The User’s Guide to
Genome-Wide Microarray Analysis

( we find what others don’t )
Dear Colleague,

This guide has been created as an educational tool to assist you with your discussion of chromosome microarray analysis with your patients. It also contains a separate section with a review of microarray testing for the practitioner. It is designed such that one side of the booklet is intended as the patient view, and the opposing page contains information for the practitioner. Pages for the patient are designated by a small P next to the page numbers.

We hope that this guide gives you and your patients a better understanding of chromosome microarray analysis.

Sincerely,

The GeneDx Team
Information for the Patient
Oligo microarray test results may provide information that will assist in diagnosing and managing your patients

Indications for ordering a microarray

A Patient presenting with:
- Multiple congenital anomalies
- Multiple dysmorphic features
- Unexplained mental retardation (MR) or global developmental delay
- Autism or unexplained autistic features
- Seizures

A patient with any of the above and/or a normal karyotype/FISH studies

To confirm and further characterize abnormal cytogenetic results
Our bodies are made up of millions of cells. Each cell typically contains 2 sets of 23 chromosomes, 1 set inherited from the mother and the other from the father. Each chromosome has many genes which contain all essential information for growth and development.
Too much or too little genetic material can cause differences in growth and development. Oligo Array is a diagnostic procedure that looks for the presence of too much or too little genetic material and is more sensitive than traditional chromosome analysis.
Traditional chromosome analysis like karyotyping, subtelomeric FISH, and targeted FISH can identify the following:

**Extra Chromosome**

**Missing Chromosome**

**Large Deletion on a Chromosome**

**Large Duplication on a Chromosome**
What Can Oligo Arrays Find?

Oligo arrays find what “traditional” chromosome studies find:
- Too few (Monosomy) or too many (Trisomy) chromosomes
- Gross Deletions (2-5 Mb in size)
- Gross Duplications (2-5Mb in size)

Oligo arrays find what “traditional” chromosome studies cannot find:
- Very small deletions (0.3Mb-0.5Mb in size, even smaller in targeted regions)
- Very small duplications (0.3Mb-0.5Mb in size, even smaller in targeted regions)
- The exact boundaries of deletions and duplications
- Specific genes in segments of genomic gain or loss that may be of clinical significance

What Can Oligo Arrays Not Find?

- Balanced rearrangements such as reciprocal translocations & inversions
  These abnormalities are only detectable by traditional cytogenetic methods (karyotyping, FISH)
- Low level mosaicism
- Genomic imbalances in regions that are not represented on the microarray
- Small DNA mutations such as point mutations, small intragenic deletions or insertions
  Detectable only by DNA sequencing
What Can Oligo Arrays Detect?

- Too few or too many chromosomes
- Deletions ranging from very large to very small
- Duplications ranging from very large to very small
- The exact boundaries of deletions and duplications
- Specific genes that may be involved in a disorder

What Can Oligo Arrays Not Detect?

- Changes that do not result in a gain or loss of genomic material
**How Is It Done? Step 1:**

- Whole blood in EDTA (purple/lavender top tube) sample is drawn from the patient
- Patient’s DNA is isolated from the white blood cells
- Patient DNA is tagged with **red** fluorescent dye
- Control DNA is tagged with **green** fluorescent dye
How Is It Done?

Patient’s DNA is extracted from their blood sample

Step 1

Patient’s DNA is labeled or “tagged” with red fluorescent label

Control DNA is labeled or “tagged” with green fluorescent label
How Is It Done? Step 2:

- The patient's DNA is combined with the control DNA sample
- The combined DNA is hybridized to the microarray
- The microarray is a glass slide coated with 44,000-105,000 specifically selected probes placed across the unique regions of the human genome
- During hybridization, “tagged” pieces of DNA from patient and control attach to the probes
How Is It Done?

**Step 2**

**A.**
At each spot, custom-made DNA probes are fixed to the microarray slide

**B.**
Tagged DNA is applied to the microarray slide

**C.**
Top view of microarray slide

- Side view of DNA probes attached at each spot
- Pipette with combined patient and control DNA
- "tagged" pieces of DNA from patient and control attach to the probes
How Is It Done?  Step 3:

After incubation, the analysis instrument determines how much red (patient DNA) and green (control DNA) is attached to each of the microscopic spots on the array.

The ratio of red to green signals are interpreted by the instrument and displayed:

- **Yellow** = Equal amounts of patient and control DNA → Normal
- **Red >> Green** → Genomic gain in patient DNA
- **Red << Green** → Genomic loss in patient DNA
How Is It Done?

Analysis

Actual microarray slide has 44,000-105,000 spots

Analysis instrument

Normal Result
Genomic Gain (duplication)
Genomic Loss (deletion)
Possible Result Outcomes
Negative Microarray Results

Negative result is illustrated by:

- Similar amount of patient and control DNA present
- Yellow dots on the array
- Even signal along the entire chromosome on array chromosome graph
Typical Pattern of Negative Microarray Result

Normal amount of DNA (2 copies) appears as “yellow” dots on array

- Normal amount of DNA (2 copies) appears as even signals around baseline
- Normal amount of DNA (2 copies) appears as yellow dots on array
- Hundreds of spots are graphically lined up to depict one whole chromosome

Copy Number 1, 2, 3

Normal results seen as even signals around baseline

Gain ↑

Loss ↓
Negative Test Result

Many genetic conditions cannot be diagnosed by oligo microarray CGH

- Discuss further testing to look for mutations within a single gene (sequencing) if a specific disorder/syndrome is suspected
- Discuss karyotype analysis to look for balanced or other rare chromosome abnormalities
- Some disorders are multi-factorial, and other genetic and environmental factors could be considered
- Not all causes of an individual’s physical / developmental features can be identified with today’s testing technologies

- Continued follow-up with your health care team
- Appropriate clinical follow-up and management
- Discuss emerging new testing options with genetic professionals

What’s Next
Typical Pattern of a Genomic Deletion

Genomic deletion is illustrated by:

- Loss of patient DNA compared to the control DNA
- Green dots on the array diagram
- “Dip” on the chromosome array graph
Typical Pattern of a Genomic Deletion

Probes in deleted region appear as “green” dots on array

Deletion seen as “dip” in signal intensity

Copy Number 1 2 3

Loss

Gain

Deletion Normal
Typical Pattern of a Genomic Duplication

Genomic duplication is illustrated by:

- Additional copies of patient DNA compared to the control DNA
- Red dots on the array diagram
- “Jump” in the chromosome array graph
Typical Pattern of a Genomic Duplication

Probes in duplicated region appear as “red” dots on array

Duplication seen as “jump” in signal intensity
Positive Test Result

Deletion/duplication of genetic material identified

Diagnosis Made

- Testing of parents recommended to establish recurrence risk
- Disease specific management and treatment may be available
- Testing of other family members (if deletion/duplication is inherited) is available

What’s Next
Copy Number Variant of Unknown Significance

Definition

Deletion/duplication of DNA identified that may or may not be associated with the clinical features because:

- There are no previous reports of deletions/duplications in this region
- The abnormality is very small
- The abnormality might be a normal variation in the family and/or general population
- The relationship between the genes in the deletion/duplication region and the clinical features is unknown

Need more information

(Continued on next page)
Copy Number Variant of Unknown Significance

**What’s Next**

**Need more information**

- **Testing of both parents is necessary**
  - Unaffected parent also has the same del/dup
    - Del/dup is most likely a normal variant in the family and is not associated with the child’s condition
  - Parents do not have the del/dup
    - Del/dup has newly occurred in the child and is more likely to be associated with the child’s condition
  - Parent is affected and also has the same del/dup
    - Del/dup is inherited and is likely to be associated with the condition occurring in the family
  - Parents not available for testing
    - No further interpretation possible

- Future research and/or case studies may provide a better understanding of this result
- Continued clinical follow-up and management is recommended
A Mock Example

- A patient presented with developmental delay, short stature, hypotonia, preaxial polysyndactyly, congenital heart disease, congenital renal malformation, and other dysmorphic features.
- The patient had previous normal karyotype and FISH studies
- Oligo microarray identified a large deletion on the short arm of chromosome 7
- Several specific genes in the region were identified, including GLI3

Deletion of GLI3 is known to cause

- Greig Cephalopolysyndactyly syndrome (GCPS)
- Deletion of other genes in this region may be responsible for other features
  (for example: renal malformations)
Microdeletion on the short arm of chromosome 7 associated with a known genetic disorder
Practitioner’s Section
What is an Oligo Microarray?

- The microarray is a glass slide coated with 44,000-105,000 specifically selected probes placed across the unique regions of the human genome.
- These probes are short pieces of DNA (oligonucleotides of 60 bp in length).
- These probes were selected to be spaced every 80,000 base pairs (44,000 probe array) or every 32,000 base pairs (105,000 probe array).
- In known deletion/duplication regions, probe coverage is more dense, reaching one probe every 5,000 base pairs.

GenomeDx Oligonucleotide Array Comparative Genomic Hybridization (Oligo aCGH)

- GenomeDx is a diagnostic test, offered through GeneDx, that can identify regions of gain or loss of genetic material across the entire human genome (with the exception of centromeres, telomeres, and satellites).
- GenomeDx uses a new technology called ‘Oligonucleotide Array Comparative Genomic Hybridization,’ for short ‘Oligo aCGH’.
- Oligo array is a test in which a patient’s DNA and control DNA are fluorescently labeled and hybridized to a microarray of several thousand oligonucleotides. Their signal intensities are compared and plotted against a map of the human genome.

When to use GenomeDx Oligo aCGH?

- As a primary screening test for the diagnosis of persons with unexplained dysmorphic features, birth defects, unexplained mental retardation/developmental delay, multiple congenital anomalies, autism, seizures or any suspicion of genomic imbalance.
- As a complementary or replacement test for FISH and BAC-based microarray analysis when a deletion or duplication syndrome (contiguous or single gene) is suspected.
- As a superior alternative to subtelomere FISH studies in persons with developmental disabilities/mental retardation.
- As a test to determine the presence or absence of a specific gene within a known region of genomic imbalance (contiguous gene deletion syndrome).
- As a test to detect unbalanced chromosome aberrations.
- As a complementary diagnostic test in a Mendelian disorder (single gene disorder) due to functional loss of one allele (haploinsufficiency), in particular when sequence analysis fails to identify a causative mutation and a whole gene deletion may be a cause.
What Can Oligo Arrays Find?

Oligo arrays find what “traditional” chromosome studies find:

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Oligo arrays find what karyotyping cannot:

- Very small deletions (0.3Mb-0.5Mb in size, even smaller in targeted regions)
- Very small duplications (0.3Mb-0.5Mb in size, even smaller in targeted regions)
- The exact boundaries of deletions and duplications
- Specific genes in segments of genomic gain or loss that may be of clinical significance

Oligo arrays find what targeted BAC arrays cannot:

- Deletions and duplication across the entire genome and not only in specific known regions
- Very small deletions (0.3Mb-0.5Mb in size, even smaller in targeted regions)
- Very small duplications (0.3Mb-0.5Mb in size, even smaller in targeted regions)
- The exact boundaries of deletions and duplications

What Can Oligo Arrays Not Find?

- Balanced Rearrangements (reciprocal translocations, inversions), low level mosaicism and genomic imbalances in regions that are not represented on the microarray:
  Those are only detectable by traditional cytogenetic methods (karyotyping, FISH)
- Small DNA mutations (point mutations, small intragenic deletions or insertions detectable by DNA sequencing)
Is it a large del/dup or a small del/dup imbalance?
• The larger the imbalance the more likely it is to cause a medical problem

Is it Gene Rich or Gene Poor?
• The more genes that are involved the more likely it is to cause a medical problem

Which Genes are involved?
• How important are the genes involved in a genomic imbalance?
• What is the function and clinical significance of the genes in the deleted/duplicated region?
• Are any of these genes associated with a specific genetic syndrome?
• Does the patient’s phenotype correlate with any of the genetic disorders mapped to this region?
• Are there known cytogenetic abnormalities reported for this region?

CNV (Copy Number Variation in the general population)
• Does the del/dup detected fall within a known region of copy number variation in the general population?
• Is the del/dup detected familial or de novo?

Is Parental Testing necessary?
• If the imbalance can not be definitively linked to the patient’s phenotype, parental testing may be performed to determine if either parent has the same imbalance
• If one parent has the imbalance then it is likely benign
How To Order Oligo Microarray Analysis: GenomeDx

For GenomeDx oligo microarray analysis please submit the following information:

**Forms**
- GenomeDx sample submission form (Please legibly print the reporting addresses)
- Payment option form (page 2 of sample submission form)
- Informed consent

**Clinical Information**
- It is important to provide the lab with as much clinical information as possible for optimal result interpretation
- This information is critical for us to relate the patient’s features to the genes present in a deleted/duplicated region and their associated disorders

**Specimen Requirements**
- The array requires 1-3mL of whole blood in EDTA (purple/lavender top tube) per person, including parents
- Buccal brushes will not be accepted

**Shipping Instructions**
- Ship the specimen/s overnight at ambient temperature, using a cool pack in hot weather

**Parental Samples**
- Parental samples are strongly recommended. Parental testing helps to distinguish de novo occurring del/dup associated with a disease from a benign familial variant
- Analysis of parental samples is performed at no additional charge

To download the GeneDx information sheet and submission form please visit our website at www.genedx.com and click on the GenomeDx icon on the left hand side of the main page.

For further assistance please contact us at genedx@genedx.com
Please visit our website at www.genedx.com for the following information:

- GenomeDx Information Sheet: Test indications, sensitivity and limitations, detailed technical information, CPT codes and prices, turn-around-time and specimen requirements
- GenomeDx Informed Consent (see sample form on following pages): Submission strongly encouraged
- GenomeDx Submission Form (see sample form on following pages):
  Please remember to enter billing information (page 2 of submission form)
- Frequently Asked Questions:
  - Add-on of a test
  - What is the difference between GenomeDx and CopyDx tests
  - Expedited testing
  - Insurance coverage and other billing questions
  - Shipping information
  - Many more

Other Resources
- CNV database - http://projects.tcag.ca/variation
- UCSC Genome Browser - http://genome.ucsc.edu
Sample Forms
Informed Consent for - Genome Dx Oligo Array CGH

My signature below or on page 1 of the Sample Submission Form for Genome Dx indicates that I have been informed of the following facts about the Genome Dx test and I have had the opportunity to have any questions answered.

Why is this test done?

1. In the Genome Dx [my/my child’s] DNA will be studied to look for genes or spaces between genes where the number of copies is lower or higher than usual.
2. Many genetic diseases and syndromes are caused by a deletion or duplication of one or more genes.
3. On the other hand, many genetic disorders are caused by changes in genes other than in their copy number, and this test cannot be expected to diagnose those changes.
4. This test is not the only way to look for genetic changes, and my physician may recommend this test before or after doing other genetic tests.

What might I find out from this test?

5. I might learn that no gene duplications or deletions were found. This outcome does not mean that [I/my child] does not have a genetic disease.
6. I might learn that a specific gene or genes is duplicated or deleted, explaining the cause of a disorder that I already know [I have/my child has].
7. I might learn that gene duplications or deletions were found that can predict possible long term medical problems that I do not already know about. My physician will be informed of any long term risks that become apparent through this test, according to current medical understanding.
8. This test does not have the ability to detect all the long term medical risks [I/my child] might experience.

What is learned by comparing parent and child DNA?

9. Some genes or spaces between genes tend to have duplications or deletions that do not cause medical problems. They may be normal genetic variations between individuals.
10. When a duplication or deletion is found in the patient being tested, it is important to find out if a parent also has it. If it is a spontaneous change in the child’s DNA, then it is more likely that the duplication or deletion is responsible for the child’s medical problem.
11. By sending the parents’ and child’s DNA at the same time, just in case the parents’ DNA might be needed, the test interpretation can be speeded up and the reporting of ambiguous results can be reduced.
12. If a parent is not the actual biological parent of the child, the laboratory may or may not recognize the situation when parental DNA is tested. If the laboratory was not informed in advance about any non-biological relationship and does not deduce it, false conclusions may be drawn about the significance of duplications and deletions in the child.
13. If the laboratory does deduce that there is a non-biological relationship between a parent and child, it may be necessary to disclose the fact to the physician and/or to call the child’s result inconclusive.

Sign here or on the Sample Submission (Order) Form.

Signature: ____________________________ Date: ____________________
Sample Submission Form - Genome Dx
Whole Genome Analysis using Oligonucleotide Microarray

Patient Name: ___________________________ Last Name: ___________________________
First Name: ___________________________ MI: ___________________________
Gender: □ Male □ Female □ Unknown
Date of Birth: ______/______/______(mm/dd/yy)
Patient Address: ___________________________ Number and Street: _____________ Apt: ______
City: ___________________________ ST: ______ Zip Code: ______
Patient Phone: Home: ( ) ___________________________ Work: ( ) ___________________________
Medical Record Number: ___________________________
Other Submitter’s Patient Id(s): ___________________________

Specimen Requirements:
1-3 mL of whole blood in lavender top (EDTA) tube per person. Samples from parents are highly recommended for the most accurate and rapid interpretation of the child’s result.

Specimen Submitted:
<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood in EDTA</td>
<td>□</td>
<td>☑</td>
<td>☑</td>
</tr>
<tr>
<td>Date Obtained</td>
<td>☑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s Name</td>
<td>___________________________</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>Father’s Name</td>
<td>___________________________</td>
<td>______</td>
<td>______</td>
</tr>
</tbody>
</table>

Clinical diagnosis (Check all that apply):
This information is crucial for the accurate interpretation of the test results.

General:
□ Developmental delay
□ FTT
□ IUGR
□ Short stature
□ Neurological:
□ Agenesis of the corpus callosum
□ Autism
□ Chiari malformation
□ Holoprosencephaly
□ Hypertonia
□ Hypotonia
□ Lissencephaly
□ Seizures
□ Head:
□ Cleft lip/palate
□ Macrocephaly
□ Microcephaly
□ Eyes:
□ Aniridia
□ Cataract
□ Coloboma
□ Cardio:
□ Congenital heart disease
□ Dysmorphic features:
□ Other medically significant problems:
Prev. cyto. result (attach if avail.):
Suspected specific syndrome(s):

Other:
□ Bilary atresia
□ Choanal atresia
□ Cong. Diaphragm. Hernia
□ Malrotation
□ Tracheoesophageal fistula

Skeletal:
□ Rib anomalies
□ Scoliosis
□ Skeletal abnormalities
□ Hand/feet:
□ Clinodactyly
□ Club foot
□ Missing digit(s)
□ Polydactyly/Syndactyly
□ rocker bottom feet
□ Uro-Genital:
□ Ambiguous genitalia
□ Horseshoe kidney
□ Hydrenephrosis
□ Hypospadias
□ Renal agenesis

PAYMENT: Please Complete Page 2

Ordering Checklist
☐ Sample Submission form(s) (page1)
☐ Completed Payment Option form (page 2)
☐ Specimen Tube(s), appropriately labeled

Informed Consent:
I have read the consent document and give GeneDx permission to perform oligo aCGH testing as described.
(Sign here): ___________________________
Payment Options

1. Institutional Billing Information:

GeneDx Account #: ____________________________

Contact Name: ________________________________

Address: ______________________________________

City: ___________ State: ______ Zip Code: ________

Phone: (____) _______ Fax: (____) _______

Hospital/Lab Name: ____________________________

BILLING STAMP

2. Payment by credit card: The full amount of the test fee is charged at the time of sample submission.

Name as it appears on card: ______________________________

Billing address: _______________________________________

City: ___________ State: ______ Zip Code: ________

Phone: (____) _______

☐ Mastercard ☐ Visa ☐ Discover ☐ American Express

Account Number: ____________________________

Expiration date: Month __/Year ______

3/4 Digit Security Code: __________

Please bill my credit card in the amount of $___________ for diagnostic laboratory tests performed by GeneDx, Inc.

Signature (Required) __________________ Date __________

3. Payment by check or money order: Minimum of 75% of the cost of the test is required at the time of sample submission, with the remainder of the fee billed at the time of test completion.

Check or money order enclosed in the amount of $_________

* For patients from outside the United States, 100% of the fee is due at the time of sample submission

4. Insurance Billing:

GeneDx does not bill insurance companies directly unless all of the following is submitted:

• Credit card information (complete part 2) to which any outstanding balance may be billed;

• An authorization number or letter of agreement from the insurance company.

The letter of agreement should be directed to GeneDx and detail the reimbursement rate, the name of the department or individual to whom the bill will be sent (including address, phone and fax numbers) and the patient’s name and policy number.

• Copy of both sides of the insurance card.

• ICD9 codes, if insurance submission is requested:

I UNDERSTAND THAT I AM RESPONSIBLE IN ALL CASES FOR ALL FEES NOT COVERED BY INSURANCE. __________________ Signature (Required)

Note: If you plan to apply to your insurance carrier for reimbursement of your expenses for this test, the following information may be helpful in the case that GeneDx is requested by the carrier to prepare supporting documentation for you to use in your insurance claim:

Insurance Carrier: ____________________________ Is this a Blue Cross/Blue Shield Plan? ☐ YES ☐ NO

Subscriber Name: ____________________________ Is this a Medicaid plan? ☐ YES ☐ NO

Subscriber DOB: ____ / ____ / _______ ID#: ____________________________

Sample Submission (Requisition) Form © GeneDx 03/07 Page 2 of 2

www.genedx.com
The User’s Guide to

Genome-wide Microarray Analysis

Authored by Bradley Joel Williams, MGC, and the clinical staff of GeneDx.

We gratefully acknowledge the invaluable assistance of the senior staff of GeneDx, and Ushta Davar Canteenwalla, MS.