Summary Report

Microarray Use in Canadian Clinical Cytogenetics Laboratories

Hosted by The Centre for Applied Genomics
Tuesday July 29, 2008
The Faculty Club, University of Toronto
41 Willcocks St., Toronto

8:30-8:50	Opening Remarks Moderators: Dr. Steve Scherer, The Centre for Applied Genomics Dr. Charles Lee, Brigham and Women's Hospital
8:50-9:10	Roche NimbleGen Dr. Vanessa Ott, Product Manager for CGH
9:10-9:30	Signature Genomics Dr. Lisa Shaffer, President and CEO and/or Dr. Bassem Bejjani, CMO
9:30-9:50	CombiMatrix Molecular Diagnostics Dr. Mercedes Gorre, VP, Scientific Affairs
9:50-10:10	BlueGnome Ltd. Dr. David Chrimes, Business Development Manager
10:10-10:30	Oxford Gene Technology Ltd. Dr. Mike Evans, Chief Executive
10:30-10:50	Coffee Break
10:50-11:10	Agilent Technologies Dr. Garrick Peters, Program Manager, Microarrays
11:10-11:30	Illumina Inc. Dr. Dan Peiffer, Product Manager, DNA Analysis Products
11:30-11:50	Affymetrix Inc. Dr. Richard Shippy, Director of Business Development, Emerging Markets
11:50-12:10	US Academic Laboratory Consortium for aCGH Dr. David Ledbetter
12:10-1:15	Lunch
1:15-1:45	Discussion and clarification on morning presentations
1:45-2:20	Software and data interpretation
2:20-2:40	Coffee Break
2:40-3:15	Array resolution and reporting standards for clinical labs
3:15-4:00	Requirements for result confirmation
4:00-4:30	Meeting wrap up Moderators: Dr. Steve Scherer, The Centre for Applied Genomics Dr. Charles Lee, Brigham and Women's Hospital

Morning Session (Steve Scherer, Moderator)

Opening Remarks

Dr. Scherer welcomed participants on behalf of The Centre for Applied Genomics (TCAG), the sponsor of the meeting's venue. Representatives of 24 Canadian laboratories were registered, as well as those from companies and organizations that currently market products or services relevant to the subject of the conference. Vendors were invited for opening remarks and their own presentation, but withdrew from further participation. Each had been asked to format the presentation according to the following:

Overview

1. Brief overview of business plan on long-term commitment to products, emphasizing clinical genetics applications.

Design and Performance

2. Is the platform already being used in clinical labs for diagnostics, and what resolution is being utilized?
3. Has the platform been designed to cover clinically relevant loci and sub-telomeres; and what is the resolution of coverage in these areas compared to the rest of the genome?
4. Are you able to quote data regarding expected false positive/negative calls for specific levels of resolution?
5. Details regarding the advantages of using a particular vendor's software in a clinical laboratory.

Pricing and Costs

6. How many array experiments can be performed per lab tech per week?
7. What are the capital expenses for the platform? How much does it cost in reagents/consumables on a per sample basis?
8. Are you willing to provide arrays and reagents at a discounted price for validation purposes - typically about 40 or so arrays would be required?

Future Products and Support

9. Long-term commitment to support Canadian laboratories. For example, are there "user groups" available for your platform to facilitate lab interactions? Are they freely available or at a cost?
10. Are there new versions of arrays, equipment, and/or software we should know about?

Workshop Objective: To catalyze implementation of chromosome microarray analysis in Canadian clinical laboratories

Aims:

1. Facilitate education on available technologies and suppliers
2. Facilitate informed discussion relevant to local, provincial, and national decision-making
3. Facilitate sharing of research experiences with clinical laboratories

Presentations by vendors

Participants in the morning segment (listed in the agenda) represented a spectrum of offerings for diagnostic cytogenetics using microarray technologies. Some market infrastructure equipment and focus on the platform technology. Array types include those based on bacterial artificial chromosomes (BACs), synthetic oligonucleotides, or single nucleotide polymorphisms (SNPs) with quantitation. Some emphasize arrays designed for standardized approaches to clinical service, whereas others emphasize customization. Some have developed analytical software, which may have a more research or clinical focus. Some are primarily service laboratories, and offer options from analytical support for their platforms to full diagnostic reports. Expertise varies with respect to prenatal, postnatal and hematological foci. All provided hard copy materials for distribution to the participants, and fielded questions.

The International aCGH Consortium

Dr. David Ledbetter presented his recent activity with a proposal for a uniform, evidence-based molecular karyotype and international public database, following a meeting at Emory University (Atlanta, GA) June 23-24, 2008. Initially envisioned as a U.S. Consortium for Academic Laboratories, they are encouraging international participation in their initiative. Following the model of standardization of a newborn screening test panel for the U.S., the consortium is advocating a standard oligo-based array panel, optimized for clinical diagnostics, with analytical software, and data sharing to support analysis and interpretation. Participation would allow access to volume discounts from the company supplying the array platforms (Agilent).

Afternoon Discussion (Charles Lee, Moderator)

Points for Discussion

A. Summary of Morning Session

- Providers of service vs providers of arrays / reagents
- Arrays
  - BAC arrays vs oligo arrays
  - Targeted array vs whole-genome array
  - Copy-neutral detection
  - Capital expenses
  - Automation (very little mentioned)
  - Costs - single array vs dye-swap
  - Sublicense to Abbott (Agilent)
  - Number of array experiments / FTE: 40 to 192 arrays per week (~2,000 – 10,000 arrays per year)
  - Discounted price for validation
  - Canadian purchasing consortium - should we join the US consortium?

B. Software and Data Interpretation

- Associated software
- Quality control?
  - Homebrew testing / within laboratory validation of arrays
  - Nationwide / Regional training?
  - Inter-laboratory competency testing?
- What to do about “benign” CNVs?
  - Database of Genomic Variants
  - Access to company databases
  - Access to “consortium” databases
  - For prenatal / postnatal cases – parental bloods (when to obtain?)

- For hematologic cases?

C. Array Resolution and Reporting Standards for Clinical Labs

- BAC-based vs oligo-based arrays
  - Will BAC-based arrays miss imbalances picked up by oligo-based arrays?
- Targeted array vs whole-genome array (coverage in “critical” regions / “backbone”)
  - postnatal, prenatal (can the same arrays be used?)
  - hematological disorders
- Mosaicism
  - e.g. Do we need 3 consecutive probes in a row gained/lost by 1 copy to make a diagnostic call? What about a situation where 5 consecutive calls all with a 0.4x gain?
- Technology platform comparisons (Lars Feuk)?
- Patient confidentiality? (e.g. web-based software / reporting)

D. Requirements for Result Confirmation

- Follow-up FISH testing?
- What about imbalances smaller than a BAC clone? qPCR? MLPA?

E. Miscellaneous

- aCGH before / after / instead of G-banded karyotyping?

Discussion

Throughput and turnaround time

- Labs may need to be somewhat cautious about claims by manufacturers. More than wet lab work, the analysis and interpretation are rate-limiting aspects for service, and currently more time-consuming than for karyotypes. This bottleneck may ease with time and experience. Effective software tools are important in this regard.

Research vs clinical needs

- Thresholds - Clinical questions generally require higher thresholds (lower resolution) to filter pathogenic changes without identifying undue numbers of small variants that may be of unknown significance. All array platforms will likely detect all such large events (e.g. >200kb). Research questions, however, may require resolution of variants in the range of 5kb, and for such experiments, the various platforms perform very differently (Lars Feuk has comparative data from analyses using Affymetrix 6.0, Illumina, Agilent and Nimblegen platforms, soon to be published). Resolution thresholds need to be set according to the nature of information needed. Size of the variant is not the only determinant of clinical significance. Relative to the ~5Mb resolution of karyotypes which have served for years, 500kb should seem a great improvement.
- Analysis - Dr. Feuk described data for array platforms analysed with different programs. There is significant discrepancy among the programs in how they "call" CNVs, such that concordance from the same raw data may be as low as 70%. The smaller the variant, the greater the discrepancy, and regions with segmental duplications are more problematic than unique sequences. Again, this is more relevant for research analyses than for clinical diagnostics.
- Platforms - There clearly will not be consensus on a single array platform to recommend, and the community is probably better-served with a competitive market. There needs to be rapprochement with clinicians about minimal level of resolution for initial arrays as well as referral criteria – consistent across the board. Various considerations of BAC vs oligo arrays in clinical diagnostics were discussed. Despite some current advantages of BAC arrays, such as ease and speed of validation with FISH probes, the technology is moving toward oligonucleotide arrays for clinical applications (this shift seems to be dictated by economics of reproducibly make high-quality arrays). Though labs are likely to work with various platforms, it would help, for data comparison, to have markers in common.
- Software - Products with a clinical focus are needed, rather than those designed primarily for research applications.

- Standard samples - For validation and platform comparison, standard samples are required. A few such samples (NA15510 and NA10851) have been assayed at TCAG on multiple platforms; samples are in the public domain, and data are available for sharing. These samples and other experimental standards are outlined in a special review (Scherer et al., Challenges and Standards in Integrating Surveys of Structural Variation; *Nature Genetics Perspectives* 39, S7-S15, 2007).

Mosaicism

- Setting algorithms with high thresholds will also limit the detection of true mosaicism, which may be important in postnatal diagnostics. On the other hand, neither would the standard guidelines for karyotype analysis facilitate detection of tissue-specific or low-level mosaicism.

Current status of arrays in Canadian diagnostic laboratories

- Of the laboratories in attendance, the following indicated they are currently validating or offering arrays for diagnostic service: The Hospital for Sick Children (Toronto), McMaster University Medical Centre (Hamilton), Credit Valley Hospital (Mississauga) and Mount Sinai (Toronto)
- Other centres are sending samples out for commercial analysis, but they themselves continue to use G banding for oncology and prenatal cases.

Array vs karyotype for postnatal diagnostics

- At a meeting in June 2008, the US Academic Laboratory Consortium for aCGH resolved to replace karyotype with microarray testing as the first line clinical test in individuals with abnormal phenotypes including unexplained mental retardation, developmental delay, multiple congenital anomalies or autism. A manuscript (white paper) with arguments for this is being prepared (Michael Watson) for the American College of Medical Genetics (ACMG) and publication in Genetics in Medicine.
- The Canadian College of Medical Geneticists (CCMG) Clinical Practice committee (Alessandra Duncan) is currently working on guidelines concerning this. The Canadian situation is somewhat different than for many US labs, which provide whatever service the user is prepared to pay for.
- Arrays may not completely replace karyotypes, particularly for situations at risk for balanced rearrangements. Data are needed concerning the yield of karyotype analysis following negative array results, in order to develop policy about follow-up.
- Many European labs are replacing karyotypes with arrays, as costs lower and yields improve.

Changing work roles

- With decreasing demand for karyotype analysis, some concern has been expressed that the demand for cytogenetics technologists may be less. At the same time, the burden of work is shifting heavily toward analysis and interpretation of microarray data. Lab directors described the different roles being undertaken by technologists during this evolution, and are not concerned that their staff will be redundant.
- Analysis of arrays is more time-consuming for the cytogeneticist, and is likely to involve consultation with referring physicians concerning phenotypes and follow-up recommendations. The more innocuous the finding, the more time is likely to be taken in interpretation.
- Much time would be saved with better referral information.

Validation and follow-up

- Guidelines are needed. FISH validation may not needed, for example, to confirm a deletion involving 100 oligos on an array. On the other hand, at least one lab currently will report only array results that can be FISH-validated, though CNVs detected in an area of known clinical significance will prompt consultation with the referring clinician, and possible follow-up by qPCR or MPLA assays.
- Some physicians are requesting oligo arrays in the case of negative results from BAC arrays.

- FISH may be used, not just for validation, but also for follow up in family members.

Clinical Reports

- Standards and practices are highly variable among reports from commercial laboratories. Some anecdotal examples of problems were mentioned, particularly with respect to interpretation of results. Some reports are signed out by technologists, rather than certified directors.
- At least one cytogeneticist is currently reviewing reports from outside labs for the local referring clinicians, adding a written expert opinion.
- It is particularly important to document the date of analysis, and genome assembly build used for interpreting data, as well as dates and details of any other databases or resources used for reference.

In-house vs outside laboratories

- Currently, the cost of array tests can be reimbursed only if sent to a laboratory outside Canada. In Ontario, the Ministry of Health is aware of the extent of expenditure in this realm, and will soon solicit proposals for local laboratory service. Important arguments for repatriating this service will have to do with turnaround time, quality of service, and accommodation to local issues. Also, the ability to teach skills for health professionals is tied to having local expertise.
- It may or may not be appropriate for all current cytogenetics laboratories to set up their own array analysis. Mechanisms will need to tie funding to the numbers of samples assayed.

Data storage

- Policies will be needed with respect to local or public database storage of raw data.
- Coordinating with existing infrastructure such as DECIPHER or the US Consortium is likely more realistic that an independent Canadian initiative.

Education and training issues

- The US consortium is planning workshops for health professionals who need to use and deliver results of arrays (including counsellors).
- Training in array technology is not currently covered by the Canadian Society of Medical Laboratory Technologists (CSMLT) programs at the BC Institute of Technology or the Michener Institute, but may be in progress in both institutes.

Concluding Consensus Statements

1. In principle, the Canadian centres wish to work together and coordinately, to share technologies, training and standards, within the limitations of resources and provincial jurisdictions. Research laboratories have a role to facilitate the process of implementation into clinical service.
2. Both national and international standards should be advocated.
3. No single array platform is to be proposed, but individual choices should conform to agreed standards. Oligonucleotide arrays are expected to supersede BAC arrays, and possibly be followed later by whole-genome DNA sequencing.
4. A process is needed for setting thresholds for array variants. The standard of care tends to be set by majority practice in the US, and it is in Canadian interests to conform to that. The US Academic Laboratory Consortium will advocate a whole-genome array backbone with a minimum 500kb resolution (or perhaps 250kb) superimposed with clinically-relevant target regions at higher density. Today's discussion favoured a minimum average target density in the range of 200-300kb, but would accept a threshold of 500kb to start.
5. Guidelines concerning this technology transition for clinical service are urgently needed from the CCMG, who should be encouraged to expedite the generation and approval of such guidelines.
6. The group wishes to pursue inroads to the possibility of joining the US Academic Laboratory Consortium, with the proposal that it become a North American or International Consortium.