Tbx5 is a transcription factor which is required for the normal transcriptional regulation and development of the heart and limbs. Tbx5 is encoded by the TBX5 gene, which is composed of 10 alternatively spliced exons and is located at 12q24.1. Multiple mutations in TBX5 have been identified in patients with Holt-Oram syndrome, which is the most common heart and limb syndrome. Holt-Oram syndrome is characterized by congenital heart defects, including atrial or ventricular septal defects, hypoplastic left heart syndrome, mitral valve prolapse, and persistent superior vena cava, as well as bilateral forelimb deformities, which are primarily in the preaxial array. These limb defects can include upper extremity phocomelia, digit aplasia, triphalangeal thumb, and carpal bone defects. Holt-Oram syndrome demonstrates autosomal dominant inheritance with a broad range of clinical severity both within and between families. Definitive genotype/phenotype correlations have not been described.

The John Welsh Cardiovascular Diagnostic Laboratory offers molecular genetic testing for TBX5 mutations. Individuals will be tested by automatic fluorescent DNA sequencing of the 8 coding exons (exons 3-10) of the TBX5 gene. Exons 1 and 2 are non-coding. We strongly recommend initial testing of a clearly affected individual, if available, in order to provide the greatest test sensitivity and clearest interpretation of results for subsequent family members. Genetic counseling is recommended for all individuals.

**REASONS FOR REFERRAL**

- Molecular confirmation of the diagnosis of Holt-Oram syndrome

**METHODOLOGY**

Genomic DNA will be analyzed for TBX5 mutations by automatic fluorescent DNA sequencing of the 8 coding exons of the TBX5 gene, as well as the exon/intron junctions and a portion of the 3’ untranslated region. Patient DNA will be sequenced in both the forward and reverse orientations. If a mutation is identified, additional family members will be analyzed only for the familial mutation by automatic fluorescent DNA sequencing.

**SERVICE FEES**

<table>
<thead>
<tr>
<th>Index Case (Male or Female)</th>
<th>Direct and Institutional Billing</th>
<th>CPT Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$750 per sample</td>
<td>83891, 83898x9, 83904x18, 83912</td>
</tr>
<tr>
<td>Additional Family Members</td>
<td>$200 per sample; known familial mutation only</td>
<td>83891, 83898, 83904x2, 83912</td>
</tr>
</tbody>
</table>

**SENSITIVITY**

DNA Sequencing Analysis: Approximately 96% detection of mutations in exons 3-10 of TBX5

**SPECIMEN REQUIREMENTS**

- **Blood** (preferred): EDTA (purple-top) tubes: 
  - Adult: 5 cc 
  - Child: 5 cc 
  - Infant: 2-3 cc

- **Tissue**: Frozen (preferred), RNAlater, Formalin-fixed, Paraffin embedded

- **Other Body Fluids**: Call to inquire