# Exploration of Intellectual Disability and Neurodegenerative Diseases with Exome Sequencing (Reference 2014.01.009)

Notice of Assessment

June 2014

# 1 GENERAL INFORMATION

**1.1 Requestor:** CHU Sainte-Justine

**1.2 Application for Review Submitted to MSSS:** January 28, 2014

**1.3 Application Received by INESSS:** March 1, 2014

**1.4 Notice Issued:** June 30, 2014

> **Note**
> This notice is based on the scientific and commercial information submitted by the requestor and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

# 2 TECHNOLOGY, COMPANY, AND LICENCE(S)

## 2.1 Name of the Technology

Exome sequencing on the Illumina HiSeq 2500 platform following exome capture with the Agilent kit *SureSelect Human All Exon V4* (soon to be V5).

The called variants are validated by Sanger sequencing of the proband and the proband's parents. Clinical relevance of identified variants is then determined with a genetic association test (trio design).

## 2.2 Brief Description of the Technology, and Clinical and Technical Specifications

Sequencing the complete set of encoding regions (the exome) with next-generation sequencing (NGS) detects mutations responsible for intellectual disability (ID) (or mental retardation) and neurodegenerative diseases (ND). This technology can obtain millions of sequences quickly and in parallel. Once compiled, they provide the complete sequence of an individual's genes. It thus enables detection of variants of genes previously associated with disease and of variants not yet identified as causal.

Exome sequencing is performed after the capture (or enrichment) of approximately 200,000 exons [Mamanova et al., 2010; Metzker, 2010; Turner et al., 2009]. During this step, the genomic DNA is fragmented and ligated to oligonucleotide linkers, and the DNA fragments are then hybridized with thousands of biotinylated oligonucleotide baits to ensure the entire exome is covered. The DNA-fragments/biotinylated-oligonucleotides complexes are purified using streptavidin-coated magnetic beads (Appendix A). Following elution of the captured DNA, the fragments are fixed on a solid support and bridge amplified, which generates clusters of multiple copies of the same DNA fragment. Sequencing is then performed on a basis similar to Sanger sequencing: the nucleotides of the DNA fragments are detected during the synthesis of a strand complementary to the template. The nucleotide added at each cycle is identified by its fluorescence (type and intensity) (Appendix B).

The resulting sequences are then evaluated for quality, assembled and compared with a reference sequence. Assembling is performed using the Burrows-Wheeler Aligner (BWA 0.6.2) program and the Genome Analysis Toolkit (GATK v2.6) from Broad Institute. Called variations are filtered both automatically and manually in order to retain only the variants that have a proven, suspected or unknown role in the etiology of the disease. The automated

analysis is performed using the GATK Unified Genotyper (Broad Institute), and the variants are annotated with Annovar.[1]

The called variants are confirmed by Sanger sequencing, as recommended by the American College of Medical Genetics and Genomics (ACMG) [Rehm et al., 2013]. DNA from the proband's parents is also sequenced to evaluate the relevance of the called variants. The requestor estimates it has validated ten or so variations per case [information provided by the requestor; Yang et al., 2013].

### 2.3 Company or Developer

The SureSelect Human All Exon v4 50Mb kit is used to capture the exon sequences. It was designed by Agilent (Mississauga, Ontario).

The HiSeq 2500 platform is a technology developed and produced by Illumina (San Diego, CA, USA).

**2.4 Licence(s):** Not applicable.

**2.5 Patent, If Any:** The sequencing technology is patented by Illumina.

### 2.6 Approval Status (Health Canada, FDA)

This test and the technologies used for performing this test have not been approved by Health Canada or the FDA.

**2.7 Weighted Value:** 2,967.48

## 3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

### 3.1 Targeted Patient Group

The test will be available for adult and pediatric populations, based on a specific decision algorithm (Appendix C).

1. For patients with a developmental delay or an intellectual disability, the test will be performed under the following conditions:
    - The patient shows an overall moderate to severe developmental delay or intellectual disability.
    - The clinical investigation could not establish an etiology from the clinical history (for example, newborn anoxia, prenatal exposure to alcohol) or clinical presentation (for example, single-gene syndrome) that could be the focus of a targeted investigation.
    - The absence of pathogenic abnormalities is established by DNA-chip-based comparative genomic hybridization (CGH).
    - Relevant phenotypic screening has been completed (for example, brain imaging).

[1] ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data [website]. Available at: http://www.openbioinformatics.org/annovar/.

2. For patients with a neurodegenerative disease, the test is performed under the following conditions:
    - Failure to reach developmental milestones or progression of a particular neurological symptom (excluding epilepsy).
    - Lack of a specific diagnosis that could be explored effectively by targeted sequencing.
    - Relevant phenotypic screening has been completed.
    - Exclusion of sporadic cases of neurodegenerative conditions characterized by substantial genetic complexity (for example, Parkinson disease).

## 3.2 Targeted Disease(s)

**Intellectual Disability (ID)**

Intellectual disability (ID) is the most common neurological disorder. Its prevalence is estimated to be between 1% and 3% of the population [Héron and Jacquette, 2009; Shevell et al., 2003; Leonard and Wen, 2002; Yeargin-Allsopp et al., 1997; Baroff, 1982]. It has wide-ranging consequences on society by virtue of its prevalence, the extent of required support services, and the social and emotional effects on the individual, his/her family and society. In 2003, the US Centers for Disease Control and Prevention (CDC) estimated the cost of care per patient over a lifetime to be approximately $1 million [Topper et al., 2011]. Managing ID requires an early diagnosis and treatment combined with access to the health services required for treatment and preventive or follow-up monitoring of the associated physical and mental comorbidities [Einfeld et al., 2011; Van Schrojenstein Lantman-de Valk and Walsh, 2008; Kwok and Cheung, 2007].

ID has been categorized into four phenotypic subtypes based on intelligence quotient (IQ). Slight, moderate and severe IDs are associated respectively with IQ ranging from 50 to 70, 35 to 50 and 20 to 35. In some cases, patients with an IQ below 20 are considered to be profoundly disabled [Chiurazzi and Oostra, 2000; Battaglia et al., 1999]. The American Psychiatric Association [APA, 2003] reports that 85% of all ID patients have mild ID, whereas 10% have moderate ID. Severe ID accounts for 3% to 4% of all ID patients. Lastly, 1% to 2% of all ID patients are classified as having profound ID.

ID is a clinical feature associated with more than 2,000 of the diseases and syndromes described in OMIM.[2] This phenotypic heterogeneity complicates diagnosis and establishment of etiology. The causes of ID can be acquired or genetic. Acquired cases are associated with, among other things, environmental exposure to infection, trauma or teratogens. In developed countries, it is estimated that most of the moderate and severe forms of ID (IQ < 50) are single gene disorders, and that the milder forms result from the interplay of genetic and environmental factors [Ropers, 2010]. ID is often sporadic, with no hereditary basis, which suggests that a large proportion of cases are caused by *de novo* mutations [De Ligt et al., 2012].

The genetic causes of ID can be roughly divided into three groups: chromosomal aberrations, single gene disorders and epigenetic disorders. An additional subgroup could be disorders caused by mitochondrial DNA defects. Two-thirds of these cases are caused by trisomy 21, the prevalence of which is approximately 1 case per 770 live births in Quebec.[3] The second

[2] OMIM website consulted on 26 March 2014 (http://www.ncbi.nlm.nih.gov/omim).
[3] Ministère de la Santé et des Services sociaux du Québec (MSSS). Trisomy 21 [website], available at http://www.msss.gouv.qc.ca/sujets/santepub/depistage-prenatal/professionnels/index.php?Trisomie-21.

leading cause, accounting for 0.5% of ID cases, is associated with a *FMR1* gene mutation (fragile X syndrome).[4] Several other genes on the X chromosome, but also on the autosomes, have been associated with syndromic ID [Campeau et al., 2013; Carss et al., 2013; Mefford et al., 2012; Coffee et al., 2009; Crawford et al., 2001; Amir et al., 1999; Hamel et al., 1999]. A specific genetic cause remains unknown for 55% to 60% of ID patients [Mefford et al., 2012; Topper et al., 2011; Rauch et al., 2006], and several authors have suggested that 450 to 1,000 genes may be associated with ID [Schuurs-Hoeijmakers et al., 2013; Van Bokhoven, 2011].

**Neurodegenerative Disease (ND)**

Neurodegenerative diseases comprise more than 600 disorders that affect primarily brain neurons. Alzheimer disease (AD), Parkinson disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington disease (HD) are the disorders affecting the majority of ND patients [Inglese and Petracca, 2013; Coppodè, 2012; Williams, 2002]. NDs are incurable and debilitating conditions that result in degeneration or loss of neurons (or of their myelin sheath), culminating in impaired movement (ataxia) or mental function (dementia). NDs can be severe and often fatal[5] [Gbaya et al., 2012]. Several NDs are characterized by a gradual accumulation of particular protein aggregates in specific brain areas. These aggregates contribute to the etiology of the disease and its diagnosis [Yankner et al., 2008].

Neurodegenerative diseases can be caused by certain conditions such as alcoholism, brain injuries, tumours or strokes. The role of environmental factors such as metals and pesticides has also been reported [review by Modgil et al., 2014]. Family clusters of several neurodegenerative disorders and association studies have led to the identification of genes associated with the most common forms of neurodegenerative diseases. However, the majority of cases appear sporadic [Al-Chalabi et al., 2013; Cruts et al., 2012; Sellbach et al., 2006; Campion et al., 1999]. A genetic component seems to influence the onset of phenotypes, but the number of genes involved, their interactions and the contribution of epigenetic factors make the etiology complex and the transmission different from the classical Mendelian pattern [Cruts et al., 2012; Coppodè, 2012; Parròn et al., 2011; Williams, 2002].

People with an ND form sizeable populations. For example, Alzheimer disease is the most common ND and the most prevalent form of dementia among the elderly. In Quebec, it is estimated that in 2009, 100,000 people had Alzheimer's disease and associated comorbidities.[6] For persons 65 years of age or older, 1 in 11 is affected, and prevalence doubles every 5 years on average [Gbaya et al., 2012]. The second leading ND in Quebec is Parkinson disease. In the early 2000s, Parkinson disease affected approximately 100,000 people 65 years of age or older (approximately 1 in 100) [Gbaya et al., 2012].

As is the case for ID, determining the etiology is complex, not only because of the substantial genetic heterogeneity, but also because of the influence of environmental factors. Thus far, for Alzheimer disease alone, 695 genes — whose protein roles range from lipid transport (*APOE*, *CLU*) to immune response (*CD33*, *EPHA1*) — have been associated with this disease.[7]

[4] Centers for Disease Control and Prevention. Fragile X syndrome (FXS) [website], available at http://www.cdc.gov/ncbddd/fxs/data.html.
[5] European Commission. Neurodegenerative disorders [website]. Available at http://ec.europa.eu/health/major_chronic_diseases/diseases/brain_neurological/index_en.htm
[6] Statistics from La Société Alzheimer de Québec (website: http://www.societealzheimerdequebec.com/).
[7] Alzforum. AlzGene – Field Synopsis of Genetic Association Studies in AD [website], available at http://www.alzgene.org/.

It is estimated that 90% to 95% of the cases among persons 65 years of age or older are sporadic [Coppodè, 2012; Campion et al., 1999].

### 3.3 Number of Patients Targeted

**Intellectual Disability**

The requestors estimate the incidence of moderate to severe forms of ID to be 0.5%. There are approximately 400 incident cases every year in Quebec. However, because the diagnostic yield of the conventional approaches (karyotyping, CGH and candidate-gene testing) is about 50%, the forecasted number of tests is about 200 cases annually.

It should be noted that, for each proband, Sanger sequencing of about 10 loci will also be performed to genotype the parents (400 samples).

**Neurodegenerative Disease**

Based on previous experience, the requestor estimates the annual demand to be 100 cases.

### 3.4 Medical Specialities and Other Professions Involved

Molecular diagnostics, genetic counselling, genetics, neurology and pediatrics.

### 3.5 Testing Procedure

The requestor has established a precise algorithm describing the conditions in which exome sequencing may be requested (Appendix C). Only a medical geneticist may request exome sequencing to establish the etiology of an intellectual disability; a neurodegenerative disease requires a joint request from a medical geneticist and a neurologist. The test may be performed only after the request has been reviewed by an expert committee comprised of medical geneticists and neurologists specializing in neurodevelopmental and neurodegenerative conditions.

## 4 TECHNOLOGY BACKGROUND

### 4.1 Nature of the Diagnostic Technology

Exome sequencing will be performed for patients in whom disease etiology could not be established using the first-line tests. In the case of genetic screening, this test will thus complement CGH and the sequencing of certain particular genes when a syndromic disease is suspected.

This is a new test that will not replace any test currently in the Index. The Index includes screening for mutations in certain individual genes associated with ID and ND, namely:

- Detection of *FMR1* trinucleotide expansion in fragile X syndrome using Southern blot – code 50340
- Detection of *FMR2* trinucleotide expansion in *FRAXE* intellectual disability – code 50140
- Detection of CAG trinucleotide expansion in Huntington disease - code 50161
- Screening for *SACS* mutations in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) using allele-specific oligonucleotide (ASO) – code 50010
- Screening for mutations in the *FXN* gene using NAAT and Southern blot in Friedreich ataxia (detection of trinucleotide expansion) – code 50011
- Screening for mutations in Prader-Willi syndrome, Angelman syndrome using MS-MLPA – code 50301

When screening for *FMR2* and *FRAXE* mutations in fragile X syndrome (code 50140), screening must be performed prior to exome sequencing. Screening for all other mutations should continue in cases with a family history or in which the clinical presentation indicates targeted genetic testing. The codes could also be used for screening for the carrier of the mutant allele.

## 4.2 Brief Description of the Current Technological Context

Diagnosing and establishing the etiology of an ID involves a phenotypic investigation and genetic screening. Several organizations and expert groups have issued recommendations in this regard, including the American Academy of Pediatrics and the American Academy of Neurology [Héron and Jacquette, 2009; Moeschler and Shevell, 2006; Shevell et al., 2003]. The set of tests selected often depends on the patient's particular phenotype. The requested tests include biomedical tests, brain imaging, electrophysiology, and genetic and metabolic examinations.

Approximately 15% of IDs are attributable to cytogenetically visible genetic aberrations [Rauch et al., 2006; De Vries et al., 2005; Leonard and Wen, 2002], such as extra chromosomes, unbalanced translocations, and relatively substantial deletions. Conventional karyotyping and fluorescence *in situ* hybridization (FISH) can also be performed in some cases of suspected aneuploidy (such as trisomy 21 or trisomy 18) or some cases of certain well-defined syndromes, or to confirm certain results obtained by CGH.

Using CGH has led to the detection of losses or gains of smaller chromosomal segments and copy number variants undetected by conventional cytogenetic analysis. This approach can identify 15% to 20% of ID cases [Flore and Milunsky, 2012; Cooper et al., 2011; Manning and Hudgins, 2010; Miller et al., 2010; Hochstenbach et al., 2009; Zahir and Friedman, 2007]. The ACMG recommends it as a first-line diagnostic test [Kearney et al., 2010].

Sequencing of targeted genes is also performed in the presence or absence of particular features such as microcephaly or macrocephaly, autism, facial dysmorphism or seizures. This approach has limited application because it is highly dependent on the correspondence between the patient's phenotype and the clinical description of the disorder. In addition, the genetic heterogeneity associated with the diseases makes this type of test onerous and expensive. The diagnostic yield associated with screening for mutations in targeted genes is about 5% to 10% [Willemsen and Kleefstra, 2014; Topper et al., 2011].

Analysis of the gene panels associated with ID and ND and exome sequencing are currently available on the market in the United States. Several diagnostic laboratories perform sequencing of gene panels comprised of 12 to more than 100 genes. Depending on the vendor, these panels target certain subtypes of ID or ND (for example, ID associated with the X chromosome or Alzheimer disease). Exome sequencing is generally recommended for patients who have undergone advanced testing and present specific clinical features. Exome sequencing for clinical applications is available at Baylor College of Medicine, ARUP, Emory Genetics Laboratory, Cincinnati Children's Hospital Medical Center, Duke Medicine, Gene By Gene and Ambry Genetics, among others. The costs associated with this type of analysis range from $1,095 to over $15,000, depending on the type of results ordered (raw data or final reports), the nature of what is to be sequenced (the entire exome or only the 4,000 to 4,600 genes currently associated with human diseases), and the required turnaround time for obtaining results.

## 4.3 Brief Description of the Advantages Cited for the New Technology

The clinical entities targeted by this test are genetically and phenotypically very heterogeneous, and current investigation methods can determine genetic causes for only 40% to 50% of patients. However, because exome sequencing by NGS provides quick, low-cost sequencing of most exons in parallel [Dixon-Salazar et al., 2012; Need et al., 2012; Neveling et al., 2012; Shanks et al., 2012], and because an estimated 80% to 85% of the variants causing Mendelian diseases are located within the exome [Dixon-Salazar et al., 2012; Bonnefond et al., 2010], the rate of establishing genetic etiology using this approach could be significant. Furthermore, when the patient has an atypical or low-specificity clinical presentation, this test eliminates the need for a precise phenotype-genotype correspondence in order to target genetic testing.

Given the exponential rate of evolution of knowledge and in contrast to targeted Sanger sequencing, this approach permits sequencing data to be revisited later to determine whether cause can be established in undetermined cases. In 2012, De Ligt et al. sequenced the exomes of 100 ID patients. They reported establishing genetic etiology in 16% of the cases. In 2013, a new exonic sequencing test for unexplained cases increased this yield rate to 29% [Willemsen and Kleefstra, 2014].

Notwithstanding the technical limitations, the diagnostic yield reported by several groups is greater than that of other currently available diagnostic approaches [Yang et al., 2013; Dixon-Salazar et al., 2012; Rauch et al., 2012; Manning and Hudgins, 2010].

## 4.4 Cost of Technology and Options

Table 1 presents the one-time costs of launching the test, as well as the costs of providing the test on an ongoing basis. These costs do not include the human resources and paid training costs (including conferences) associated with initial training and subsequent skills maintenance.

**Table 1: Cost of launching and providing the test**

| ONE-TIME LAUNCH COSTS | | |
|---|---|---|
| ITEM | COST | COMMENTS |
| HiSeq 2500 | N/A | |
| Information technology infrastructure | N/A | To be provided by Génome Quebec under the CHUSJ-GQ partnership |
| Total one-time costs | N/A | |
| **CONTINUING COSTS** | | |
| ITEM | COST | COMMENTS |
| Quality control | | |
| CAP or other testing (annual cost) | | In 2014, CAP did not offer sequencing by NGS. |
| Reagents for external quality control testing | Nil | |
| Human resources for carrying out tests | Nil | |
| Software updates (annual cost) | N/A | Génome Quebec will provide the updates. |
| Operating costs | | |
| Reagents (exon capture, sequencing by NGS, Sanger sequencing), per patient | $1,022.51 (HiSeq) + $877.69 (Sanger) + $102 (repeat) | |
| Human resources, per patient | $828.05 | $35 per hour in 2014 |
| *Total continuing costs* | *$2,934.20* | |

Abbreviations: CAP = College of American Pathologists; CHUSJ-GQ = CHU Sainte-Justine-Génome Québec; NGS = next-generation sequencing; N/A = Not applicable.

# 5 EVIDENCE

## 5.1 Clinical Relevance

### 5.1.1 Other Tests Replaced: none.

### 5.1.2 Diagnostic or Prognostic Value

Inability to establish the genetic bases of neurological disorders like ID and ND has clinical ramifications including unnecessary invasive testing, a lack of information on prognosis, and a lack of advice on improving quality of life and initiating helpful treatment (or limited access to such resources) [De Ligt et al., 2012; Romano, 2010; Stevenson and Carey, 2009; Shea, 2006; Moeschler and Shevell, 2006]. Lack of knowledge of the etiology also hinders recurrence management, reproductive counselling, and possible prenatal diagnosis. Furthermore, getting an explanation often has significant implications for the patient and the patient's family [Willemsen and Kleefstra, 2014; Flore and Milunsky, 2012].

Since it was introduced, exome sequencing has proven useful in research to determine the causes of Mendelian diseases within families, among cohorts presenting the same phenotype, and even in individual patients [Need et al., 2012; Vissers et al., 2010; Choi et al., 2009; Ng et al., 2009]. However, few currently published studies focus on the clinical application of this technology, as most examine instead the feasibility of introducing the approach in a clinical setting. These studies are often conducted on restricted, stringently selected cohorts in whom traditional diagnostic approaches have failed to determine etiology. The purpose of these studies is often to discover new genes, with applicability generally a secondary issue. However, interestingly, Topper et al. already pointed out in 2011 that exome sequencing has had some major implications for the discovery of genes involved in ID.

### 5.1.3 Therapeutic Value

The therapeutic value associated with exome sequencing is limited; genes and their mutations are highly heterogeneous and rarely a therapeutic target. In two exome sequencing studies assessing 218 individuals with moderate to severe ID, De Ligt [2012] and Dixon-Salazar et al. [2012] report four cases in which they could take action based on the detection of genetic mutations.

Recently, in a study of 18 patients from 13 families, Foley et al. [2014] reported having identified mutations in the *SLC52A2* gene that can cause Brown-Vialetto-Van Laere syndrome (OMIM 211530), an incurable neurodegenerative disease. They demonstrated that the mutations reduce riboflavin uptake and the expression of its transporter protein. For 16 patients carrying these mutations, high-dose riboflavin therapy resulted in a significant clinical and biochemical improvement in two cases and a preliminary clinical response in 13 cases.

Some groups also report that the detection of causal mutations by exome sequencing led to a change in diagnosis for several patients with atypical presentations. The therapeutic value of the test can at present be evaluated only on an individual basis, depending on the disease and the affected gene.

## 5.2 Clinical Validity

Exome sequencing enables screening for the genetic etiology of phenotypically complex diseases or diseases involving multiple genes, some of which might be unknown. This approach can be used for both inherited disease and sporadic cases (*de novo* mutations). X chromosome mutations, autosomal recessive inheritance, autosomal dominant inheritance and somatic mosaicism have been detected through exome sequencing [Choi et al., 2011; Lindhurst et al., 2011; O'Roak et al., 2011; Bilgüvar et al., 2010; Choate et al., 2010; Vissers et al., 2010]. Taken together, the results of various studies suggest that this approach can contribute significantly to establishing the etiology of various diseases [e.g., Gonzaga-Jauregui et al., 2012; Bamshad et al., 2011; Gilissen et al., 2011].

However, several authors emphasize the difficulties associated with instituting exome sequencing in a clinical setting. Sanger sequencing of targeted genes is a common type of test in diagnostic laboratories. In this limited context, it leads to the detection of a small number of variants per targeted gene. The called variants can be considered causal and can help establish an accurate diagnosis. In the absence of sufficiently substantiated scientific evidence, other variants can be declared "potentially causal," while the role of other variants may be uncertain or unknown. An analysis of every individual's exome finds approximately

20,000 to 30,000 specific variants. Approximately 90% of all variants are found in more than 5% of the population and may not be directly involved in the diseases that may be investigated; every person carries an excess of 76 to 190 deleterious missense variants and up to 20 mutations responsible for the complete loss of function of the protein produced or associated with diseases (nonsense, splice-site, or frameshift mutations) [Liu et al., 2013; Foo et al., 2012; MacArthur et al., 2012; The 1000 Genomes Project Consortium, 2012]. Imputing a disease to one or more variants can thus be complex.

Furthermore, many (about 2,000) databases are used to compile the different variants and their associations with diseases, and they frequently contain errors (more than 25% of entries are erroneous) [Kenna et al., 2013; Marshall, 2013; Bell et al., 2011]. Recently, Marshall [2013] and Brownstein et al. [2014] reported two independent studies that aimed to compare the results received from 33 clinics asked to analyze and interpret exome sequencing data and genome sequencing data, respectively. The participants were asked to determine, from the same samples, the genetic etiology of the diseases. Although there was a general consensus on analysis and interpretation processes, the identified causal variants were mostly inconsistent. In fact, the function of the majority of the genes of the genome and their association with the various diseases are not very well known, which contributed to the inconsistencies. Researchers must frequently examine expression patterns, residue conservation, protein structure predictions, and *in vitro* and *in vivo* studies, as well as various other tools to evaluate the relevance and possible role of a gene and its variants in the disease. Characterizing each of the candidate variants quickly becomes an onerous, difficult task in this context.

Despite these shortcomings, the American College of Medical Genetics and Genomics (ACMG) considers that the benefits of exome sequencing outweigh the risks under certain conditions (see section 5.4, Recommendations from Other Organizations).

**Intellectual Disability**

For 50% to 60% of patients with ID, the genetic etiology remains unknown after the currently recommended diagnostic tests are carried out (CGH, karyotyping, FISH, MLPA and targeted-gene analyses) [Willemsen and Kleefstra, 2014; De Ligt et al., 2012]. A few studies conducted as part of projects researching new genes associated with ID have demonstrated that exome sequencing can, for a significant number of patients, detect etiologies that were previously unknown despite extensive testing.

CHU Sainte-Justine has launched a pilot project in which researchers have analyzed the exomes of 70 individuals with rare diseases. Of this cohort, 49 patients present an intellectual disability. The diagnostic yield is 36% overall (25/70) and 37% for ID cases (18/49).

**Neurodegenerative Diseases**

Current literature indicates that the use of exome sequencing to determine the genetic etiology of NDs is for the most part limited to the discovery of new genes in cases of familial neurological disorders. For example, for patients with a familial form of Parkinson disease, exome sequencing led to an association with the *EIF4G1* and *VPS35* genes [Chartier-Harlin et al., 2011; Vilariño-Güell et al., 2011; Zimprich et al., 2011]. Guerreiro et al. [2011] associated mutations in *Notch3* with some familial cases of Alzheimer disease. Furthermore, Ramagopalan et al. [2011] identified rare variants in *CYP27B1* in a cohort of patients with multiple sclerosis (MS).

No study on clinical implementation, diagnostic yield or clinical validation specific to neurodegenerative diseases has been found.

**Incidental Findings**

As the Canadian College of Medical Geneticists (CCMG) has not yet issued a recommendation, the requestor has set the following guidelines for incidental findings:

- In all cases, an incidental finding with no known consequence on health, according to current knowledge, is not disclosed.
- In the pediatric population, an incidental finding is reported only if the mutation is associated with a known health consequence during childhood or adolescence; those with consequences only during adulthood are not disclosed;
- In the adult population, patients decide whether they wish to learn of incidental findings that might have a known health consequence, according to current knowledge, if treatment or preventive monitoring are available for that consequence.

**Table 2: Clinical validation studies**

| AUTHOR | NGS PLATFORM / ENRICHMENT KIT | COVERAGE / DEPTH | PHENOTYPE | NUMBER OF PATIENTS | NUMBER OF MUTATIONS / NUMBER OF PATIENTS | MUTATION TYPE | % RESOLVED | PRIOR NEGATIVE INVESTIGATIONS |
|---|---|---|---|---|---|---|---|---|
| Vissers, 2010 | SOLiD 3 | 90%/10× | Moderate to severe ID | 10 trios | 10/8: 3 known mutations and 4 in genes with a neurodevelopmental role | *De novo* | 30 | Karyotyping/CGH/ family history |
| Need, 2012 | HiSeq 2000/SureSelect Human All Exon 50 Mb, Agilent | 88%/71× | ID or developmental delay | 12 trios | 6 causal + 1 suggestive | *De novo* | 50 | Chip (Affymetrix 6.0) |
| Najmabadi, 2011 | Agilent SureSelect and sequencing with Illumina Genome Analyser | | ID | 136 families | 23 known genes<br>50 unknown genes | Hereditary/ homozygous | | |
| De Ligt, 2012* | SOLiD 4/ SureSelect Human Exome Kit, version 2, 50 Mb, Agilent | | ID with IQ < 50 | 100 trios | 79 mutations (53 patients)<br>18 in known genes (16 patients) | *De novo* | 16 | Clinical investigation and genetic testing |
| Rauch, 2012 | HiSeq 2000 / SureSelect XT Human, Agilent | 90%/20× | ID with IQ < 60 | 51 patients | 16 variants in genes previously associated with ID<br>6 candidate mutations | *De novo* | 31 | No major chromosomal abnormalities |
| Dixon-Salazar, 2012 | $GA_{IIx}$ or HiSeq200 / SureSelect Human All Exome 50 Mb, Agilent | 96%/10× | Developmental delay | 118 patients | One mutation in a gene associated with the identified disease in 10 cases (8%).<br>In 19% of the cases (22 patients), one or more mutations identified in 22 other | Recessive inheritance | 27 | Genetic etiology |

| AUTHOR | NGS PLATFORM / ENRICHMENT KIT | COVERAGE / DEPTH | PHENOTYPE | NUMBER OF PATIENTS | NUMBER OF MUTATIONS / NUMBER OF PATIENTS | MUTATION TYPE | % RESOLVED | PRIOR NEGATIVE INVESTIGATIONS |
|---|---|---|---|---|---|---|---|---|
| | | | | | previously unassociated genes whose mutations segregated with the phenotype. | | | |
| Schuurs-Hoeijmakers, 2013 | SOLiD 4/SureSelect Human All Exome 50 Mb, Agilent | | | 19 families | 8 genes: 3 previously associated with ID and 5 with pathogenic potential. | | 16 | Karyotyping and CGH |
| Yang, 2013 | Illumina Genome Analyzer$_{IIx}$ Platform (Illumina) and HiSeq 2000 | 95%/20× Mean coverage of 130× | Diverse (80% with neurological disorders) | 250 patients | 86/62 | *De novo* Autosomal dominant or recessive | 25 | Comment: incidental findings for 30 of 250 patients tested by exome sequencing |

Abbreviations: CGH = comparative genomic hybridization; ID = intellectual disability; NGS = next-generation sequencing.

*The researchers described clinical sensitivity with exome sequencing in terms of diagnostic yield or determination of genetic etiology. Sensitivity was highly variable among studies, ranging from 16% to over 60%.

**Table 3: Summary table of clinical validity**

| COMPONENT | PRESENCE | ABSENCE | NOT APPLICABLE |
|---|---|---|---|
| Sensitivity | X | | |
| Specificity | | X | |
| Positive predictive value (PPV) | | X | |
| Negative predictive value (NPV) | | X | |
| Likelihood ratio (LR) | | X | |
| ROC curve | | X | |
| Accuracy | | X | |

## 5.3 Analytical (or Technical) Validity

The sensitivity and specificity of the detection of disease-causing genetic variants is such that validation is required at every step of the analytic process, from the extraction of genetic material to the computer-assisted analysis of variants [Rehm et al., 2013; Gargis et al., 2012]. The approach used must optimize the uniformity of coverage, read depth and quality of sequences and allow for the elimination of a maximum of non-causal variants without eliminating those responsible for the etiology.

It must be kept in mind that the exome sequencing technology is rapidly evolving. Chemistries and technologies are frequently changed to achieve better performance in terms of sequencing coverage, speed and adaptability for various applications. Several studies with the goal of comparing the performances of various sequencing platforms and exome capture kits were initially carried out when the various NGS tools were put on the market. However, there are few formal studies in the literature of the updates or optimized versions. The original studies provide proof of concept, and the laboratories responsible for the analyses must validate the performance parameters of the various components of their platform.

**Validity of the HiSeq2500 platform**

The utility and validity of each of the NGS platforms have been reported in recent years as part of technical validation studies, etiological studies of various diseases, and studies of NGS implementation in clinical settings.

The requestors plan to use the Illumina HiSeq 2500 platform for their analyses. This platform is an upgrade of the HiSeq 2000 platform, with the difference being that it offers a rapid-run mode and can accommodate a smaller number of samples.

Table 4 summarizes a few comparative studies highlighting the performance of the HiSeq2000.

**Table 4: Summary of a few recent comparative studies of NGS platforms**

| STUDY | TYPE OF SAMPLE (NUMBER) | TYPE OF TEST | CRITERIA STUDIED | COMMENTS |
|---|---|---|---|---|
| Quail, 2012 | 4 microbial genomes (GC content from 19.3% to 67.7%) | Whole genome (read depth of 15×) | **Error rates**: < 0.26<br>**Specificity:** 99.9% (Q30)<br>**Sensitivity:** 68% to 76% of SNPs detected | The analysis software was that of the manufacturer, which may account for some of the observed variability. |
| Sivakumaran, 2013 | Human DNA (8) | Panel of 24 hearing-loss genes (read depth 40×) | **Specificity: 99.99%**<br>**Sensitivity: 100%**<br>**Concordance:** > 99.99% | ▪ 29% of the regions were compared with Sanger sequencing results.<br>▪ The test could detect SNPs and indels (up to 22 nucleotides).<br>▪ NextGENe software used for sequence analysis. |
| Sule, 2013 | Human DNA (10) | Panel of 34 genes associated with low and high bone mineral density (average read depth of 900×) | **Sensitivity:** 100% for the 6 known patients. For 4 other patients, detection of previously unknown causal mutations confirmed by Sanger sequencing. | ▪ Poor coverage was observed in genes having paralogs and in GC-rich regions.<br>▪ NextGENe software used for sequence analysis |
| Umbarger, 2014 | Human DNA (194 immortal-ized cell lines (Coriell*) and 59 samples derived from whole blood) | Panel of 15 genes | **Specificity:** > 99.99%<br>**Sensitivity:** 99.97% for SNV; 95.3% for indels<br>**Reproducibility:** Concordance rate: 0.999997.<br>Pearson correlation coefficient: 0.868 | ▪ The genotypes observed by NGS and compared with the HapMapProject data and the discordances were evaluated by Sanger sequencing.<br>▪ Sequences were analyzed using the Burrows-Wheeler Aligner (version 0.5.7) and GATK (version 1.0.5083). |

Abbreviations: DNA = deoxyribonucleic acid; GC = guanine-cytosine; NGS = next-generation sequencing; Q = quality score indicating probability of error; SNP = single-nucleotide polymorphism; SNV = single-nucleotide variant.

***** Coriell Cell Repositories, Coriell Institute for Medical Research (Camden, NJ, USA)

Only one study assessing the performance of the HiSeq2500 platform was identified. Linderman et al. [2014] validated several performance parameters of their platform comprising exome capture kit Nimblegen SeqCap EZ Human Exome Library v3.0, HiSeq 2500, and their bioinformatics pipeline based on the 1000 Genomes Project pipeline. Five samples

of human DNA (obtained from the Coriell Institute for Medical Research) were sequenced. The results were compared with the genotypes obtained using the in-house Illumina Human Omni2.5 BeadChip chip test, with the data obtained through the 1000 Genomes Project and the International HapMap Project. The authors used a read depth of 20× to assess the performance parameters.

**Reproducibility and Repeatability**

Concordance between the intra- or inter-assay sequencing replicates is approximately 89.5% for insertions/deletions (indels) and 97.9% for single-nucleotide variants (SNVs).

**Sensitivity**

The concordance of observed genotypes between chip data, data from the 1000 Genomes Project and HapMap, and exome sequencing exceeds 97%. However, the ability to detect pathogenic variants was assessed to be approximately 92% to 93%.

**Table 5: Summary table of analytical validity of the HiSeq platform**

| PARAMETER | PRESENCE | ABSENCE | NOT APPLICABLE |
|---|---|---|---|
| Repeatability | X | | |
| Reproducibility | X | | |
| Analytical sensitivity | X | | |
| Analytical specificity | X | | |
| Matrix effect | | X | |
| Concordance | X | | |
| Correlation between test and comparator | X | | |

**Validity of Exon Capture Kit**

A few characterization and comparative studies provide an analytic characterization of the various kits used for capturing the exome.

Asan et al. [2011] assessed the performance of the Nimblegen Human Exome 2.1 M Array, the Nimblegen SeqCapEZ (v 1.0) and the Agilent SureSelect Human All Exon Kit platforms. To that end, they conducted exome sequencing of a DNA sample from an immortalized cell line from an individual sequenced in the YanHuang whole-genome sequencing analysis[8] after conducting duplicate exome capture for each platform. The sequencing platform used was the HiSeq2000.

*Targeted regions*

A compilation of the data from the CCDS (release of March 27, 2009), refGen (release of April 21, 2009) and EnsemblGen databases showed that the SureSelect kit can cover approximately 1,100 more genes than the Nimblegen kits, i.e., 17,199 protein-coding genes comprising 80.6% of the protein-coding sequences versus 16,188 genes (75.9%).

A similar compilation carried out using the genes in the Online Mendelian Inheritance in Man (OMIM; release of March 10, 2011), Human Gene Mutation Database (HGMD; Professional

[8] YanHuang Project [website], available at http://yh.genomics.org.cn/.

2009.2), Genome-Wide Association Study (GWAS; release of March 3, 2011) and Cancer Genome Project (CGP; release of December 1, 2010) databases showed that the Agilent kit targeted 4,871 genes (86%), in comparison with Nimblegen's 4,642 genes (83%).

*Coverage*

The three kits cover 98% to 99% of their targeted regions at a low read depth. The level of coverage drops significantly within 10× to 50× read depths. In this range, the Agilent platform covers 59% of the targeted regions.

*Sensitivity*

Platform sensitivity was assessed at different read depths in comparison with the results obtained from whole genome sequencing:

At 10× (Q30), the Nimblegen platform showed sensitivity >83% whereas the Agilent platform showed sensitivity of approximately 77%.

From 20× to 50×, the sensitivity of the Agilent platform ranged from 64% to 85%.

*Specificity*

At a read depth of 30×, 99.88% of the homozygous sites were specifically detected versus 99.7% of the heterozygous sites.

*Repeatability*

An assessment of intra-platform reproducibility obtained correlation coefficients greater than 0.65 for coverage rate and greater than 0.90 for read depth.

*Bias*

The authors reported that all three platforms showed biases when GC content is < 20% or > 75%. A capture bias favouring the reference allele is also observed.

Parla et al. [2011] evaluated the first versions of the Nimblegen SeqCap EZ Exome Library SR and Agilent SureSelect Human All Exon Kit by re-sequencing two trios (father, mother, and child) that had been characterized in the 1000 Genomes Project. DNA was obtained from cell lines (Coriell Cell Repositories), and the sequences were analyzed on the Genome Analyzer$_{IIx}$ (Illumina). The reference sequence used was the human hg18 reference, and the Burrows-Wheeler Aligner (BWA) was used to align the sequences.

*Targeted regions*

The Agilent kit covers 98.3% of the CCDS database sequences and the Nimblegen kit covers 89.8%.

*Coverage*

The NimbleGen platform covered 86.58% to 88.04% of the CCDS database sequences at a read depth of 1×, whereas SureSelect covered 95.94% to 96.11% of the sequences. At a read depth of 20×, and by optimizing the volume of data generated, sequence coverage ranged from 85.81% to 85.98% with the Nimblegen platform and from 90.16% to 90.59% with the Agilent platform.

*Read depth*

Nimblegen covers the exome with a mean depth from 61× to 93×, compared with 39× to 53× with the Agilent platform.

*Sensitivity*

Sensitivity greater than 97% was established when comparing the SNPs obtained by sequencing with those identified by the International HapMap Project and the 1000 Genomes Project.

*Specificity*

The specificity of the data generated by the platforms was assessed to be > 99.9965% in comparison with the 1000 Genomes Project standard, and > 99.35% in comparison with the HapMap standard.

Sulonen et al. [2011] carried out a comparative study of the Agilent SureSelect All Exon kit and its updated version Human All Exon 50Mb, and the Nimblegen SeqCap EZ Exome kit and its version 2.0. The sequencing platform was $GA_{IIx}$ (Illumina). The human reference sequence was hg19 (GRCh37).

*Targeted regions*

The Agilent SureSelect and SureSelect 50Mb respectively targeted 274,264 and 300,040 exons covering 20,699 and 23,031 genes, while the Nimblegen SeqCap and SeqCap v2 kits targeted 252,479 and 298,304 exons from 18,865 and 23,028 genes.

*Coverage*

The sequences covered by the Agilent and Nimblegen first-version kits mapped respectively 81.4% and 84.4% of reference sequence hg19 (GRCh37). The updated kits covered 94.2% (Agilent) and 75.3% (NimbleGen) of hg19.

At a read depth of 20×, 69.4% of bases were covered with SureSelect compared with 78.8% covered with Nimblegen. The updated kits covered 60.3% and 81.2%, respectively.

*Concordance*

The SNPs identified by sequencing were validated using an Illumina Human660W-Quad v1 SNP chip. Genotype correlation was 97.44%, 98.75%, 97.25% and 99.39%, respectively, for Agilent kits versions 1 and 2 and Nimblegen kits versions 1 and 2.

Clark et al. [2011] compared the performance of the Nimblegen SeqCap EZ Exome Library v2.0, the Agilent SureSelect Human All Exon 50Mb, and the Illumina TruSeq Exome Enrichment using DNA from a blood sample and the HiSeq2000 platform. Sequence alignment was performed with the BWA and the variants were analyzed with GATK.

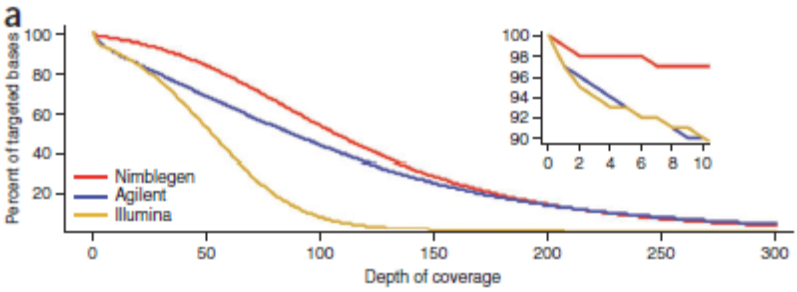
*Capture efficiency*

Capture efficacy was assessed by measuring coverage of all bases read. With Nimblegen, 98.6% of the targeted bases were covered at least once, and 96.8% were covered at least 10 times. With Illumina, 97.1% of bases were covered at least once, and 90.0% at 10×. With Agilent, 96.6% of bases were covered at 1× and 89.6% at 10×.

*Coverage*

Figure 1 shows the depth of coverage based on number of bases sequenced.

**Figure 1: Read depth based on number of bases sequenced**




Source: Clark et al., 2011.

*Concordance*

The observed SNPs were validated with the Illumina Human 1M-Duo SNP chip. Concordance rates were 99.3% for Agilent, 99.5% for Nimblegen, and de 99.2% for Illumina.

*Mutation types detected*

The detected mutations included small indels ranging in size from -84 to +18 bases.

More recent validation studies using the latest kit upgrades could not be identified in the literature. However, version 5 of the Agilent SureSelect kit is now available. The company reports that 21,522 genes (375,999 exons) are now covered by their strategy based on CCDS, RefSeq, Gencode, MiRbase, TCGA and UCSC data.

**Table 6: Summary table of capture kit analytical validity**

| PARAMETER | PRESENCE | ABSENCE | NOT APPLICABLE |
|---|---|---|---|
| Repeatability | X | | |
| Reproducibility | | X | |
| Analytical sensitivity | X | | |
| Analytical specificity | X | | |
| Matrix effect | | X | |
| Concordance | X | | |
| Correlation between test and comparator | | X | |

**Requestor's General Validation of the Test**

As part of its research, pilot projects and clinical activities, the requestor has performed exome sequencing of over 1,000 research samples and 100 clinical samples.

*Coverage of targets*

According to the requestor's experience, 98% and 95% of exons are covered, with minimum read depths, respectively, of 10× and 20×, if using the Agilent SureSelect v4 kit.

The requestor indicates that it is currently drafting a bioinformatics pipeline for gene panels through which, for each disease investigated, only the variants found in associated genes are assessed. Instances of insufficient coverage for certain regions will be flagged to the clinician for subsequent diagnostic examination, as required.

*Read depth*

Mean depth coverage of 137× is observed.

*Specificity*

The requestor has evaluated the specificity of its approach by Sanger sequencing 128 variants called by NGS. No false-positives have been detected.

*Exome capture kit v5*

According to several of the requestor's collaborators, version v5 of the Agilent capture kit offers better exon coverage than the previous version. The requestor is currently validating the latest version.

**External Quality Control**

At present, there is no external quality control program for clinical exome sequencing. The requestor plans to exchange samples with the Baylor Medical Genetics Laboratories to mitigate this limitation. Baylor uses a sequencing and analysis strategy similar to the requestor's strategy.

## 5.4 Recommendations from Other Organizations

In a policy statement issued in 2012,[9] the ACMG states that exome sequencing (and genome sequencing) should be considered for an affected individual when:

- The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis.
- A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making whole exome or genome sequencing analysis of multiple genes simultaneously a more practical approach.
- A patient presents with a likely genetic disorder but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.
- A fetus with a likely genetic disorder in which specific genetic tests , including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.

[9] American College of Medical Genetics and Genomics (ACMG). Policy Statement: Points to Consider in the Clinical Application of Genomic Sequencing [website]. Bethesda, MD: ACMG; 2012. Available at https://www.acmg.net/StaticContent/PPG/Clinical_Application_of_Genomic_Sequencing.pdf.

In March 2013, the ACMG finalized its recommendations for reporting incidental findings in exome sequencing. It recommends that certain incidental findings must be reported for the clinical benefit of the patient and the patient's family. It goes even further by recommending that variants linked to certain medical conditions be actively sought. The CCMG recommendations in this regard are expected to be issued in 2014.

In addition, a number of experts and national and international organizations (ACMG, CAP, CCMG, CDC, CMGS, etc.) have issued recommendations for methodology and other issues to be considered when implementing next-generation sequencing in a diagnostic setting; quality assurance protocols for this technology; and establishment of interpretation criteria.

# 6 ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

## 6.1 Impact on Material and Human Resources

Significant costs are associated with the acquisition, validation and necessary maintenance of an NGS platform and required IT resources (analysis programs, data storage). It is also essential to have specialized staff with access to regularly updated resources and initial and continuous training appropriate for analyzing and interpreting results.

## 6.2 Economic Consequences of Introducing Test Into Quebec's Health Care and Social Services System

Exome sequencing does not replace any other test. It thus represents an additional cost for the health care system at the analytical level. In a small proportion of cases, determining the etiology of the disease will enable early treatment, if any.

The test might also generate incidental findings that will incur additional patient management to treat or monitor a previously undiagnosed disease.

## 6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues

Exome sequencing (as is the case with the other NGS tests) lies at the borderline between diagnostic utility and basic research. Mutations of variable, probable or uncertain pathogenicity can influence clinical and personal decisions [Evans and Rothschild, 2012; Makrythanasis and Antonarakis, 2012]. In this regard, some organizations have published recommendations on whether incidental findings must be reported or which ones must be recorded. In addition, the rapid evolution of current knowledge requires suppliers of a test like the one proposed by the requestor to establish rules governing the subsequent re-analysis of results obtained for a patient.

# 7 IN BRIEF

## 7.1 Clinical Relevance

In specific cases, establishing the genetic etiology of the neurological disorders targeted by the requestor confirms the diagnosis, contributes to the establishment of a prognosis or treatment, or limits the proliferation of diagnostic tests. The results obtained using the test in question could also play a role in certain reproductive decisions.

## 7.2 Clinical Validity

A review of the literature revealed few validation studies that used exome sequencing approaches for clinical purposes. Exome sequencing has the potential to change clinical practice for genetic diseases. However, more knowledge is needed about the pathogenicity of variants [Rauch et al., 2012]. Advances in scientific knowledge will lead to a higher rate of resolution.

## 7.3 Analytical Validity

Exome sequencing is a technique that has demonstrated a high level of analytical sensitivity. However, this approach still cannot be used to investigate certain regions. The ACMG recommends using Sanger sequencing to confirm the called variants.

## 7.4 Recommendations from Other Organizations

At this time there are no recommendations on using this approach to investigate cases of ID or ND. However, a number of experts and national and international organizations (ACMG, CAP, CCMG, CDC, CMGS, etc.) have issued recommendations for methodology and other issues to consider when implementing next-generation sequencing in a diagnostic setting; quality assurance protocols for this technology; and establishment of interpretation criteria.

# 8 INESSS NOTICE IN BRIEF

Exploration of intellectual disability and neurodegenerative diseases with exome sequencing

**Status of the Diagnostic Technology**

- ☐ Established
- ☐ Innovative
- ☒ Experimental (for research purposes only)
- ☐ Replacement for technology_____, which becomes obsolete

**INESSS Recommendation**

- ☐ Include test in the Index
- ☐ Do not include test in the Index
- ☒ Reassess test once:
  - Validation has been completed, especially of the bioinformatics pipeline;
  - An external quality control mechanism is put in place;
  - The laboratory complies with the MSSS guidelines on the organization of public molecular-diagnosis services in Quebec.

**Additional Recommendation**

- ☐ Draw connection with listing of drugs, if companion test
- ☐ Produce an optimal use manual
- ☐ Identify indicators, when monitoring is required

**Note:** This test has considerable clinical potential but is still experimental.

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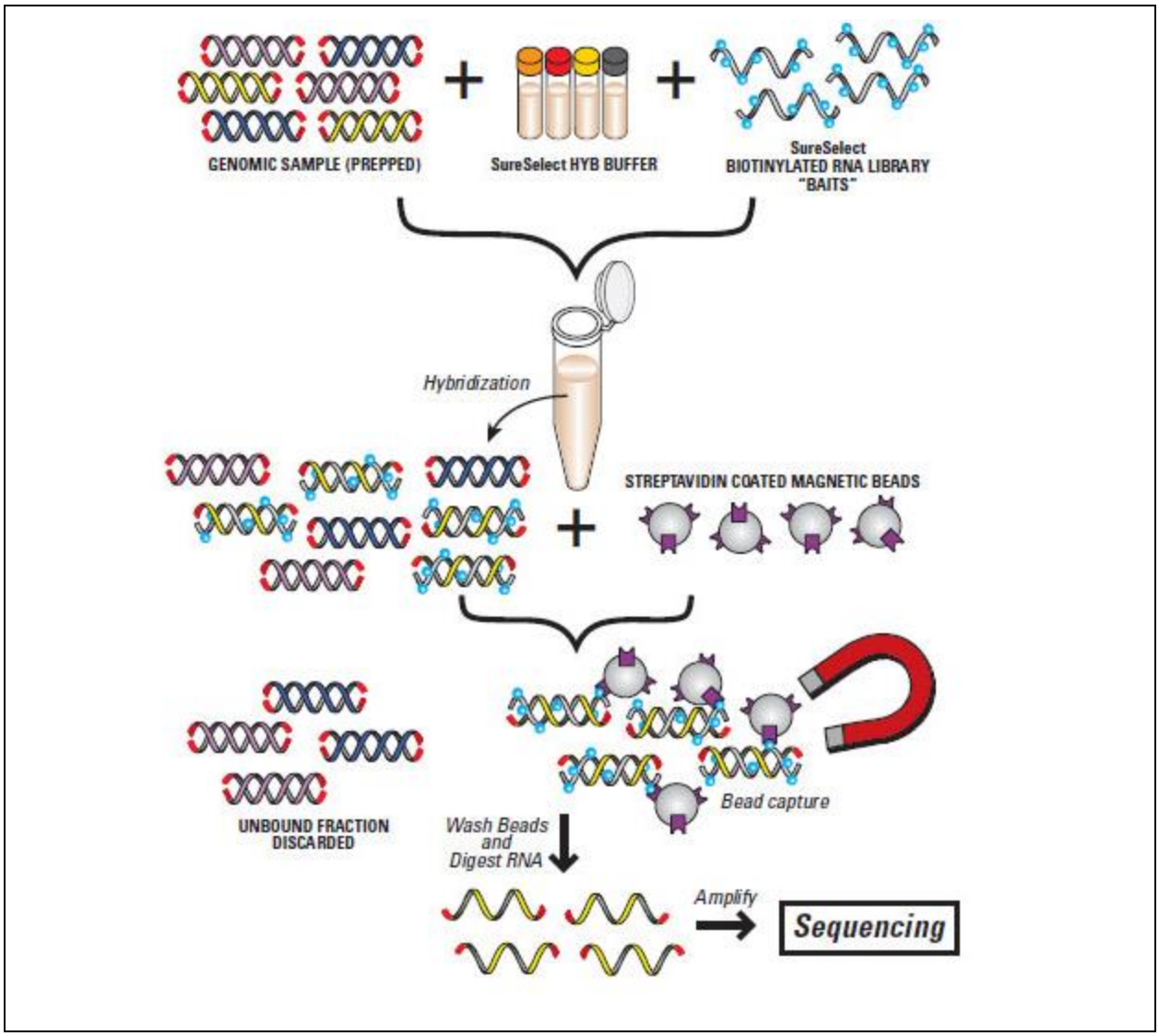
# APPENDIX A

Overview of exon capture process

**Figure A1: SureSelect Human All Exon (Agilent) kit**

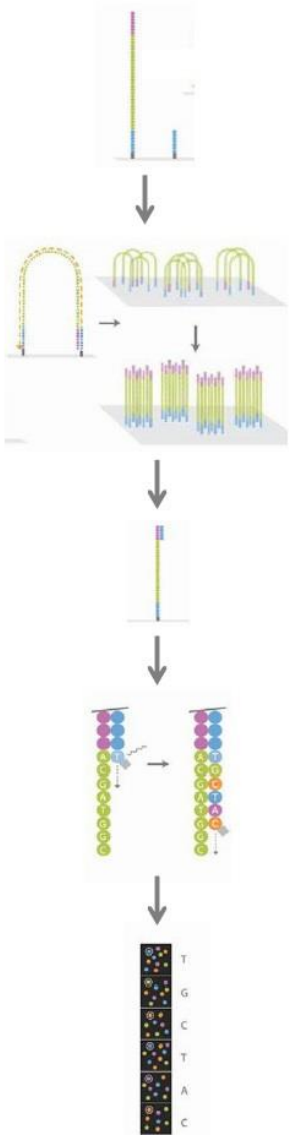



Adapted from http://www.genomics.agilent.com/.

# APPENDIX B

Next-generation sequencing

**Figure B1: Illumina sequencing technology**




DNA fragments attached to solid support

Bridge amplification and cluster generation

Primer hybridization

Addition of nucleotides complementary to the template (extension)

Sequence generation

# APPENDIX C

Clinical exome sequencing framework

**Criteria for submitting a sample for exome sequencing. Document submitted by the requestor**

We propose that requests for clinical exome sequencing be first submitted to the laboratory for approval. To submit a request, clinicians must complete a questionnaire concerning the patient's clinical presentation and family history and summarizing the outcome of prior investigations. A group of experts consisting of medical geneticists and neurologists specializing in neurodevelopmental and neurodegenerative disorders then reviews the request. If the request is approved, the clinicians must submit the sample along with the duly completed consent form.

**A. Development delay or intellectual disability**

At least initially, a medical geneticist must submit the request for clinical exome sequencing for the indication development delay or intellectual disability. The selection criteria are as follows:

1. Global developmental delay or moderate or severe intellectual disability;
2. Absence of history-specific etiology (e.g., newborn anoxia, prenatal exposure to alcohol, family history relevant to an established etiological diagnosis, etc.);
3. Relevant phenotypic screening completed (e.g., brain imaging);
4. No pathogenic abnormalities established by CGH;
5. Fragile X syndrome excluded, where applicable;
6. Absence of clinical presentation (e.g., specific single-gene syndrome) that could be the focus of a targeted investigation using a high-performance diagnostic test at a reasonable cost.

**B. Neurodegenerative diseases**

At least initially, a medical geneticist and a neurologist must submit a joint request for clinical exome sequencing for the indication of neurodegenerative diseases. The selection criteria are as follows:

1. Failure to meet developmental milestones or progression of a particular neurological symptom (excluding epilepsy);
2. Relevant phenotypic screening has been completed;
3. Lack of accurate diagnosis that could be explored effectively by targeted sequencing of one or more genes;
4. Exclusion of sporadic cases of neurodegenerative conditions characterized by substantial genetic complexity (e.g., Parkinson disease).