Development of a Fluorescent *in situ* hybridization assay (FISH) for the diagnosis of Velocardiofacial Syndrome (VCFS) in Sri Lanka

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Velocardiofacial Syndrome is associated with orofacial clefting, congenital heart defects, and developmental delay. It is caused by a 3Mb deletion of chromosome 22q11.2. The gold standard test is FISH but using commercial probes incurs high costs which are beyond the means of most patients. This study was aimed at developing a FISH based assay. Bacterial Artificial Chromosome (BAC) clones (FISH mapped and end-sequenced) spanning a region within the deleted and control regions were identified using publicly available databases and obtained from BACPAC Resource Center, Children's Hospital Oklahoma, USA. Bacterial cultures of the BAC clones, RP11-1057H19 (173,947bp) for the target region (22q11.2), and CH17-338N2 (205,938bp) for the control region (22q13.3) were grown and plasmid DNA was prepared using optimized methods. The isolated BAC DNA was further confirmed by PCR using 3 sets of primers spanning the two regions. BAC DNA (target and control) were fluorescently labelled by degenerate oligonucleotide PCR (DOP- PCR) using Fluorescien-12-dUTP (Green) and Chroma Tide Alexa Fluor 546-14-dUTP (Orange) respectively. The labeled DNA was purified by size exclusion chromatography and a small aliquot run on an agarose gel and visualized. If necessary, the labeled DNA fragments were DNase treated using an optimized protocol to obtain labeled fragments in the range of 200-500bp. The labeled DNA probes were used to perform FISH analysis of prepared metaphase spreads from three patients whose samples had already been tested using a validated, commercial probe. One patient had a deletion in the target region while two were negative. These results were in agreement with those obtained using commercial probes. The developed FISH probe can distinguish deleted from non-deleted cases. The preliminary data supports this as a useful diagnostic test for VCFS. Analysis of more samples is ongoing.

Keywords: Fluorescent in situ Hybridization, Velocardiofacial syndrome.