

**Nebulin (NEB) Gene Sequencing (Test #355)**  
**Nemaline Myopathy (NEM2)**

**Brief Description of Clinical Features:** Mutations in the nebulin gene (*NEB*; OMIM #161650) are one cause of autosomal recessive nemaline myopathy (NEM) (OMIM #256030). NEM is a genetically and clinically heterogeneous disorder characterized by muscle weakness, hypotonia and the presence of nemaline bodies in skeletal muscle fibers. Muscle weakness is typically observed in affected neonates or infants, although later onset cases are reported (Ryan et al. *Ann Neurol* 50:312-320, 2001). The most severely affected muscle groups are proximal limb, facial, bulbar, and respiratory muscles. Deep tendon reflexes are absent or depressed. Histologically, NEM is characterized by type 1 fiber predominance and the presence of rod-like structures called nemaline bodies upon Gomori trichrome staining of skeletal muscle (Ryan et al. *Neurol* 60:665-673, 2003). Six clinical types of NEM have been delineated based on age of onset, severity and distribution of weakness, and respiratory function (Ryan et al. 2001; North and Ryan, *GeneReviews*, 2006). Nebulin gene mutations more often cause typical neonatal onset disease, although *NEB* mutations have been found in every clinical form of NEM (Lehtokari et al. *Hum Mut* 27:946-956, 2006). Overlap among the six clinical groups is significant, and adults are sometimes diagnosed only after another family member has presented with typical signs.

**Genetics:** To date, mutations in six genes have been shown to cause nemaline myopathy. Mutations in *ACTA1* (NEM3) and *NEB* (NEM2) are the only relatively common causes (Ryan et al. 2001). All reported cases of *NEB*-associated NEM demonstrate autosomal recessive inheritance (Lehtokari et al. 2006). In the largest cohort published to date, Lehtokari et al. (2006) identified 64 *NEB* mutations segregating with autosomal recessive NEM in 55 families. A small majority of the mutations were point mutations resulting in either altered intron-exon splicing, nonsense mutations or missense mutations. Small deletions accounted for 31% of all mutations. The only common *NEB* mutation is an exon 55 deletion found at a carrier frequency of about 1% among people of Ashkenazi Jewish ancestry (Anderson et. al. *Hum Genet* 115:185-190, 2004).

**Description of This Particular Test:** Testing is accomplished by amplifying all coding exons (3-183) and ~50 bp of adjacent noncoding sequence, then determining the nucleotide sequence using standard dideoxy sequencing methods and a capillary electrophoresis instrument. Exons 82-105 are organized in three repetitive blocks of 8 exons each and, because these blocks are nearly identical in sequence, it is not possible to amplify and sequence the 24 exons independently. Our strategy is to co-amplify the identical exons (*e.g.*, exons 82, 90, and 98), then sequence the *pool* of DNA fragments (each pool consists of six alleles). As all *NEB* mutations reported to date occur outside of the repetitive region, these exons will be sequenced only if indicated by results from the non repetitive exons.

**Reference Sequence:** NM\_004543.3

**Indication for Testing:** Individuals with symptoms consistent with the typical congenital form of nemaline myopathy. Individuals whose muscle biopsies show predominance of type 1 fibers and nemaline bodies.

**Sensitivity of test:** As determined by our validation study, analytical sensitivity for detecting insertions and deletions in the repetitive region is nearly 100%, and ~70% for base substitutions. This test will not detect the exon 55 deletion found in Ashkenazi Jews. A separate test (Test #356) is available to diagnose this mutation.

**Turnaround Time:** Maximum of 80 days.

**Specimen Requirements:** See page 4 of the Requisition Form.

**Price:** Sequencing of the *NEB* Gene **\$ 6,490 maximum**

CPT Codes	Description	Exons 3-81	Exons 3-81 & 106-183	Exons 3-183
83890	Ascertainment	\$30 (x1)	\$30 (x1)	\$30 (x1)
83891	DNA Isolation	\$40 (x1)	\$40 (x1)	\$40 (x1)
83898	Amplification	\$909 (x70)	\$1539 (x138)	\$1810 (x146)
83904	Mutation ID by Sequencing	\$1,846 (x70)	\$3,532 (x138)	\$3870 (x146)
83894	Separation	\$200 (x1)	\$400 (x1)	\$420 (x1)
83912	Interpretation and Report	\$220 (x1)	\$300 (x1)	\$320 (x1)
<b>Totals:</b>		<b>\$3,245</b>	<b>\$5,841</b>	<b>\$6,490</b>

**Accreditation Info.** CLIA ID #: 52D1027685 (expires 1/18/13) (CAP#: 7185561, AU ID: 1407125 expires 12/20/12)

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