NEW YORK STATE OF OPPORTUNITY. | Office for People With Developmental Disabilities

INTAKE PROCEDURES FOR PRENATAL FRAGILE X DNA TESTING

NEW YORK STATE
INSTITUTE FOR BASIC RESEARCH
IN DEVELOPMENTAL DISABILITIES

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The Fragile X Laboratory provides molecular analysis of the fragile X gene *FMR1* by PCR and Southern analysis

OUR REQUIREMENTS FOR PRENATAL SAMPLES

- Patient's Information including: demographic data and LMP or EDC
- 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're asking patients to sign the second consent line, which allows us to retain the DNA for use in future research on developmental disabilities. Additionally, stored parental DNA can be used in all future fragile X analyses of subsequent pregnancies, eliminating the need for additional blood collection.

- Blood: 5-10 ml (in lavender-top vacutainer) for each parent
- Prenatal sample:
  1. Collection date
  2. Sample type: chorionic villus sample (CVS) or amniotic fluid (AF)
  3. Ship by overnight delivery in sterile medium at room temperature in an insulated container.

INFORMATION FOR THE REFERRING HEALTH PROFESSIONAL
Requirements for samples

CVS

1. We request a minimum of 5-10 mg of **dissected** tissue.
2. The cytogenetic analysis on the CVS sample must be completed elsewhere by a licensed laboratory and the patient must be informed of this requirement.
3. 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 direct fragile X analysis on uncultured cells, the fluid is preferably clear. Even a“tinge” of blood may introduce a background of maternal alleles.
2. The cytogenetic analysis of the prenatal specimen must be completed elsewhere by a licensed laboratory and the patient must be informed of this requirement.
3. Three 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 within 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 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. The assay we are currently using is ~99% accurate and able to detect the presence of full mutation alleles in both males and females. PCR is, however, very sensitive to contamination, of which maternal cell contamination is the most problematic.

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 (please see p.4 for the diagrams). 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: $750 includes analysis of the prenatal sample and parental blood specimens.
CPT codes: 81243, 81265

For all prenatal cases, we offer post-natal fragile X analysis at no additional cost to our clients.

In case of financial hardship, please telephone Tatyana Adayev, Ph.D. (718-494-5314).

Fragile X DNA Analysis

Instructions for Shipping Prenatal Samples

Samples requirements:

1. CVS 5 – 10 mg of dissected tissue in sterile medium for PCR
2. AF 10 ml amniotic fluid for PCR
3. Blood samples (5 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 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

Forms to include with the specimens:

1. Requisition form 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

A fillable version of the requisition forms (pdf) are available:

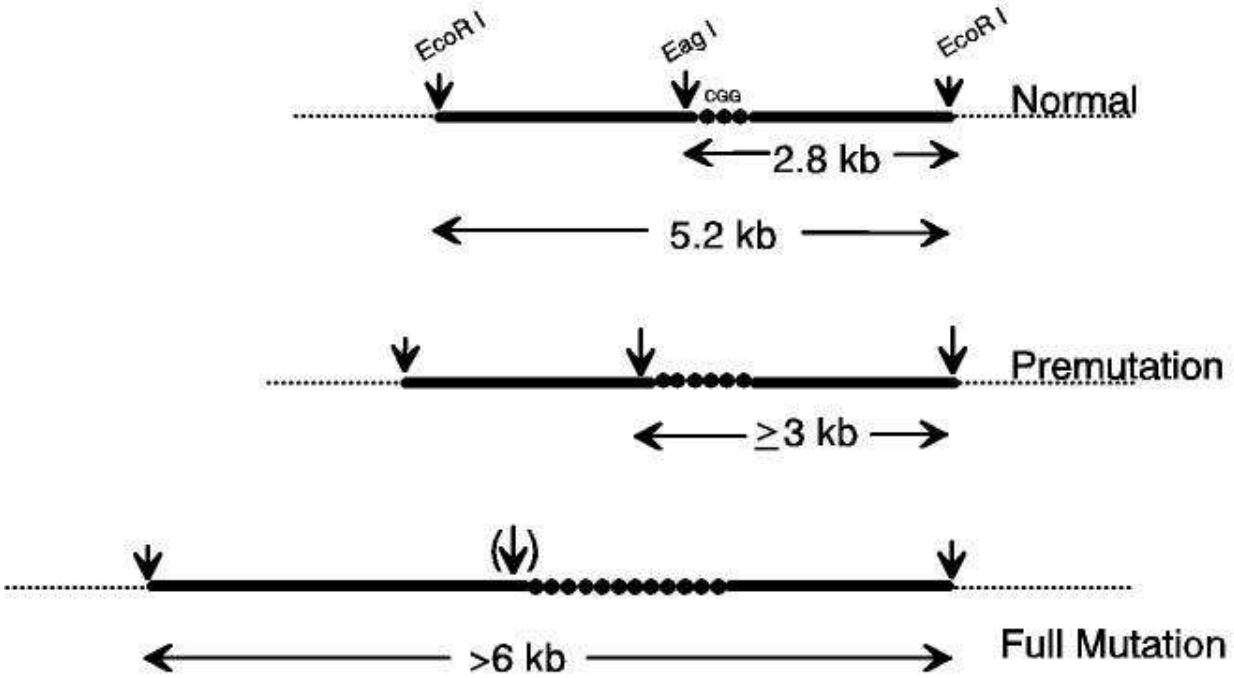
- on web at https://opwdd.ny.gov/ibr/molecular-diagnostic-laboratory
- by request fxlab@opwdd.ny.gov
- can be obtained from Dr. Tatyana Adayev at tatyana.adayev@opwdd.ny.gov

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

Fees:	$750 includes analysis of prenatal and parental samples
Methodology:	PCR and Southern analysis

We offer free post-natal fragile X analysis for all prenatal testing.

Please telephone Dr. Tatyana Adayev with any questions at 718-494-5314.



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