



**NEW YORK STATE
INSTITUTE FOR BASIC RESEARCH
IN DEVELOPMENTAL DISABILITIES**

1050 Forest Hill Road, Staten Island, New York 10314
(718) 494-5363 / FAX (718) 494-4835

W. Ted Brown, M.D., Ph.D., Director

INTAKE PROCEDURES FOR PRENATAL FRAGILE X DNA TESTING

Our requirements:

Name of patient and LMP

Pedigree

Forms:

1. Requisition forms with consent signed for mother and father
2. Requisition form with consent signed for the prenatal sample
(If possible we request patients sign the second consent which allows us to retain the DNA for use in future research in developmental disabilities).

Blood: 10 ml (in lavender top vacutainer) for each parent

Prenatal sample:

1. Date scheduled
2. Sample: chorionic villus sample (CVS) or amniotic fluid (AF)
3. Ship by overnight delivery in sterile medium at room temperature in an insulated container.

TECHNICAL INFORMATION FOR THE REFERRING HEALTH PROFESSIONAL:

Requirements for samples:

CVS

1. We request a minimum of 5-10 mg of **dissected** tissue.
2. After the sample is obtained, the contact person should telephone us to confirm that the procedure was carried out.
3. The cytogenetic analysis on the CVS sample must be completed elsewhere by a licensed laboratory and the patient must be informed of this requirement.
4. Three T-25 flasks should be grown by the cytogenetics laboratory and shipped to the Institute for Basic Research for Southern analysis.

AF

1. We request 10 ml of amniotic fluid to carry out PCR for fragile X.
2. After the amniocentesis is performed, the contact person should telephone to let us know the condition and amount of fluid obtained. For direct analysis on uncultured cells, the fluid is preferably clear. Even a “tinge” of blood may introduce a background of maternal alleles.
3. Four T-25 flasks should be grown by the cytogenetics laboratory and shipped to the Institute for Basic Research for Southern analysis.

Results:

PCR: Results are generally available in 1 week. This analysis is carried out on DNA from the prenatal sample in parallel with maternal and paternal DNA. Preliminary results are given by telephone after interpretable results have been obtained. A preliminary report will be issued if the results are unambiguous.

Southern analysis: Results are available in 2-4 weeks. This analysis is carried out to confirm or clarify the PCR studies. We usually harvest 3-4 confluent T25 flasks for DNA isolation. A final report is issued upon completion of these studies.

Limitations of the assays

PCR: With this technique, copies of the CGG triplet region are made from a small sample of DNA. Thus, PCR is very sensitive to contamination, of which maternal cell contamination is the most problematic.

1. For a male fetus, this assay is highly accurate because either the mother's normal allele or the expanded allele is inherited.
2. When the mother's fragile X allele is inherited in a male fetus as a full mutation, we are usually able to amplify the expanded allele. The large repeat number may present a technical challenge both because of the high GC composition and the repetitive nature of the sequence.
3. For a female fetus, if the parents' normal alleles are distinguishable (e.g., 20 and 30 repeats), the diagnosis is highly accurate barring any maternal cell contamination. We are usually able to amplify both the normal and fragile X alleles in a female fetus.
4. For a female fetus if the parents have the same size normal alleles and the fetal analysis reveals a single allele, the data must be interpreted with caution until the Southern analysis has confirmed the results.

Southern analysis: These data are less subject to artifacts because the analysis is carried out on a large amount of genomic DNA isolated from fetal cells. Nevertheless, this technique does have some limitations.

1. With a CV sample, methylation of the CpG island 5' to the gene is

incomplete. In an EcoR I – Eag I digestion of DNA from a postnatal individual, a 2.8 kb band is seen in a normal male, a 2.8 kb and 5.2 kb band in a normal female, >5.2 kb band in full mutation male and a 2.8, 5.2 and >5.2 kb band in a full mutation female (see illustration). Because methylation is incomplete in a CVS sample, the full mutation bands may be shifted and be visible between 2.8 and 5.2 kb. There may be rare cases in which a follow-up amniocentesis might be indicated.

2. With an AF sample, methylation in the fragile X region is complete and the problem of incomplete methylation does not occur. An obvious disadvantage of an amniocentesis, however, is one of time. Furthermore, amniocytes can sometimes be difficult to grow to generate sufficient cells for Southern analysis.

Fee: \$460 includes analysis of the prenatal sample and parental blood specimens. In case of financial hardship, please telephone Sarah L. Nolin, Ph.D. (718-494-5293).



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1050 Forest Hill Road, Staten Island, New York 10314
(718) 494-0600 / FAX (718) 698-3803

W. Ted Brown, M.D., Ph.D., Director

Fragile X DNA Analysis

Instructions for Shipping Prenatal Samples

Samples requirements:

1. CVS 5 – 10 mg dissected tissue in sterile medium for PCR
2. AF 10 ml amniotic fluid for PCR
3. Blood samples (5 - 10 ml in lavender tube) from mother and father should accompany the prenatal sample. If possible, we ask parents to sign the second consent that allows us to retain the DNA for research.
4. 3-4 flasks of cultured cells to be sent at a later date for Southern analysis

Place the specimens in a zip-lock bag with ample packing material for protection. Ship Monday through Thursday in a crush-proof container by overnight express to:

Fragile X DNA Laboratory
Human Genetics Department
Institute for Basic Research
1050 Forest Hill Road
Staten Island, NY 10314

Three forms must accompany the samples:

1. Requisition sheet for the prenatal sample with consent signed
2. Requisition forms for each blood sample from mother and father with consents signed
3. Pedigree, if available

Please use the requisitions form. An electronic file (pdf) of the requisition form can be obtained on our web site

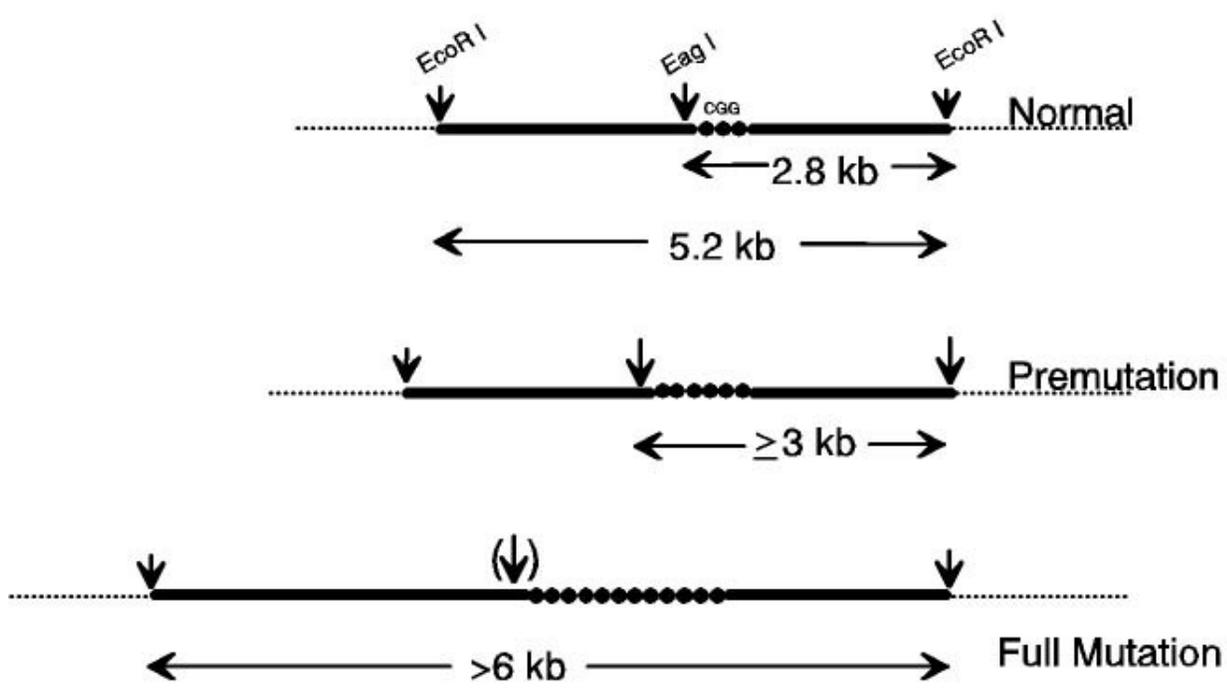
<http://www.opwdd.ny.gov/institute-for-basic-research/research/departments/human-genetics/fxl>

or from Dr. Sarah L. Nolin (sally.nolin@opwdd.ny.gov).

A written report of the results of this study is provided upon completion of all diagnostic tests within two to six weeks.

Fees: \$460 includes analysis of prenatal and parental samples
Methodology: PCR and Southern analysis

Please telephone Dr. Sarah L. Nolin with any questions (718-494-5293 or 718-494-5333).



Fragile X Gene: EcoR I-Eag I Map. CVS full mutation is usually cleaved at the Eag I site (↓) (incomplete methylation).

Diagnostic EcoR I-Eag I Patterns in Southern Analysis

