

**Heart Institute Diagnostic Lab**

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## ***CFC1, FOXH1, NODAL* and *ZIC3* Heterotaxy Syndrome**

Heterotaxy syndrome is a multiple congenital anomaly syndrome characterized by complex cardiovascular malformations and visceral situs anomalies. Autosomal recessive, autosomal dominant, and X-linked inheritance occur, although heterotaxy is most commonly sporadic. The *ZIC3* gene is a zinc finger transcription factor that causes the X-linked form of heterotaxy. The *ZIC3* gene contains 4 exons and is located at chromosome Xq26.2. *ZIC3* mutations have been identified in approximately 75% of families with X-linked heterotaxy and in 1% of sporadic patients with congenital heart disease (1,2). *ZIC3* mutations have also been identified in females with cardiovascular malformations.

*NODAL* encodes a TGF $\beta$  ligand that is critical for establishing left-right asymmetry during early development. The *NODAL* gene contains 3 exons and is located at chromosome 10q22.1. *CFC1* encodes a co-factor required for *NODAL* signaling through the TGF $\beta$  pathway to generate left-right asymmetry. The *CFC1* gene contains 6 exons and is located at chromosome 2q21.1. *FOXH1* encodes a forkhead transcription factor. The forkhead protein domain binds DNA and the C-terminal domain interacts with phosphorylated Smad proteins to mediate TGF $\beta$  signaling. The *FOXH1* gene contains 3 exons and is located at chromosome 8q24.3. Mutations in *NODAL* cause heterotaxy or heterotaxy spectrum cardiovascular malformations in up to 10% of sporadic cases (2-4). In addition to heterotaxy, mutations in these 3 laterality genes have been identified in isolated cardiovascular malformations including transposition of the great arteries (TGA), double outlet right ventricle (DORV), unbalanced atrioventricular canal defects, conotruncal defects such as tetralogy of Fallot (TOF) and septal defects in 5-10% of isolated cases (2,4,5). Mutations in these genes exhibit autosomal dominant inheritance with reduced penetrance and variable expressivity. Missense variants have been identified that appear to act as susceptibility alleles (4,6,7). Mutations are identified in one or more of these genes in a higher percent of familial cases (6). Mutations in *NODAL* pathway genes also account for approximately 1% of holoprosencephaly cases (4).

**Shipping Instructions**

Please enclose a test requisition form with sample. All information must be complete before sample can be processed. Samples may be shipped at room temperature by overnight Federal Express to arrive Monday through Friday.

**Ship To:**

Cincinnati Children's  
Hospital Medical Center  
Attn: Heart Institute Diagnostic Lab  
240 Albert Sabin Way,  
Room S4.381  
Cincinnati, OH 45229-3039

**Indication**

To identify the molecular basis of heterotaxy syndrome or related cardiovascular malformations including TGA, DORV, TOF, and atrioventricular canal defects; determination of recurrence risk, especially in cases of X-linked heterotaxy.

## Methodology:

All coding exons as well as the exon/intron boundaries and a portion of untranslated regions of the gene(s) are amplified by PCR. Genomic DNA sequences from both forward and reverse directions are obtained by automatic fluorescent detection using an *ABI PRISM® 3730 DNA Analyzer*. Sequence variants different from National Center for Biotechnology Information GenBank references are further evaluated for genetic significance. If a mutation is identified, a known familial mutation analysis will be available for additional family members.

## Sensitivity & Accuracy:

Greater than 99% of the mutations are detectable by sequence based methods. Sequencing does not detect deletions or duplications.

## References:

1. Ware SM, Peng J, Zhu L, Fernbach S, Colicos S, Casey B, Towbin J, Belmont JW. Identification and functional analysis of *ZIC3* mutations in Heterotaxy and related congenital heart defects. *American Journal of Human Genetics*. 2004;74:93-105.
2. Sutherland MJ, Ware SM. Disorders of left-right asymmetry: Heterotaxy and situs inversus. *American Journal of Human Genetics Part C Seminars in Medical Genetics*. 2009;151C:307-317.
3. Mohapatra B, Casey B, Li H, Ho-Dawson T, Smith L, Fernbach SD, Molinari L, Niesh SR, Jefferies JL, Craigen WJ, Towbin JA, Belmont JW, Ware SM. Identification and functional characterization of *NODAL* rare variants in Heterotaxy and isolated cardiovascular malformations. *Human Molecular Genetics*. 2009;18:861-871.
4. Roessler E, Ouspenskaia MV, Karkera JD, Velez JI, Kantipong A, Lacbawan F, Bowers P, Belmont JW, Towbin JA, Goldmuntz E, Feldman B, Muenke M. Reduced *NODAL* signaling strength via mutation of several pathway members including *FOXH1* is linked to human heart defects and holoprosencephaly. *American Journal of Human Genetics*. 2008;83:18-29.
5. Goldmuntz E, Bamford R, Karkera JD, dela Cruz J, Roessler E, Muenke M. *CFC1* mutations in patients with transposition of the great arteries and double-outlet right ventricle. *American Journal of Human Genetics*. 2002;70:776-780.
6. De Luca A, Sarkozy A, Consoli F, Ferese R, Guida V, Dentici ML, Mingarelli R, Bellacchio E, Tuo G, Limongelli G, Digilio MC, Marino B, Dallapiccola B. Familial transposition of the great arteries caused by multiple mutations in laterality genes. *Heart*. 2010;96:673-677.
7. Selamet Tierney ES, Marans Z, Rutkin MB, Chung WK. Variants of the *CFC1* gene in patients with laterality defects associated with congenital cardiac disease. *Cardiology in the Young*. 2007;17:268-274.
8. Bedard JE, Haaning AM, Ware SM. Identification of a novel *zic3* isoform and mutation screening in patients with heterotaxy and congenital heart disease. *PLoS One*. 2011;6:e23755.

## Specimen:

Peripheral blood in EDTA tube

Adult: 5-10mL

Child: 3-5mL

Infant: 1-3mL

For other specimen types, please contact Amy Garrison at 513-803-3317

## Turnaround Time:

Panel 4 to 6 weeks

Full Mutation Analysis (Individual genes) 2-4 weeks

Known Mutation Analysis (Individual genes) 1-2 weeks

## CPT Codes:

Full Gene Sequencing:

*CFC1* Sequencing 81479

*FOXH1* Sequencing 81479

*NODAL* Sequencing 81479

*ZIC3* Sequencing 81479

Additional Family Members 81403

