attach report of proband)
- Prenatal diagnosis for deletion/duplication of RB1 in DNA isolated from cultured amniocytes or chorionic villi (attach report of proband)

Please indicate if enucleated RB tumor is being submitted for this test. Yes ___ No ___

Initials _____

PROBAND SEQUENCING

UNIVERSITY OF PENNSYLVANIA
DEPARTMENT OF GENETICS
GENETIC DIAGNOSTIC LABORATORY

Informed Consent for Retinoblastoma Testing/Child

I, _____, hereby request testing for the **retinoblastoma gene, RB1**, using a DNA-based test. I understand that a sample of my child's blood will be obtained from a vein, a procedure that carries very little risk. I understand that the diagnostic samples will be used for the purpose of attempting to determine if my child is a carrier of an altered RB1 gene for this genetic disease.

I understand that:

1. In some cases the DNA test directly detects an abnormality, called a mutation, in the gene and the test is >99% accurate. There is a chance that my child has a mutation which will not be detected by this testing. There is a chance that an alteration of unknown significance may be identified.
2. I understand that the DNA analysis performed at the University of Pennsylvania Genetic Diagnostic Laboratory is specific only for this disease and in no way guarantees my child's health.
3. These tests are relatively new and are subject to change periodically to improve or expand the utility of the test. The tests are not considered research but are considered to be the best and newest laboratory service available. This testing is often complex and utilizes specialized materials so that there is a small possibility that the test will not work properly or that an error will occur. There error rate is low, perhaps 1 in 1000 samples. My signature below acknowledges my voluntary participation in this test, but in no way releases the laboratory and staff from their professional and ethical responsibility to me and my child.
4. In some cases it may be possible for the laboratory to reanalyze leftover DNA samples in the future using new and improved methods. However, I understand that the Genetic Diagnostic Laboratory is not a DNA banking facility and my child's DNA sample may not be available for future clinical studies.
5. Because of the complexity of DNA based testing and the important implications of the test results, results will be reported to me only through the physician or genetic counselor who requested the testing. The results are confidential; they will only be released to other medical professionals or other parties with my written consent. Participation in DNA testing is completely voluntary.
6. I understand that my child's sample will be used only for the test requested. In some cases, DNA samples may be anonymized (stripped of all identifiers) and used as control samples or in research. Results from such testing can not be attributed to identifiable patients and the results are not reportable.

Initials _____

PROBAND SEQUENCING

7. Any blood or tissue specimens obtained for the purposes of this genetic testing become the exclusive property of the Genetic Diagnostic Laboratory. After the specific tests requested have been completed and reported, the Laboratory may dispose of, retain, or preserve these specimens and may use these specimens for research. I understand that my identity will be protected and that research results will not be provided to me or to any other party. If there are new developments in the field, my physician/genetic counselor may be contacted by the Genetic Diagnostic Laboratory staff to offer me the opportunity to have additional clinical testing. If use of this genetic material results in a scientific publication, it will not contain any identifying information.

CONSENT OF PARENT/GUARDIAN:

Print Patient's Name: _____

Signature of Parent/Guardian: _____

Patient's Birth Date: _____

Date Signed: _____

Physician's/Counselor's Statement: I have explained DNA testing to this individual. I have addressed the limitations outlined above, and I have answered this individual's questions.

Print Name: _____

Signature: _____

Date Signed: _____

Initials _____

PROBAND SEQUENCING

**UNIVERSITY OF PENNSYLVANIA
DEPARTMENT OF GENETICS
GENETIC DIAGNOSTIC LABORATORY**

Informed Consent for Retinoblastoma Testing/Adult

I, _____, hereby request testing for the **retinoblastoma gene, RB1**, using a DNA-based test. I understand that a sample of my blood will be obtained from a vein, a procedure that carries very little risk. If this is a prenatal diagnostic test, fetal cells obtained by amniocentesis, chorion villus sampling or fetal blood sampling will be used. I understand that the diagnostic samples will be used for the purpose of attempting to determine if I (or my fetus) am/is a carrier of an altered RB1 gene for this genetic disease.

I understand that:

1. In some cases the DNA test directly detects an abnormality, called a mutation, in the gene and the test is >99% accurate. There is a chance that I have a mutation which will not be detected by this testing. There is a chance that an alteration of unknown significance may be identified.
2. I understand that the DNA analysis performed at the University of Pennsylvania Genetic Diagnostic Laboratory is specific only for this disease and in no way guarantees my health (or the health of my fetus.)
3. These tests are relatively new and are subject to change periodically to improve or expand the utility of the test. The tests are not considered research but are considered to be the best and newest laboratory service available. This testing is often complex and utilizes specialized materials so that there is a small possibility that the test will not work properly or that an error will occur. There error rate is low, perhaps 1 in 1000 samples. My signature below acknowledges my voluntary participation in this test, but in no way releases the laboratory and staff from their professional and ethical responsibility to me.
4. In some cases it may be possible for the laboratory to reanalyze leftover DNA samples in the future using new and improved methods. However, I understand that the Genetic Diagnostic Laboratory is not a DNA banking facility and my DNA sample may not be available for future clinical studies.
5. Because of the complexity of DNA based testing and the important implications of the test results, results will be reported to me only through the physician or genetic counselor who requested the testing. The results are confidential; they will only be released to other medical professionals or other parties with my written consent. Participation in DNA testing is completely voluntary.
6. I understand that my sample will be used only for the test requested. In some cases, DNA samples may be anonymized (stripped of all identifiers) and used as control samples or in research. Results from such testing can not be attributed to identifiable patients and the results are not reportable.
7. When results of the laboratory testing are available, I will be given the option of postponing or declining disclosure of these results. If I choose to learn the results, I will be given this information as part of a counseling session.

Initials _____

PROBAND SEQUENCING

8. Any blood or tissue specimens obtained for the purposes of this genetic testing become the exclusive property of the Genetic Diagnostic Laboratory. After the specific tests requested have been completed and reported, the Laboratory may dispose of, retain, or preserve these specimens and may use these specimens for research. I understand that my identity will be protected and that research results will not be provided to me or to any other party. If there are new developments in the field, my physician/genetic counselor may be contacted by the Genetic Diagnostic Laboratory staff to offer me the opportunity to have additional clinical testing. If use of this genetic material results in a scientific publication, it will not contain any identifying information.

CONSENT OF PATIENT:

Print Patient's Name: _____

Signature of Patient: _____

Patient's Birth Date: _____

Date Signed: _____

Physician's/Counselor's Statement: I have explained DNA testing to this individual. I have addressed the limitations outlined above, and I have answered this individual's questions.

Print Name: _____

Signature: _____

Date Signed: _____

Initials _____

PROBAND SEQUENCING

UNIVERSITY OF PENNSYLVANIA
DEPARTMENT OF GENETICS
GENETIC DIAGNOSTIC LABORATORY

Informed Consent for Retinoblastoma Testing/Tumor

I, _____, hereby request testing for the **retinoblastoma gene, RB1**, using a DNA-based test. I understand that a sample of my/my child's frozen tumor tissue will be used. I understand that the diagnostic samples will be used for the purpose of attempting to determine if I/my child am/is a carrier of an altered RB1 gene for this genetic disease.

I understand that:

1. In some cases the DNA test directly detects an abnormality, called a mutation, in the gene and the test is >99% accurate. There is a chance that I have a mutation which will not be detected by this testing. There is a chance that an alteration of unknown significance may be identified.
2. I understand that the DNA analysis performed at the University of Pennsylvania Genetic Diagnostic Laboratory is specific only for this disease and in no way guarantees my health (or the health of my fetus.)
3. These tests are relatively new and are subject to change periodically to improve or expand the utility of the test. The tests are not considered research but are considered to be the best and newest laboratory service available. This testing is often complex and utilizes specialized materials so that there is a small possibility that the test will not work properly or that an error will occur. There error rate is low, perhaps 1 in 1000 samples. My signature below acknowledges my voluntary participation in this test, but in no way releases the laboratory and staff from their professional and ethical responsibility to me.
4. In some cases it may be possible for the laboratory to reanalyze leftover DNA samples in the future using new and improved methods. However, I understand that the Genetic Diagnostic Laboratory is not a DNA banking facility and my DNA sample may not be available for future clinical studies.
5. Because of the complexity of DNA based testing and the important implications of the test results, results will be reported to me only through the physician or genetic counselor who requested the testing. The results are confidential; they will only be released to other medical professionals or other parties with my written consent. Participation in DNA testing is completely voluntary.
6. I understand that my sample will be used only for the test requested. In some cases, DNA samples may be anonymized (stripped of all identifiers) and used as control samples or in research. Results from such testing can not be attributed to identifiable patients and the results are not reportable.
7. When results of the laboratory testing are available, I will be given the option of postponing or declining disclosure of these results. If I choose to learn the results, I will be given this information as part of a counseling session.

Initials _____

PROBAND SEQUENCING

8. Any blood or tissue specimens obtained for the purposes of this genetic testing become the exclusive property of the Genetic Diagnostic Laboratory. After the specific tests requested have been completed and reported, the Laboratory may dispose of, retain, or preserve these specimens and may use these specimens for research. I understand that my identity will be protected and that research results will not be provided to me or to any other party. If there are new developments in the field, my physician/genetic counselor may be contacted by the Genetic Diagnostic Laboratory staff to offer me the opportunity to have additional clinical testing. If use of this genetic material results in a scientific publication, it will not contain any identifying information.

CONSENT OF PATIENT:

Print Patient's Name: _____

Signature of Patient: _____

Patient's Birth Date: _____

Date Signed: _____

Physician's/Counselor's Statement: I have explained DNA testing to this individual. I have addressed the limitations outlined above, and I have answered this individual's questions.

Print Name: _____

Signature: _____

Date Signed: _____

Initials _____

FAMILIAL MUTATION

**UNIVERSITY OF PENNSYLVANIA
DEPARTMENT OF GENETICS
GENETIC DIAGNOSTIC LABORATORY**

Informed Consent for Retinoblastoma Testing

I, _____, hereby request testing for the **retinoblastoma gene**, using a DNA-based test. I understand that a sample of my or my child's blood will be obtained from a vein, a procedure that carries very little risk. I understand that the diagnostic samples will be used for the purpose of determining if I or my child is a carrier of a mutation in the RB1 gene already detected in a family member.

I understand that:

1. The DNA analysis performed at the University of Pennsylvania Genetic Diagnostic Laboratory is specific only for this disease and in no way guarantees my or my child's health.
2. These tests are relatively new and are subject to change periodically to improve or expand the utility of the test. The tests are not considered research but are considered to be the best and newest laboratory service available. This testing is often complex and utilizes specialized materials so that there is a small possibility that the test will not work properly or that an error will occur. There error rate is low, perhaps 1 in 1000 samples. My signature below acknowledges my voluntary participation in this test, but in no way releases the laboratory and staff from their professional and ethical responsibility to me and/or my child.
3. In some cases it may be possible for the laboratory to reanalyze leftover DNA samples in the future using new and improved methods. However, I understand that the Genetic Diagnostic Laboratory is not a DNA banking facility and my or my child's DNA sample may not be available for future clinical studies.
4. Because of the complexity of DNA based testing and the important implications of the test results, results will be reported to me only through the physician or genetic counselor who requested the testing. The results are confidential; they will only be released to other medical professionals or other parties with my written consent. Participation in DNA testing is completely voluntary.
5. I understand that my child's sample will be used only for the test requested. In some cases, DNA samples may be anonymized (stripped of all identifiers) and used as control samples or in research. Results from such testing can not be attributed to identifiable patients and the results are not reportable.
6. Any blood or tissue specimens obtained for the purposes of this genetic testing become the exclusive property of the Genetic Diagnostic Laboratory. After the specific tests requested have been completed and reported, the Laboratory may dispose of, retain, or preserve these specimens and may use these specimens for research. I understand that my identity will be protected and that research results will not be provided to me or to any other party. If there are new developments in the field, my physician/genetic counselor may be contacted by the Genetic Diagnostic Laboratory staff to offer me the opportunity to have additional clinical testing. If use

Initials _____

FAMILIAL MUTATION

of this genetic material results in a scientific publication, it will not contain any identifying information.

CONSENT OF PATIENT/PARENT/GUARDIAN:

Print Patient's Name: _____

Signature of Patient/Parent/Guardian: _____

Patient's Birth Date: _____

Date Signed: _____

Physician's/Counselor's Statement: I have explained DNA testing to this individual. I have addressed the limitations outlined above, and I have answered this individual's questions.

Print Name: _____

Signature: _____

Date Signed: _____

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Verification of Correctly Identified Blood Tubes

- ▶ I am a participant in genetic DNA testing.
- ▶ I have been shown the tubes containing my blood for this genetic testing and my name has been correctly placed on each one of these tubes.
- ▶ I have signed a copy of the consent form regarding this genetic testing to be sent along with my blood samples. I have been given a copy of the consent form to keep.

Participant Name: _____

Participant Signature: _____ Date: _____

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Patient Registration Form

Please provide the following information. We cannot perform your test without ALL of this information. PLEASE PRINT ALL ANSWERS.

▶ PATIENT INFORMATION

LAST FIRST MI

MAIDEN NAME / ALIAS

Date of Birth: / /

Sex: Male Female

Patient Address:

STREET/NUMBER APT NO.

CITY STATE ZIP

COUNTRY

HOME TELEPHONE

DAY TELEPHONE

▶ REFERRING PHYSICIAN/CARE PROVIDER

NAME MD DO OTHER

STREET ADDRESS

CITY STATE ZIP

TELEPHONE

▶ PAYMENT OPTIONS (MUST CHOOSE ONE)

I have enclosed a check or money order payable to the Genetic Diagnostic Laboratory for the amount of \$_____.

Please charge credit card:

VISA Master Card

Discover American Express

Credit Card Number:

- - -

Expiration Date: /

PRINT NAME AS IT APPEARS ON CARD

SIGNATURE OF CARDHOLDER

I have Pennsylvania Medicaid. A copy of my Medicaid card is attached.

The institution where my testing originated has agreed to pay all charges for the testing.

NAME OF INSTITUTION

BILLING ADDRESS 1

BILLING ADDRESS 1

TELEPHONE

CONTACT PERSON

PERSON AUTHORIZING PAYMENT