

Biotinidase Deficiency, BTD Gene Sequencing with Deletion/Duplication, Varies

Overview

Useful For

For confirming biotinidase deficiency as indicated by enzymatic testing or newborn screening

Carrier testing of individuals with a family history of biotinidase deficiency, but disease-causing variants have not been identified in an affected individual

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
_STR1	Comp Analysis using STR	No, (Bill only)	No
	(Bill only)		
_STR2	Add'l comp analysis w/STR	No, (Bill only)	No
	(Bill Only)		
CULFB	Fibroblast Culture for	Yes	No
	Genetic Test		
CULAF	Amniotic Fluid	Yes	No
	Culture/Genetic Test		
MATCC	Maternal Cell	Yes	No
	Contamination, B		

Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in the *BTD* gene associated with biotinidase deficiency. See Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, recurrence risk assessment, familial screening, and genetic counseling for biotinidase deficiency.

Testing Algorithm

Prenatal specimens:

If an amniotic fluid specimen or cultured amniocytes are received, an amniotic fluid culture will be performed at an additional charge.

If a chorionic villi specimen or cultured chorionic villi are received, a fibroblast culture will be performed at an additional charge.

For any prenatal specimen that is received, maternal cell contamination testing will be performed at an additional charge.

Skin biopsy or cultured fibroblast specimens:

For skin biopsy or cultured fibroblast specimens, a fibroblast culture will be performed at an additional charge. If viable



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cells are not obtained, the client will be notified.

Special Instructions

- Molecular Genetics: Biochemical Disorders Patient Information
- Informed Consent for Genetic Testing
- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Biochemical testing for the diagnosis of biotinidase deficiency should be considered prior to molecular confirmation with this test. The preferred test for assessment of biotinidase deficiency is BIOTS / Biotinidase, Serum.

Testing for the *BTD* gene as part of a customized panel is available. See CGPH / Custom Gene Panel, Hereditary, Next-Generation Sequencing, Varies. To modify this panel via CGPH, use the Inborn Errors of Metabolism disease state for step 1 on the Custom Gene Ordering Tool.

Targeted testing for familial variants (also called site-specific or known variants testing) is available for variants identified in the *BTD* gene. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

Additional Testing Requirements

All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen as this must be a different order number than the prenatal specimen.

Specimen Required

Patient Preparation: A previous hematopoietic stem cell transplant from an allogenic donor will interfere with testing. For information about testing patients who have received a hematopoietic stem cell transplant, call 800-533-1710.



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Submit only 1 of the following specimens:

Specimen Type: Whole blood

Container/Tube: Lavender top (EDTA) or yellow top (ACD)

Specimen Volume: 3 mL **Collection Instructions:**

1. Invert several times to mix blood.

2. Send whole blood specimen in original tube. Do not aliquot.

3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information.

Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 4 days/Frozen 4 days

Additional Information:

- 1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
- 2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
- 3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

Specimen Type: Saliva

Patient Preparation: Patient should not eat, drink, smoke, or chew gum 30 minutes prior to collection.

Supplies:

DNA Saliva Kit High Yield (T1007) Saliva Swab Collection Kit (T786)

Container/Tube:

Preferred: High-yield DNA saliva kit

Acceptable: Saliva swab

Specimen Volume: 1 Tube if using T1007 or 2 swabs if using T786 **Collection Instructions:** Collect and send specimen per kit instructions.

Specimen Stability Information: Ambient (preferred) 30 days/Refrigerated 30 days

Additional Information: Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

Specimen Type: Skin biopsy

Supplies: Fibroblast Biopsy Transport Media (T115)

Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The

solution should be supplemented with 1% penicillin and streptomycin.

Specimen Volume: 4-mm Punch

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information:

1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for



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specimens received after 24 hours and will be evaluated to determine if testing may proceed.

2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Cultured fibroblasts

Source: Skin

Container/Tube: T-25 flask Specimen Volume: 2 Flasks

Collection Instructions: Submit confluent cultured fibroblast cells from a skin biopsy. **Specimen Stability Information**: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information:

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Tissue biopsy **Supplies**: Hank's Solution (T132)

Container/Tube: Sterile container with sterile Hank's balanced salt solution, Ringer's solution, or normal saline

Specimen Volume: 0.5 to 3 cm(3) or larger

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information:

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.

Specimen Type: Blood spot

Supplies: Card-Blood Spot Collection (Filter Paper) (T493)

Container/Tube:

Preferred: Collection card (Whatman Protein Saver 903 Paper)

Acceptable: PerkinElmer 226 filter paper or blood spot collection card

Specimen Volume: 2 to 5 Blood spots

Collection Instructions:

- 1. An alternative blood collection option for a patient older than 1 year is a fingerstick. For detailed instructions, see How to Collect a Dried Blood Spot Sample.
- 2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
- 3. Do not expose specimen to heat or direct sunlight.
- 4. Do not stack wet specimens.
- 5. Keep specimen dry

Specimen Stability Information: Ambient (preferred)/Refrigerated

Additional Information:

1. Blood spot specimens are acceptable but not recommended. Multiple extractions will be required to obtain sufficient yield for supplemental analysis, and there is significant risk for test failure due to insufficient DNA.



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- 2. Due to lower concentration of DNA yielded from blood spot, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.
- 3. For collection instructions, see <u>Blood Spot Collection Instructions</u>
- 4. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777)
- 5. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800)

Specimen Type: Extracted DNA

Container/Tube:

Preferred: Screw Cap Micro Tube, 2 mL with skirted conical base

Acceptable: Matrix tube, 1 mL

Collection Instructions:

1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.

2. Include concentration and volume on tube.

Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated

Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

PRENATAL SPECIMENS

Due to its complexity, consultation with the laboratory is required for all prenatal testing; call 800-533-1710 to speak to a genetic counselor.

Specimen Type: Amniotic fluid

Container/Tube: Amniotic fluid container

Specimen Volume: 20 mL

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information: Specimen will only be tested after culture.

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid. An additional 2 to 3 weeks are required to culture amniotic fluid before genetic testing can occur.
- 3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Prenatal cultured fibroblasts (eg, products of conception), amniocytes, or other confluent cultured cells. This does not include cultured chorionic villi.

Container/Tube: T-25 flask Specimen Volume: 2 Flasks

Collection Instructions: Submit confluent cultured cells from another laboratory. **Specimen Stability Information**: Ambient (preferred) < 24 hours/Refrigerated < 24 hours



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Additional Information:

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing.
- 3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Chorionic villi

Container/Tube: 15-mL tube containing 15 mL of transport media

Specimen Volume: 20 mg

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information: Specimen will only be tested after culture.

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks are required to culture fibroblasts before genetic testing can occur.
- 3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Cultured chorionic villi

Container/Tube: T-25 flasks Specimen Volume: 2 Full flasks

Collection Instructions: Submit confluent cultured cells from another laboratory.

Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours

Additional Information:

- 1. Specimens are preferred to be received within 24 hours of collection. Culture and/or extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
- 2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing.
- 3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Forms

1. New York Clients-Informed consent is required.

Document on the request form or electronic order that a copy is on file. The following documents are available:

- -Informed Consent for Genetic Testing (T576)
- -Informed Consent for Genetic Testing (Spanish) (T826)
- Molecular Genetics: Biochemical Disorders Patient Information (T527)
- 3. If not ordering electronically, complete, print, and send a <u>Biochemical Genetics Test Request</u> (T798) with the specimen.

Specimen Minimum Volume

See Specimen Required

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.



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Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Note: Where applicable, verbiage refers to sex assigned at birth.

Biotinidase deficiency is an inherited metabolic disease caused by reduced levels of biotinidase, an enzyme that recycles biotin by releasing it from its metabolic product, biocytin, or exogenous dietary proteins. Biotin is a vitamin that serves as a coenzyme for 4 carboxylases that are essential for amino acid catabolism, gluconeogenesis, and fatty acid synthesis. Depletion of free biotin reduces carboxylase activity, resulting in secondary carboxylase deficiency. Depending on the amount of residual biotinidase activity, individuals can have either profound or partial biotinidase deficiency. Age of onset and clinical phenotype vary among individuals. Profound biotinidase deficiency occurs in approximately 1 in 137,000 live births and partial biotinidase deficiency occurs in approximately 1 in 110,000 live births, resulting in a combined incidence of about 1 in 61,000.

Untreated profound biotinidase deficiency (<10% of normal biotinidase activity) manifests within the first decade of life as seizures, hypotonia, neurosensory hearing loss, respiratory problems, and cutaneous symptoms including skin rash, alopecia, and recurrent viral or fungal infections. Among children and adolescents with profound biotinidase deficiency, clinical features include ataxia, sensorineural hearing loss, developmental delay, and eye problems such as optic neuropathy leading to blindness. Partial biotinidase deficiency (10%-30% of normal biotinidase activity) is associated with a milder clinical presentation, which may include cutaneous symptoms without neurologic involvement.

Treatment with biotin has been successful in both preventing and reversing the clinical features associated with biotinidase deficiency. As a result, biotinidase deficiency is included in most newborn screening programs in order to prevent disease. Biotinidase deficiency exhibits a similar clinical presentation to carboxylase and holocarboxylase synthetase deficiency. Therefore, measurement of the biotinidase enzyme is required to differentiate between these diseases and ensure proper diagnosis. Newborn screening for biotinidase deficiency involves direct analysis of the biotinidase enzyme from blood spots obtained shortly after birth. This enables early identification of potentially affected individuals and quick follow-up with confirmatory biochemical and molecular testing.

Biotinidase deficiency is inherited in an autosomal recessive manner, caused by variants in the biotinidase gene (*BTD*). The carrier frequency for biotinidase deficiency in the general population is about 1:120. Several common variants in the *BTD* gene have been identified, accounting for about 60% of affected individuals. Sequencing of the entire *BTD* gene detects other, less common, disease-causing variants. While genotype-phenotype correlations are not well established, it appears that certain variants are associated with profound biotinidase deficiency, while others are associated with partial deficiency.

The preferred test to diagnose biotinidase deficiency is the enzymatic assay, BIOTS / Biotinidase, Serum. Molecular tests



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form the basis of confirmatory or carrier testing. Individuals with decreased enzyme activity are more likely to have 2 identifiable disease-causing variants in the *BTD* gene by molecular genetic testing.

Reference Values

An interpretive report will be provided.

Interpretation

All detected variants are evaluated according to American College of Medical Genetics and Genomics recommendations.(1) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Clinical Correlations:

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of a reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact Mayo Clinic Laboratories genetic counselors at 800-533-1710.

Technical Limitations:

Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of the gene that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

This analysis targets single and multi-exon deletions/duplications; however, in some instances single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic mutations and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.



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For detailed information regarding gene specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent non-leukoreduced blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants:

Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare professionals to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

Variant Evaluation:

Evaluation and categorization of variants are performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline. (1) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. Incidental findings may include, but are not limited to, results related to the sex chromosomes. These findings will be carefully reviewed to determine whether they will be reported.

Clinical Reference

- 1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424.
- 2. Wolf B. Biotinidase Deficiency. In: Adam MP, Feldman J, Mirzaa GM, et al., eds. GeneReviews [Internet]. University of Washington, Seattle; March 24, 2000. Updated May 25, 2023. Accessed March 21, 2025. Available at www.ncbi.nlm.nih.gov/books/NBK1322/
- 3. Kaye CI; Committee on Genetics, Accurso F, et al. Newborn screening fact sheets. Pediatrics. 2006;118(3):e934-e963
- 4. Nyhan WL, Barshop B, Ozand PT. Multiple carboxylase deficiency/biotinidase deficiency. In: Atlas of Metabolic Diseases. 2nd ed. Oxford University Press; 2005:42-48
- 5. Wolf B, Jensen KP, Barshop B, et al. Biotinidase deficiency: novel mutations and their biochemical and clinical correlates. Hum Mutat. 2005;25(4):413



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Performance

Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing is performed to test for the presence of variants in coding regions and intron/exon boundaries of the *BTD* gene, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 20X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletions/insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the gene analyzed.

There may be regions of the gene that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. (Unpublished Mayo method)

The reference transcript for the *BTD* gene is NM_000060.4. Reference transcript numbers may be updated due to transcript re-versioning. Always refer to the final patient report for gene transcript information referenced at the time of testing. Confirmation of select reportable variants may be performed by alternate methodologies based on internal laboratory criteria.

PDF Report

Supplemental

Day(s) Performed

Varies

Report Available

10 to 14 days

Specimen Retention Time

Whole blood/Saliva: 28 days (if available); Extracted DNA: 3 months; Blood spots: 1 year (if available)

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees

- Authorized users can sign in to <u>Test Prices</u> for detailed fee information.
- Clients without access to Test Prices can contact <u>Customer Service</u> 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact <u>Customer Service</u>.



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Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

81404

88233-Tissue culture, skin or solid tissue biopsy (if appropriate)

88240-Cryopreservation (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BTD	BTD Gene Analysis	94242-5

Result ID	Test Result Name	Result LOINC® Value
622554	Test Description	62364-5
622555	Specimen	31208-2
622556	Source	31208-2
622557	Result Summary	50397-9
622558	Result	82939-0
622559	Interpretation	69047-9
622560	Additional Results	82939-0
622561	Resources	99622-3
622562	Additional Information	48767-8
622563	Method	85069-3
622564	Genes Analyzed	48018-6
622565	Disclaimer	62364-5
622566	Released By	18771-6