

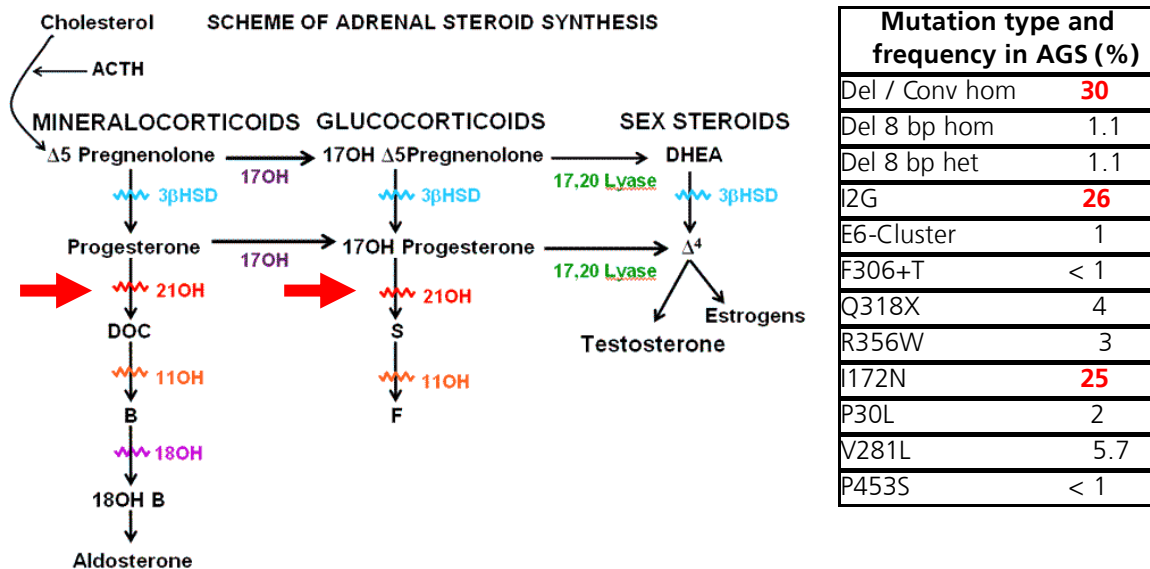
## CYP21B Steroid-21-Hydroxylase – Adrenogenital Syndrome - AGS

### Function of CYP21B

**CYP21B** – Steroid-21-Hydroxylase – is essential in adrenal steroid biosynthesis.

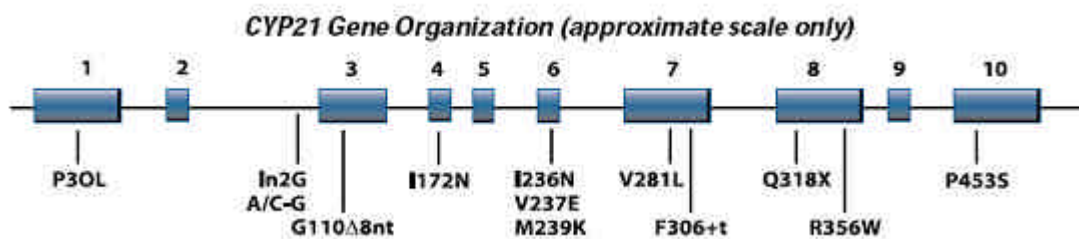
Large deletions/conversions and mutations lead to loss or impairment of enzymatic activity.

In homozygous or compound heterozygous state they lead to **Adrenogenital Syndrome**.



### Genomic organisation of CYP21B

The functional CYP21 B gene and its non-functional pseudogene CYP21A are arranged in tandem in the HLA cluster on chromosome 6. CYP21B and A are both about 3 kb in size and are > 95% homologous. Deletions, conversions and the most common mutations of the functional CYP21B gene are derived from rearrangement between the functional and the pseudogene which contains 9 of the 10 most common mutations responsible for dysfunctional 21-Hydroxylase. For analysis of CYP21B mutations, it is necessary to employ long-range PCR and primers selecting against pseudogene sequences.



## **Analysing CYP21B with Genes-4U ToolSets**

### **Step 1 : Search for presence of at least one full-length copy of the functional gene using the CYP21B fl/del ToolSet™ for LightCycler™**

The **CYP21B fl/del** ToolSet is designed for genotyping the human **CYP21B** gene (**21-Hydroxylase**) for presence of at least one full length (fl) allele versus homozygous large deletions or isolated homozygous 8 bp deletions by LightCycler PCR with Melting Curve Analysis within less than one hour.

The primer pair co-amplifies a 215 bp segment of the functional gene CYP21B and a 207 bp segment of the pseudogene CYP21A. Intact CYP21B amplicons, if present, are monitored by a LC Red 640 labelled fluorescent probe complementary to the functional gene. As internal positive control, pseudogene amplicons are monitored by a LC Red 705 labelled fluorescent probe complementary to the 8 bp deletion.

**If no full length copies are found in step 1, step 2 is not necessary (30 % of cases)**

**If a full length copy is found in step 1, continue with step 2.**

### **Step 2 : Search for the 10 most common mutations in the functional gene using the CYP21B ToolSet™ for LightCycler™**

The **CYP21B ToolSet** is designed for genotyping the functional human **CYP21B** gene (**21-Hydroxylase**) for presence of the **P30L, I2G, del8bp, I172N, E6 cluster, V281L, F306+T, Q318X, R356W and P453S** mutations by LightCycler PCR with Melting Curve Analysis within **3 hours** including about 30 minutes hands-on time.

The functional gene CYP21B is specifically amplified by primer sets selecting against pseudogene sequences yielding amplicons of  $\approx$  2kb length requiring the use of a Hot Start high fidelity polymerase with proofreading activity. A separate OligoTool with a primer set and specific detection probes is used for each mutation.

In  $\approx$  **30 %** of cases of classical adrenogenital syndrome the functional CYP21B gene is absent or grossly disturbed as a result of a large gene deletion / conversion resulting in a failure to amplify long sequences of the functional gene. It is therefore strongly advised to test a sample for large deletion / conversion before initiating analysis of point mutations and small deletions.

For this we recommend the **CYP21B fl/del ToolSet™ for LightCycler™** .

#### **References : CYP21 and Adrenogenital Syndrome**

(1) Day-JD. Detection of steroid 21-Hydroxylase alleles using gene-specific PCR and a multiplexed ligation detection reaction. *Genomics* 1995; 29 : 152-162 (PMID : 8530065)

(2) Wedell-A. Steroid 21-hydroxylase deficiency: two additional mutations in salt-wasting disease and rapid screening of disease-causing mutations. *Human Molecular Genetics*, 1993; 2(5) : 499-504 (PMID : 8518786)

Use the PMID numbers to go directly to the reference citation / abstract in PubMed (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=pubmed>)