Specifications Sanquin Molecular diagnostics for CGD (X024 + X026)

Targeted sequencing with the Ion Torrent System is able to identify single nucleotide variants, small insertions and small deletions. Variants in repeat sequences, large homopolymers and large insertions/deletions are not or difficult to identify.

The **CGD AmpliSeq Panel v1** (IAD63499 185) exists of 137 amplicons and is covering 26 Kbase. 98.1% of desired areas (exons with a 10 base exon padding and the promotor region of *CYBB*) are covered in the design that includes the following seven genes: *CYBB*, *CYBA*, *NCF1*, *NCF2*, *NCF4*, *G6PD* and *RAC2.*

Coverage of the CGD Ampliseq Panel

The percentage of target bases that is covered at least 20 times is at least 97% for the recommended Mapped Reads of 250.000. The regions that are missed, either in the design or due to practical coverage, are listed Table 1 below.

Three small regions of *G6PD* are missed in the design. However, when there are specific *G6PD* requests, our RBC Enzym AmpliSeq Panel will be used and those three regions are covered well in this panel. There are some CGD patients known to have a pathogenic variant in *G6PD* (NM_000402:c.653C>T) and this position is covered well with the CGD panel.

NCF1 has two pseudogenes and is therefore a difficult region to sequence. We decided to include both pseudogenes in the panel, to get as many positions covered as possible with as many specific and distinguishable positions as possible. However, in the regions in *NCF1B* or *NCF1C* that are not covered, there won't be any pathogenic variants. Therefore, those regions are not listed in Table 1.

There are two frequent occurring pathogenic variants known in *NCF1*. One is a cross-over between the gene and one of its pseudogenes, resulting in a two base pair deletion at the start of exon two. This variant occurs in 80% of the CGD patients with a defect in *NCF1*. Reads with this deletion won't be aligned on exon 2 of the gene, but will be aligned to the pseudogenes. This variant will be seen in the failure of coverage in exon 2. With this technique we won't be able to detect carriers for this variant. Therefore it is needed to perform a *NCF1* genescan to exclude or confirm whether a person has only one normal *NCF1* gene. This genescan will be performed by us when an X026 is requested and for familiar NCF1 requests.

The second frequent occurring pathogenic variant is a nonsense variant located in exon 7 and occurs in 8 to 10% of the CGD patients with a defect in *NCF1*. This location is covered well and gene specifically with this Ampliseq CGD-panel.

Other variants in *NCF1* are less common and difficult to find. We try to analyse the other regions of *NCF1* on a research base (possibly with another Ampliseq panel), but cannot guarantee any findings.

Part 1: Missed in newly designed regions

Gene	chromosome	coordinate start	coordinate end	Exon	% Gene covered	Missed number of bases	HGMD 2023.1	HGMD Accession
NCF1A	chr7	74202979	74202990	10	99,1	12	No	
NCF4	chr22	37272085	37272146	9	95,7	62	No	
G6PD	chrX	153761147	153761158	9		12	Yes	CM950507
G6PD	chrX	153764346	153764360	3		15	No	
G6PD	chrX	153775048	153775095	1	96,1	48	Yes	CM2120831, CM2120832
					Total bases missed:	246		

Part 2: Missed by coverage in experiments in a few samples

Gene	chromosome	coordinate start	coordinate end	Exon	% Gene covered	Missed number of bases	HGMD 2023.1	HGMD Accession
NCF1A	chr7	74193352	74193472	3		50	Yes	CM961018
NCF1A	chr7	74197338	74197341	6	95,3	4	No	
					Total bases missed:	54		

Table 1: Missed regions in design (Part 1) and possibly by practical coverage (part 2).

Read more about the technique and reporting in the background information on the Sanquin website.