INTAKE PROCEDURES FOR
PREGNATAL FRAGILE X DNA TESTING

OUR REQUIREMENTS:

- Name of patient and LMP
- Pedigree

Forms:
1. Requisition form for mother and father with signed consent
2. Requisition form for the prenatal sample with signed consent
   (If possible we request patients sign the second consent line 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:**
- Date scheduled
- Sample: chorionic villus sample (CVS) or amniotic fluid (AF)
  - 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 fragile X.
2. For direct analysis on uncultured cells, the fluid is preferably clear. Even a “tinge” of blood may introduce a background of maternal alleles.
3. Three T-25 flasks should be grown by the cytogenetics laboratory and shipped to the Institute for Basic Research for Southern analysis.

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
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. 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, 81244, 81265
Tax ID # 146013200
NPI # 1962579235

In case of financial hardship, please telephone Sarah L. Nolin, Ph.D. (718-494-5293).
Fragile X Gene: EcoR I-Eag I Map. CVS full mutation is usually cleaved at the Eag I site (Ψ) (incomplete methylation).

Diagnostic EcoR I-Eag I Patterns in Southern Analysis

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<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Premutation</th>
<th>Full Mutation</th>
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<tbody>
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<td><img src="image3" alt="Diagram" /></td>
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<td><img src="image4" alt="Diagram" /></td>
<td><img src="image5" alt="Diagram" /></td>
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2.8 kb → → → 
5.2 kb → → →