# RETINAL DYSTROPHY XPANDED® PANEL

A targeted test for genetic causes of retinal dystrophy using a trio approach

## OVERVIEW:

The retinal dystrophies are a clinically and genetically heterogeneous group of eye disorders, characterized by the degeneration of photoreceptors and retinal pigment epithelium cells leading to vision impairment (Sahel et al., 2014). In general, retinal dystrophies are classified according to the types of cells, which are primarily affected within the retina, the age of onset of first symptoms, the progression of visual impairment over time, and the presence or absence of extraocular phenotypic features (i.e., syndromic or non-syndromic retinal dystrophy). Due to the heterogeneous nature of retinal dystrophies, it can be challenging to determine the specific form of retinal dystrophy or predict the disease-causing gene based on clinical features or ancillary testing alone. It is often necessary to perform testing of multiple genes to identify the underlying genetic cause of retinal dystrophy in an individual. Moreover, new genes associated with retinal dystrophy are being discovered regularly, making it challenging for clinical laboratories to keep traditional testing panels up-to-date. Additionally, interpretation of the clinical significance of variants in these newly discovered genes is often difficult in the absence of parental testing to clarify the inheritance of identified variants.

The Retinal Dystrophy Xpanded® panel uses a trio approach that includes concurrent analysis of the affected proband and both parents, which increases the likelihood of identifying a definitive genetic explanation for retinal dystrophy in an individual. Depending on the family structure, family history of retinal dystrophy, and the availability of both parents, other family members of the affected individual may be evaluated in conjunction with the proband. Please contact GeneDx for prior approval when both parents are not available to submit samples for the Retinal Dystrophy Xpanded panel. The Retinal Dystrophy Xpanded panel is based on exome capture (EC), NextGeneration sequencing (NGS), Sanger sequencing of specific regions, and targeted analysis of a comprehensive list of approximately 780 genes currently associated with retinal dystrophy. The design of the panel allows for a comprehensive, dynamic gene list that is updated regularly to ensure inclusion of genes recently associated with retinal dystrophy.

## GENETICS OF RETINAL DYSTROPHY:

The inheritance pattern of the retinal dystrophies can be autosomal dominant, autosomal recessive, X-linked, mitochondrial, or digenic (Sahel et al., 2014). The incidence of the retinal dystrophies varies by population and is estimated to be 2.5 per 10,000 individuals (Kocur et al., 2002; Ayuso et al., 2010; Finger et al., 2011). Pathogenic variants in a certain gene may be associated with a wide range of phenotypes (clinical heterogeneity), and conversely, pathogenic variants in different genes can cause the same phenotype (genetic heterogeneity). Incomplete penetrance and/or variable expressivity have been reported in many types of retinal dystrophy. In some instances, molecular confirmation of a clinical diagnosis of retinal dystrophy may have implications for treatment and management of the specific form of disease.

## TEST METHOD:

Using genomic DNA from the submitted specimen(s), the exonic regions and flanking splice junctions of the genome are captured using a proprietary system developed by GeneDx and sequenced by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100bp or greater paired-end reads. Reads are aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants in targeted genes using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing or another appropriate method

is used to confirm all potentially pathogenic variants identified in this individual and relative samples, if submitted. Sequence and copy number alterations are reported according to the Human Genome Variation Society (HGVS).

Capillary sequencing is used to perform targeted analysis for the recurrent c.2991+1655A>G variant in the CEP290 gene associated with Leber's Congenital Amaurosis (den Hollander et al., 2006) and for select deep intronic variants of ABCA4 previously reported in the literature in association with Stargardt disease (5196+1216C>A, 5196+1159G>A, 5196+1137G>A, 5196+1136C>A, 5196+1056A>G, 570+1798A>G, 1938-619A>G, 2160+584A>G, 3050+370C>T, 4540-2036C>A, 4539+2064C>T, 4539+2028C>T, 4539+2001G>A, 4539+1729G>T) (Braun et al., 2013; Zernant et al., 2014; Bauwens et al., 2015; Schulz et al., 2017).

Please note that while the Retinal Dystrophy Xpanded panel captures and sequences the exome, analysis is targeted to the specific phenotype-driven gene list for this panel. The Retinal Dystrophy Xpanded Panel gene list includes approximately 780 genes. The list was developed by searching for genes associated with retinal dystrophy in multiple sources, including OMIM, HGMD, and Human Phenotype Ontology (HPO) terms. The gene list is systematically updated at least quarterly. The current gene list is available on our website.

## RESULT REPORTING:

The Retinal Dystrophy Xpanded Panel is performed on the proband and the parental samples (and/or additional relatives) when submitted together for analysis. A single report will be issued on the affected proband in the family. A separate report will not be issued for unaffected parents or other family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results from the affected individual. If additional reports are requested for other affected family members, extra fees will apply.

The report that is issued for the affected individual will include reportable variants in genes that have been previously associated with retinal dystrophy in the published or emerging literature. The report will include pathogenic or likely pathogenic variants in genes associated or likely associated with the patient's phenotype. In some instances, the report may also include specific variants of uncertain significance (VUS) in genes that are possibly associated with the patient's phenotype. Variants that are considered to be benign or likely benign will not be reported. As the Retinal Dystrophy Xpanded Panel includes approximately 780 genes, the report will not include a comprehensive list of all observed variants. If desired, clinicians can request a separate file containing a list of identified variants that will be provided upon completion of testing.

## TEST SENSITIVITY:

The clinical sensitivity of the Retinal Dystrophy Xpanded Panel depends in part on the patient's clinical phenotype. Previous exome sequencing studies evaluating individuals with retinal dystrophy have demonstrated a diagnostic rate of 49-71% (Beryozkin et al., 2015; Lee et al., 2015; de Castro-Miro et al., 2016; Haer-Wigman et al., 2017; Riera et al., 2017; Carss et al., 2017). The sensitivity of this test is expected to be comparable to trio-based exome sequencing since it uses a trio approach to test a comprehensive list of genes known to be associated with retinal dystrophy. The clinical sensitivity is expected to be significantly lower for singleton testing when only the affected proband is tested (Retterer et al., 2015).

The average coverage of all genes on the panel is greater than 99.69% at 10X (with a depth of 10 or more reads), and approximately 95.31% of the genes on the panel have an average coverage of greater than 99.0% coverage at 10X. Several genes with a high clinical sensitivity have an average coverage of less than 90% at 10X, including *GRK1* (85.64%), *PRDM5* (89.42%), *PROM1* (85.09%), *RAB18* (87.27%), *RAB3GAP2* (82.59%), *OPN1LW* (85.85%), PRPH2 (79.5%). Note that these numbers represent the average coverage for the genes on

the panel, derived by combining data from a large number of patients. The coverage of each gene on the panel for a specific patient may vary, and the actual coverage for each gene is included in the final report.

**LIMITATIONS:**

Some types of genetic disorders may not be detectable with this test. Small sections of a few individual genes have inherent sequence properties that yield suboptimal data and pathogenic variants in those regions may not be reliably detected. In addition, mitochondrial genome sequencing and sequencing of noncoding RNA molecules (microRNA) is not performed as part of the Retinal Dystrophy Xpanded Panel.

The scientific knowledge available about the function of all genes in the human genome is incomplete at this time. It is possible that the Retinal Dystrophy Xpanded Panel may identify the presence of a variant in the sequence of an affected individual, but that we will not recognize it as the cause of their disease due to insufficient knowledge about the gene and its function. Even if the Retinal Dystrophy Xpanded Panel identifies the underlying genetic cause of a disorder in an affected individual, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

For test codes, CPT codes, and turn-around-times, please refer to the “Retinal Dystrophy Xpanded Panel” page on our website: www.genedx.com

**REFERENCES:**

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