# EBSeq

## Epidermolysis Bullosa Genetic Testing by Next-Generation Sequencing

## Genes Tested

| Gene | Protein |
|---|---|
| *CD151* | CD151 Antigen |
| *CDSN* | Corneodesmosin |
| *CHST8* | Carbohydrate sulfotransferase 8 |
| *COL7A1* | Collagen alpha-1(VII) chain |
| *COL17A1* | Collagen alpha-1(XVII) chain |
| *DSP* | Desmoplakin |
| *DST* | Dystonin |
| *EXPH5* | Exophilin 5 |
| *FERMT1* | Fermitin family homolog 1 |
| *ITGA3* | Integrin alpha-3 |
| *ITGA6* | Integrin alpha-6 |
| *ITGB4* | Integrin beta-4 |
| *JUP* | Junction plakoglobin |
| *KRT5* | Keratin, type II cytoskeletal 5 |
| *KRT14* | Keratin, type I cytoskeletal 14 |
| *LAMA3* | Laminin subunit alpha-3 |
| *LAMB3* | Laminin subunit beta-3 |
| *LAMC2* | Laminin subunit gamma-2 |
| *PKP1* | Plakophilin-1 |
| *PLEC1* | Plectin |
| *TGM5* | Protein-glutamine gamma-glutamyltransferase 5 |

*Each of the genes on this panel can also be ordered as a single gene test. Deletion/duplication analysis may also be available for the genes on this panel.*

This panel detects the most common causes of Epidermolysis Bullosa (EB). EB is a genetically heterogeneous disorder of skin fragility, manifested by blistering and/or erosions with little or no trauma. The incidence of EB is estimated to be one in 20,000, but this may be an underestimate due to patients with mild presentation. EB can be inherited in autosomal dominant or autosomal recessive manners. There are many different subtypes of EB with clearly defined hallmark symptoms, but clinical overlap makes it hard to distinguish between subtypes, especially in infancy.

| EB Subtypes | Location of blistering | Inheritance* | Genes |
|---|---|---|---|
| EB Simplex | Epidermis | AD, rarely AR | *DSP, DST, JUP, KRT5, KRT14, PKP1, PLEC1, TGM5* |
| Junctional EB | Lamina Lucida | AR | *COL17A1, ITGA3, ITGA6, ITGB4, LAMA3, LAMB3, LAMC2* |
| Dystrophic EB | Sub-lamina densa | AR or AD | *COL7A1* |
| Kindler Syndrome | Multiple layers | AR | *FERMT1* |

*AD: Autosomal Dominant; AR: Autosomal Recessive

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A clinical diagnosis of EB can be confirmed by immunofluorescence mapping (IFM), transmission electron microscopy (EM), or mutation analysis. However, mutation analysis is important to determine the inheritance pattern and subclassification, which are critical for accurate genetic counseling.

The current approach for classifying patients with EB begins with determining the major EB subtype by the level of skin cleavage, characterizing the phenotype by distribution and severity of disease, determining the mode of inheritance, the gene involved, and the type of mutation present.

**EB simplex (EBS)** is associated with skin cleavage at the epidermal level. The severity can vary and blistering can be generalized or localized. EB simplex is most commonly caused by autosomal dominant mutations in *KRT4* and *KRT14.* Rare phenotypes of EBS include:

- Acral peeling skin syndrome - *TGM5, CDSN, CHST8*
- Acantholytic EBS - *DSP, JUP*
- Skin fragility syndromes - *DSP, JUP, PKP1*
- EBS with mottled pigmentation - *KRT5*
- Migratory circinate EBS - *KRT5*
- EBS with muscular dystrophay - *PLEC1*
- EBS with pyloric atresia - *PLEC1, ITGA6, ITGB4*
- EBS-Ogna - *PLEC1*
- Autosomal recessive EBS - *DST, KRT14, EXPH5*

**Junctional EB (JEB)** is associated with skin cleavage at the lamina lucida and is autosomal recessive. The severity can vary and blistering can be generalized or localized. Rare phenotypes of JEB include:

- JEB with pyloric atresia - *COL17A1, ITGB4, ITGA6*
- Late Onset JEB - *COL17A1*
- JEB with respiratory and renal involvement - *ITGA3*
- JEB inversa - *LAMA3, LAMB3, LAMC2*

**Dystrophic EB (DEB)** is caused by mutations in *COL7A1* resulting in skin cleavage at the sublamina densa and can be inherited in an autosomal dominant (DDEB) or autosomal recessive (RDEB) manner. There are many phenotypes associated with DEB which are classified by inheritance, severity, and localization of skin findings.

**Kindler Syndrome** is an autosomal recessive genodermatosis caused by mutations in the *FERMT1 (KIND1)* gene. Symptoms include trauma induced blistering, poikiloderma, and skin atrophy. Some patients also have photosensitivity, which lessens with age, dental problems, gastrointestinal symptoms, squamous cell carcinoma, finger webbing, pseudosyndactyly, and nail dystrophy.

Mutations in *CD151* have been reported in two children with pretibial EB, nephropathy, and deafness.

## Indications:

**EBSeq panel by NGS**

- Blistering, peeling, or erosions on the skin and/or mucous membranes
- Immunofluorescence mapping (IFM), transmission electron microscopy (EM) suggestive of EB

**Single Gene Sequencing**

- Confirmation of a genetic diagnosis in a patient with symptoms, immunofluorescence antigen mapping and/or transmission electron microscopy suggestive of a specific subtype of EB in a specific gene

**Mutation Specific Analysis:**

- Presymptomatic testing of at-risk siblings and parents
- Carrier identification in individuals in whom specific mutation(s) have been identified in the proband with EB
- Prenatal diagnosis of an at-risk fetus, after confirmation of mutation(s) in the parent(s) and by prior arrangement only

## Specimen:

At least 3 mLs whole blood in a lavender top (EDTA) tube.

Label tube with patient's name, birth date, and date of collection.

Saliva samples are also acceptable for patients who cannot have blood drawn safely. Please call 513-636-4474 for a free saliva collection kit.

## Testing Methodology:

• **Next Generation Sequencing Panel:** This test is performed by enrichment of the exons, flanking intronic and un-translated regions (5' and 3') of the genes specified above using oligonucleotide probe hybridization followed by next-generation sequencing with > 20 fold coverage at every target base. All pathogenic and novel variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing.

• **Single Gene Sequencing/Mutation Specific Analysis:** Sanger sequencing following PCR amplification of the coding and exon/intron boundaries of the gene.

## Test Sensitivity:

**Clinical Sensitivity:** The clinical sensitivity of *KRT5* and *KRT14* sequencing in patients with biopsy-diagnosed EBS is 75%. The clinical sensitivity of *COL17A1, LAMA3, LAMB3,* and *LAMC2* in patients with JEB is greater than 98%. The clinical sensitivity of *COL7A1* sequencing in patients with biopsy-diagnosed DEB is 95%. The clinical sensitivity of *FERMT1* sequencing in patients with Kindler syndrome EBS is 75%.

**Analytical Sensitivity:** The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed.

**Limitations:** Mutations in regulatory regions or other untranslated regions are not detected by this test. Large deletions involving entire single exons or multiple exons, large insertions and other complex genetic events have been reported in *COL17A1, COL7A1, DSP, DST, FERMT1, ITGB4, LAMB3, LAMC2, PKP1,* and *PLEC1* and will not be identified using this test methodology. Rare primer site variants may lead to erroneous results.

## Turn-Around Time:

Four to six weeks for the next generation sequencing panel or single gene sequencing.

## Cost:

Please call 1-866-450-4198 for current pricing, insurance precertification, or with any billing questions.

## CPT Codes:

| | |
|---|---|
| • EBSeq NGS Panel: | 81406 (x2), 81479 (x19) |
| • Single gene sequencing (except *DSP* and *JUP):* | 81479 |
| • *DSP* or *JUP* single gene sequencing: | 81406 |
| • Targeted mutation analysis: | 81403 |

## Results:

Results will be reported to the referring physician or health care provider as specified on the requisition form.

## Shipping Instructions

Please enclose **test requisition** with sample.
**All information must be completed before sample can be processed.**

Place samples in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday

## Ship to:

Cytogenetics and Molecular Genetics Laboratories
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

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