Multiplex Ligation-Dependent Probe Amplification (MLPA) for Detection of Deletions and Duplications of MECP2
Jennifer Sanmann, B.S., CLSp(MB,CG) and Warren G. Sanger, Ph.D.

Multiplex ligation-dependent probe amplification (MLPA) is a technique used to identify deletions and/or duplications of one or more exons of a target gene. Following hybridization of adjacent probes to the target DNA sequences, a ligation reaction joins the adjacent primer pairs into individual target sequences of various sizes, which are all amplified utilizing a single primer set. The amplified products are then separated and quantified by capillary electrophoresis. Each specific probe creates a peak, and the difference in relative peak height or peak area between a patient sample and a normal control indicates a copy number change of the probe’s target sequence.

Our laboratory has recently validated an assay designed to identify deletions and duplications of the methyl CpG binding protein 2 (MECP2) gene region. Current literature reports that approximately 33% of classical and 5% of atypical Rett syndrome (RTT) patients with negative MECP2 sequencing have deletions of one or more exons of the MECP2 gene. Therefore, we are currently reflexing to MECP2 MLPA upon negative MECP2 sequencing results. Additionally, large-scale duplications of MECP2 have been identified in male patients with some or all of the following clinical features: developmental delay/mental retardation (MR), limited or lack of speech, hypotonia, swallowing dysfunction, limited or lack of ambulation, GI reflux, a history of recurrent infections, spasticity, and a family history of X-linked MR. The MECP2 MLPA testing can be performed to identify duplications of the MECP2 gene in these patients as well. For reference, the turn around time for MECP2 MLPA is 21-28 days. As for MECP2 sequencing, our laboratory requests 1-3 ml of peripheral blood in an EDTA tube for testing.

Research & Educational Initiatives of the Human Genetics Laboratory
Bhavana J. Dave, Ph.D., FACMG, Julia A. Bridge, M.D., FACMG, and Warren G. Sanger, Ph.D., FACMG

The Human Genetics Laboratory is actively involved in research and educational programs. Our laboratory is the central reference laboratory for all Children’s Oncology Group (COG) investigations in pediatric lymphoma. Investigators throughout the United States send specimens to our laboratory for specific studies. Our laboratory currently has several collaborative projects where the Director and/or Associate Directors serve as principal or co-investigators; the majority of these are funded by various national agencies, including the National Institutes of Health. In 2007, our research contributions have resulted in nineteen publications in peer-reviewed journals, with several faculty and staff as authors / co-authors. Additionally, eleven abstracts were presented at various national and international meetings in 2007 and thirty-one invited national and international research and educational seminar presentations were made by the Director and Associate Directors.

Research opportunities are available for in-depth investigation of a variety of genetic changes. Cytogenetics, fluorescence in situ hybridization (FISH), multi-color FISH, and array-based comparative genomic hybridization (aCGH) techniques are applied to examine several constitutional and acquired genetic abnormalities. Prospective and retrospective studies can be performed on different tissue types including peripheral blood, products of conception, amniotic fluid, bone marrows, lymph nodes and other solid tumors. Current research projects include investigations on genetic changes that have diagnostic and prognostic implications in hematologic malignancies, bone and soft tissue malignancies, and various micro-deletion/duplication syndromes.

Faculty and staff would also welcome the opportunity to participate in case reports, chart reviews or other clinical research questions.
Hybridization (aCGH) for the Detection of Microdeletions and Microduplications
Warren Sanger, Ph.D. and Diane Pickering, M.S., CLSp(CG)

Array-based comparative genomic hybridization (aCGH) has proven to be a powerful supplement to routine cytogenetic studies for the detection of small deletions and duplications in individuals with developmental delay and/or multiple congenital anomalies. Our laboratory, as well as others, have realized an approximate 8-15 percent detection rate of abnormalities among individuals with DD and/or MCA who had previously normal cytogenetic studies. The aCGH technologies have evolved during the last several years because of the ultimate objective of developing an optimal aCGH platform which will maximize detection of clinically relevant genetic imbalances. Our current routine clinical platform includes the utilization of two BAC-based chips (constitutional and 1MG) with targeted areas specific for known microdeletion and microduplication syndromes. The second available platform is a whole-genome array which is primarily used for delineation of breakpoints identified by other methods and for the discovery of new microdeletions and microduplications.

Recently, our laboratory began assessing and validating a BAC-system with 5,400 BAC loci, which includes loci targeted both in the constitutional and 1MB chips as well as loci recently identified as clinically relevant. Additionally, we are assessing a customized 44,000 oligo system which targets primarily known clinically relevant loci but also includes genome-wide coverage to detect new abnormalities. Our laboratory’s primary objective is to determine which new platform will provide the most optimum coverage for the detection of clinically relevant genomic imbalances.

Overall, aCGH has proven to be a very powerful tool to be used in conjunction with high resolution cytogenetics. The aCGH procedures should not replace conventional high resolution chromosome techniques, however, because they will not detect low-grade mosaicism or balanced structural abnormalities which can be clinically important.

1st Trimester Screening for Chromosome Abnormalities
Sara Fisher, M.S., Hope Chipman, M.S., and Warren G. Sanger, Ph.D.

First Trimester Screening is a newer screening protocol to estimate risk for Down syndrome, Trisomy 18 and Trisomy 13 during the third month of pregnancy. First Trimester Screening uses a combination of an ultrasound measurement behind the neck of a fetus (nuchal translucency), maternal serum markers and maternal age to estimate risks for a singleton or twin gestation. Screening using this particular method is available for all pregnant mothers between the gestational ages of 11 weeks 1 day and 13 weeks 6 days (CRL between 45-84 mm). First Trimester Screening is currently available in select centers.

The most common protocol for first trimester screening includes an abdominal ultrasound to ensure proper dating parameters, a measurement of nuchal translucency and a fetal heart rate. The ultrasound is performed by professionals certified to perform the nuchal translucency measurement. The maternal serum markers are measured from a dried blood sample taken by fingerstick on the same day as the nuchal translucency. Results typically take 7-10 days to complete.

Results from First Trimester Screening provide two risk estimates, a Down syndrome risk and a combined Trisomy18/Trisomy 13 risk for a current pregnancy. “Negative” results indicate the patient is at low risk. It is recommended that pregnancies with a negative/low risk be offered a blood draw for alpha-feto protein (AFP) around 15-16 weeks in pregnancy to evaluate for spina bifida and a targeted ultrasound around 20 weeks of gestation. “Positive” results indicate a higher risk for a particular diagnosis. If a patient receives positive/high risk test results, additional testing is offered at the appropriate time to further evaluate the pregnancy. Diagnostic testing may include chorionic villus sampling (CVS) or amniocentesis, depending on the gestational age of the pregnancy.

First Trimester Screening offers a higher detection rate for Down syndrome (91 percent detection with 5 percent false positive) and Trisomy 18 (95-98 percent detection with 1 percent false positive) than with quad screen or ultrasound alone. It is also completed earlier in a pregnancy and in some cases allows for two diagnostic testing options if results are received at an early gestational age.
**Technical Updates from the Lab**
Warren G. Sanger, Ph.D.

1. CAP requires that **two identifiers** be present on the request form and accompanying specimen container(s) in order to be initiated for diagnostic services. Identifiers are ideally patient’s name and birth date, but can include the hospital or clinic number. Specifically, the name and one additional identifier are required.

2. New “Test Requisition Forms” have been established for different indications. Separate test requisition forms are available for Postnatal, Prenatal and Oncology. These have been developed to make the understanding of desired testing more clear to both the clinical and laboratory staff. These forms can be accessed on our Web site (www.unmc.edu/dept/geneticslab) or you can call our office (402) 559-5070, and we will be happy to send you forms.

3. Our CGH microarray and FISH strategies are continually changing, as we have ongoing research and development programs to provide “state-of-the-art” diagnostic services for your patients. If you have a question about current lab diagnostic capabilities, please call (402) 559-5070 and ask for one of the Lab Supervisors or Directors. Also, if your clinic or hospital would like to have us provide an in-service educational presentation regarding available genetic diagnostic services, one of our Directors or other Clinical Staff would be happy to provide this.

4. We are always appreciative of any suggestions or advice about how we might improve our services to you and your patients.

*Optimal Diagnostic Care for Your Patients is Our Primary Goal.*

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**Lab Turn-Around-Times**
*(Calendar days from receipt of specimen to report)*

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<thead>
<tr>
<th>Specimen Type</th>
<th>Minimum</th>
<th>Mean</th>
<th>Specimen Type</th>
<th>Minimum</th>
<th>Mean</th>
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<tr>
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<td>8</td>
<td>Blood (newborns)</td>
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SPECIMEN SHIPPING & HANDLING

1. Notify HGL in advance of specimen arrival and/or to arrange for specimen pickup (pickup provided within the Omaha-Council Bluffs metropolitan area only). Outside of this area, the transport of specimens by overnight express courier service is available and will be paid by HGL. Specimens should arrive within 24 hours of collection.

2. Specimens should be collected under sterile conditions and transported at room temperature unless otherwise indicated. Special arrangements are required if extremes in temperatures or if extended transport times are anticipated. **DO NOT FREEZE.**

3. Label specimen with two patient identifiers (i.e., patient’s full name & date of birth) as well as date/time of specimen collection. Please use indelible ink or gummed patient labels to label specimens.

4. Send a completed cytogenetics patient information form with the specimen. Include the patient’s name, date of birth, diagnosis, physician’s name and phone number, billing/insurance information and tissue type. If these forms are not available, please call us at (402) 559-5070, and we will fax these to you, or they can be retrieved by accessing our website.

More information is available on our website:  [www.unmc.edu/services/geneticslab](http://www.unmc.edu/services/geneticslab)