Cystic Fibrosis (*CFTR*), 165 Pathogenic Variants with Reflex to Sequencing and Reflex to Deletion/Duplication

Indications for Ordering

For individuals with suspected cystic fibrosis (CF) or a *CFTR*-related disorder

Test Description

- Polymerase chain reaction followed by fluorescence monitoring of 165 pathogenic *CFTR* gene variants (see table)
- Bidirectional sequencing of the *CFTR* coding regions and intron/exon boundaries
- Deletion/duplication analysis by multiplex ligation-dependent probe amplification

Tests to Consider

Primary tests

Cystic Fibrosis (*CFTR*) 165 Pathogenic Variants with Reflex to Sequencing and Reflex to Deletion/Duplication 2013664
- For individuals with suspected CF
- This test is NOT indicated for routine obstetric carrier screening
- If individual is not symptomatic, order the CF 165 pathogenic variants test

Cystic Fibrosis (*CFTR*) 165 Pathogenic Variants with Reflex to Sequencing 2013663
- For individuals with suspected CF
- This test is NOT indicated for routine obstetric carrier screening
- If individual is not symptomatic, order the CF 165 pathogenic variants test

Related tests

Cystic Fibrosis (*CFTR*) 165 Pathogenic Variants 2013661
- Carrier screening for expectant individuals and those planning a pregnancy
- Diagnostic testing for individuals with symptoms of classic CF

Cystic Fibrosis (*CFTR*) Sequencing 0051110
- For individuals with suspected CF but without 2 pathogenic variants detected by the CF 165 pathogenic variants test
- This test is NOT indicated for routine obstetric carrier screening

Cystic Fibrosis (*CFTR*) Sequencing with Reflex to Deletion/Duplication 0051640
- For individuals with suspected CF but without 2 pathogenic variants detected by the CF 165 pathogenic variants test
- This test is NOT indicated for routine obstetric carrier screening

Cystic Fibrosis (CFTR) 165 Pathogenic Variants, Fetal 2013662
- For fetal testing when both parents are known carriers of one of the variants on the CF 165 pathogenic variants test or fetus has an echogenic bowel

Familial Mutation, Targeted Sequencing 2001961
- Useful when a pathogenic familial variant identifiable by sequencing is known

Disease Overview

Incidence

- Classic CF by ethnicity (Abeliovich, 1992)
  - Ashkenazi Jewish: 1/2,300
  - White: 1/2,500
  - Hispanic American: 1/13,500
  - African American: 1/15,100
  - Asian American: 1/35,100
- Other *CFTR*-related disorders: unknown

Symptoms

- Classic CF
  - Chronic sinopulmonary disease and infections
  - Pancreatic insufficiency (endocrine and exocrine)
  - Hepatic disease: biliary obstruction and portal fibrosis
  - Prolapsed rectum
  - Failure to thrive
  - Meconium ileus
  - Obstructive azoospermia
  - Salt loss syndromes
  - Life expectancy: ~41 years
- *CFTR*-related disorders
  - Idiopathic pancreatitis
  - Bilateral absence of the vas deferens (BAVD)
  - Bronchiectasis
  - Nasal polyposis
  - Typically presents in adulthood
    - May not decrease life expectancy

Genetics

Gene: *CFTR*

Inheritance: autosomal recessive

Penetrance

- Severe pathogenic variants: high
- Mild pathogenic variants: variable

Variants

- >2,000 variants in *CFTR* gene
  - Most are very rare and not well characterized
  - 2.6% are large insertions/deletions
  - *CFTR* is the only gene known to be causative for CF
- BAVD
  - At least one pathogenic *CFTR* variant: ~75%
    - Two pathogenic *CFTR* variants: ~20%
    - One pathogenic *CFTR* variant and one 5T variant: 25%
    - One pathogenic *CFTR* variant: 20%
    - One 5T variant: 10%
- Idiopathic pancreatitis
  - Up to 40% are predicted to have at least one pathogenic CFTR variant

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity: 99%
  - Sequencing: 97% (Strom, 2003)
  - Deletion/duplication: 2.5% (Cystic Fibrosis Mutation Database, 2011)
- Analytical sensitivity/specificity
  - Sequencing: 99%
  - MLPA: 90%

Results

- Two severe pathogenic *CFTR* variants on opposite chromosomes
  - Predicted to be affected with classic CF
- One mild pathogenic non-CF-causing variant in combination with a CF-causing variant or another mild non-CF-causing variant on the opposite chromosome
  - Increased risk for a *CFTR*-related disorder such as pancreatitis, BAVD, and respiratory disease
- Only one severe variant
  - At least a CF carrier, but may be affected if a promoter or deep intronic variant is present that was not identified
- No pathogenic variants identified
  - Unlikely to be either affected with, or a carrier of, CF

Limitations

- Diagnostic errors can occur due to rare sequence variations
- Not detected
  - Breakpoints of large deletions/duplications
  - Regulatory region and deep intronic variants
- *CFTR* gene sequencing or deletion/duplication testing may identify variants of unknown clinical significance

References

- Abeliovich D, Lavon IP, et al. Screening for five mutations detects 97% of cystic fibrosis (CF) chromosomes and predicts a carrier frequency of 1:29 in the Jewish Ashkenazi population. Am J Hum Genet. 1992;51(5): 951- 956
- Bobadilla JL, Macek M Jr, et al. Cystic fibrosis: a worldwide analysis of CFTR mutations: correlation with incidence data and application to screening. Hum Mutat. 2002;19; 575-606
- Cystic Fibrosis Mutation Database. SickKids, Toronto, Canada. 2011. (www.genet.sickkids.on.ca/ StatisticsPage.html)
- Heim RA, Sugarman EA, et al. Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation test. Genet Med. 2001;3:168-176
- Moskowitz SM, Chmiel JF, et al. Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. Genet Med. 2008 Dec;10(12):851-68
- Strom CM, Huang D, et al. Extensive sequencing of the cystic fibrosis transmembrane regulator gene: assay validation and unexpected benefits of developing a comprehensive test. Genet Med. 2003 Jan-Feb;5(1):9-14
- Sugarman EA, Rohlfs EM, et al. *CFTR* mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med. 2004;6:392-399
- Update on carrier screening for cystic fibrosis. Committee Opinion No. 486. American College of Obstetricians and Gynecologists. Obstet Gynecol. 2011;117:1028-1031
- Watson MS, Cutting GR, et al. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. Genet Med. 2004;6(5):387-391

CFTR 165 Pathogenic Variants Tested		
Legacy Name	**cDNA Name**	**Protein Name**
M1V	c.1A>G	p.Met1Val
CFTRdele2,3 (deletion of exons 2 and 3)	c.54-5940_273+10250del	Exons 2-3del
Q39X	c.115C>T	p.Gln39X
E60X	c.178G>T	p.Glu60X
P67L	c.200C>T	p.Pro67Leu
R75X	c.223C>T	p.Arg75X
➢ **G85E**	**c.254G>A**	**p.Gly85Glu**
394delTT	c.262_263delTT	p.Leu88IlefsX22 aka p.Leu88fs
405+1G>A	c.273+1G>A	**Intronic**
405+3A>C	C.273+3A>C	Intronic
406-1G>A	c.274-1G>A	Intronic
E92K	c.274G>A	p.Glu92Lys
E92X	c.274G>T	p.Glu92X
Q98X	c.292C>T	p.Gln98X
444delA	c.313delA	p.Ile105SerfsX2 aka p.Ile105fs
457TAT>G	c.325_327delTATinsG	p.Tyr109GlyfsX4 aka p.Tyr109fs
D110H	c.328G>C	p.Asp110His
R117C	c.349C>T	p.Arg117Cys
➢ **R117H**	**c.350G>A**	**p.Arg117His**
Y122X	c.366T>A	p.Tyr122X
574delA	c.442delA	p.Ile148LeufsX5 aka p.Ile148fs
➢ **621+1G>T**	**c.489+1G>T**	**Intronic**
663delT	c.531delT	p.Ile177MetfsX12 aka p.Ile177fs
G178R	c.532G>A	p.Gly178Arg
➢ **711+1G>T**	**c.579+1G>T**	**Intronic**
711+5G>A	c.579+5G>A	Intronic
711+3A>G	c.579+3A>G	Intronic
712-1G>T	c.580-1G>T	Intronic
H199Y	c.595C>T	p.His199Tyr
P205S	c.613C>T	p.Pro205Ser
L206W	c.617T>G	p.Leu206Trp
Q220X	c.658C>T	p.Gln220X
L227R	c.680T>G	p.Leu227Arg
852del22	c.720_741delAGGGAGAATGATGATGAAGTAC	p.Gly241GlufsX13 aka p.Gly241fs
935delA	c.803delA	p.Asn268IlefsX17 aka p.Asn268fs
936delTA	c.805_806delAT	p.Ile269ProfsX4 aka p.Ile269fs
F311del	c.933_935delCTT	p.Phe312del
1078delT	c.948delT	p.Phe316LeufsX12 aka p.Phe316fs
G330X	c.988G>T	p.Gly330X
➢ **R334W**	**c.1000C>T**	**p.Arg334Trp**

CFTR 165 Pathogenic Variants Tested		
Legacy Name	**cDNA Name**	**Protein Name**
I336K	c.1007T>A	p.Ile336Lys
S341P	c.1021T>C	p.Ser341Pro
1154insTC	c.1022_1023insTC	p.Phe342HisfsX28 aka p.Phe342fs
R347H	c.1040G>A	p.Arg347His
➢ **R347P**	**c.1040G>C**	**p.Arg347Pro**
R352Q	c.1055G>A	p.Arg352Gln
1213delT	c.1081delT	p.Trp361GlyfsX8 aka p.Trp361fs
1248+1G>A	c.1116+1G>A	Intronic
1259insA	c.1127_1128insA	p.Gln378AlafsX4 aka p.Gln378fs
1288insTA	c.1153_1154insAT	p.Asn386IlefsX3 aka p.Asn386fs
W401X(TAG)	c.1202G>A	p.Trp401X
W401X(TGA)	c.1203G>A	p.Trp401X
1341+1G>A	c.1209+1G>A	Intronic
IVS8 5T[a]	c.1210−12[5]	Intronic
1461ins4	c.1329_1330insAGAT	p.Ile444ArgfsX3 aka p.Ile444fs
1471delA	c.1340delA	p.Lys447ArgfsX2 aka p.Lys447fs
➢ **A455E**	**c.1364C>A**	**p.Ala455Glu**
1525-1G>A	c.1393-1G>A	Intronic
S466X(TAA)	c.1397C>A	p.Ser466X
S466X(TAG)	c.1397C>G	p.Ser466X
L467P	c.1400T>C	p.Leu467Pro
1548delG	c.1418delG	p.Gly473GlufsX54 aka p.Gly473fs
G480C	c.1438G>T	p.Gly480Cys
S489X	c.1466C>A	p.Ser489X
S492F	c.1475C>T	p.Ser492Phe
Q493X	c.1477C>T	p.Gln493X
➢ **I507del**	**c.1519_1521delATC**	**p.Ile507del**
➢ **F508del**	**c.1521_1523delCTT**	**p.Phe508del**
1677delTA	c.1545_1546delTA	p.Tyr515X
V520F	c.1558G>T	p.Val520Phe
C524X	c.1572C>A	p.Cys524X
Q525X	c.1573C>T	p.Gln525X
➢ **1717-1G>A**	**c.1585-1G>A**	**Intronic**
1717-8G>A	c.1585-8G>A	Intronic
➢ **G542X**	**c.1624G>T**	**p.Gly542X**
S549R(A>C)	c.1645A>C	p.Ser549Arg
S549N	c.1646G>A	p.Ser549Asn
S549R(T>G)	c.1647T>G	p.Ser549Arg
G551S	c.1651G>A	p.Gly551Ser
➢ **G551D**	**c.1652G>A**	**p.Gly551Asp**
Q552X	c.1654C>T	p.Gln552X

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Legacy Name	**cDNA Name**	**Protein Name**
➢ **R553X**	**c.1657C>T**	**p.Arg553X**
A559T	c.1675G>A	p.Ala559Thr
R560K	c.1679G>A	p.Arg560Lys
➢ **R560T**	**c.1679G>C**	**p.Arg560Thr**
1811+1.6kbA>G	c.1679+1.6kbA>G aka c.1679+1.6kbAG	Intronic
1812-1G>A	c.1680-1G>A	Intronic
1833delT	c.1703delT	p.Leu568CysfsX4 aka p.Leu568fs
Y569D	c.1705T>G	p.Tyr569Asp
P574H	c.1721C>A	p.Pro574His
E585X	c.1753G>T	p.Glu585X
➢ **1898+1G>A**	**c.1766+1G>A**	**Intronic**
1898+3A>G	c.1766+3A>G	Intronic
1924del7	c.1792_1798delAAAACTA	p.Lys598GlyfsX11 aka p.Lys598fs
2043delG	c.1911delG	p.Gln637HisfsX26 aka p.Gln637fs
2055del9>A	c.1923_1931del9insA	p.Ser641ArgfsX5 aka p.Ser641fs
2105-2117del13insAGAAA	c.1973_1985del13insAGAAA	p.Arg658LysfsX4 aka p.Arg658fs
2108delA	c.1976delA	p.Asn659IlefsX4 aka p.Asn659fs
2143delT	c.2012delT	p.Leu671X
2183delAA	c.2051_2052del	p.Lys684ThrfsX4
2183AA>G	c.2051_2052delinsG aka c.2051_2delinsG	p.Lys684SerfsX38
➢ **2184delA**	**c.2052delA**	**p.Lys684AsnfsX38**
R709X	c.2125C>T	p.Arg709X
K710X	c.2128A>T	p.Lys710X
2307insA	c.2175_2176insA	p.Glu726ArgfsX4 aka p.Glu726fs
L732X	c.2195T>G	p.Leu732X
2347delG	c.2215delG	p.Val739TyrfsX16 aka p.Val739fs
R764X	c.2290C>T	p.Arg764Ter
2585delT	c.2453delT	p.Leu818TrpfsX3 aka p.Leu818fs
E822X	c.2464G>T	p.Glu822X
2622+1G>A	c.2490+1G>A	Intronic
E831X	c.2491G>T	p.Glu831X
W846X	c.2537G>A	p.Trp846X
W846X(2670TGG>TGA	c.2538G>A	p.Trp846X
R851X	c.2551C>T	p.Arg851X
2711delT	c.2583delT	p.Phe861LeufsX3 aka p.Phe861fs
➢ **2789+5G>A**	**c.2657+5G>A**	**Intronic**
Q890X	c.2668C>T	p.Gln890X
2869insG	c.2737_2738insG	p.Tyr913X

CFTR 165 Pathogenic Variants Tested		
Legacy Name	**cDNA Name**	**Protein Name**
L927P	c.2780T>C	p.Leu927Pro
2942insT	c.2810_2811insT	p.Val938GlyfsX37 aka p.Val938fs
S945L	c.2834C>T	p.Ser945Leu
3007delG	c.2875delG	p.Ala959HisfsX9 aka p.Ala959fs
G970R	c.2908G>C	p.Gly970Arg
➢ **3120+1G>A**	**c.2988+1G>A**	**Intronic**
3120G>A	c.2988G>A	Intronic
3121-1G>A	c.2989-1G>A	Intronic
3171delC	c.3039delC	p.Tyr1014ThrfsX9 aka p.Tyr1014fs
3199del6	c.3067_3072delATAGTG	p.Ile1023_Val1024del aka I1023_V1024del
3272-26A>G	c.3140-26A>G	Intronic
L1065P	c.3194T>C	p.Leu1065Pro
R1066C	c.3196C>T	p.Arg1066Cys
R1066H	c.3197G>A	p.Arg1066His
L1077P	c.3230T>C	p.Leu1077Pro
W1089X	c.3266G>A	p.Trp1089X
Y1092X(C>A)	c.3276C>A	p.Tyr1092X
Y1092X(C>G)	c.3276C>G	p.Tyr1092X
M1101K	c.3302T>A	p.Met1101Lys
E1104X	c.3310G>T	p.Glu1104X
R1158X	c.3472C>T	p.Arg1158X
➢ **R1162X**	**c.3484C>T**	**p.Arg1162X**
➢ **3659delC**	**c.3528delC**	**p.Lys1177SerfsX15** **aka p.Lys1177fs**
3667del4	c.3536_3539del	p.Thr1179AsnfsX12 aka p.Thr1179fs
S1196X	c.3587C>G	p.Ser1196X
W1204X(3743G>A)	c.3611G>A	p.Trp1204X
W1204X(3744G>A)	c.3612G>A	p.Trp1204X
3791delC	c.3659delC	p.Thr1220LysfsX8 aka p.Thr1220fs
3821delT	c.3691delT	p.Ser1231ProfsX4 aka p.Ser1231fs
Q1238X	c.3712C>T	p.Gln1238X
➢ **3849+10kbC>T**	**c.3718-2477C>T**	**Intronic**
G1244E	c.3731G>A	p.Gly1244Glu
3876delA	c.3744delA	p.Lys1250ArgfsX9 aka p.Lys1250fs
S1251N	c.3752G>A	p.Ser1251Asn
S1255P	c.3763T>C	p.Ser1255Pro
S1255X	c.3764C>A	p.Ser1255X
3905insT	c.3773_3774insT	p.Leu1258PhefsX7 aka p.Leu1258fs
➢ **W1282X**	**c.3846G>A**	**p.Trp1282X**

CFTR 165 Pathogenic Variants Tested		
Legacy Name	**cDNA Name**	**Protein Name**
4005+1G>A	c.3873+1G>A	Intronic
➢ **N1303K**	**c.3909C>G**	**p.Asn1303Lys**
Q1313X	c.3937C>T	p.Gln1313X
CFTRdele22,23	c.3964-78_4242+577del	Exons 22-23del
G1343Afs	c.4028delG	p.Gly1343AlafsX4 aka p.Gly1343fs
G1349D	c.4046G>A	p.Gly1349Asp
4209TGTT>AA	c.4077_4080delTGTTinsAA	p.Val1360delfsX3 aka p.Val1360fs
E1371X	c.4111G>T	p.Glu1371X
4382delA	c.4251delA	p.Glu1418ArgfsX14 aka p.Glu1418fs
➢ **23 variants recommended for carrier screening by ACMG/ACOG** [a]The IVS8 5T variant, c.1210-12[5], will be reported when R117H is detected and in individuals who are reported to be symptomatic		