

Screening of muscular disease genes with the Access Array™ System of Fluidigm and Roche's GS Junior INSTITUT FÜR HUMANGENETIK

J. E. Bach, S. Rost, A. Gehrig, C. R. Müller

Department of Human Genetics, University of Wuerzburg, Germany

INTRODUCTION

Sequencing of large genes like the *DMD* gene or of a group of genes, e.g. muscular disease genes, for many patients in parallel is a major application in our diagnostic laboratory. In addition to Sanger sequencing, we have established next generation sequencing (NGS) with the 454 GS Junior System (Roche). For target enrichment, the Access Array[™] System of Fluidigm is used

which allows parallel amplification of 48 target regions for 48 samples in one single PCR setup. By the combination of target-specific primers and individual-specific barcode primers with 454 sequencer-adaptors, sequencer-ready amplicon libraries can be produced in a single step.

METHODS AND RESULTS

The heart of the Access Array System is the 48.48 Access Array Integrated Fluidic Circuit (IFC; Figure 1) which allows massively parallel probe amplification in nanoliter volumes. This chip allows to combine 48 samples with 48 primer pairs in order to obtain 2304 (48x48) reactions in one run. Thermal cycling is performed on the chip and thereafter PCR products can be harvested as 48 pools (according to 48



Figure 1: Fluidigm's 48.48 Access Array IFC (Integrated Fluidic Circuit) with 48 sample inlets on the left and 48 primer inlets on the right side. PCR is performed on the chip in the middle of the IFC by combining each sample with each primer. PCR products are pooled sample by sample and can be harvested from the sample inlets (approx. 10µl per sample). An 8-channel pipette can be used for loading and harvesting. (Source: Fluidigm Corporation, South San Francisco, USA)

samples) of 48 amplicons each. Amplicons can be tagged with sample-specific barcode sequences and sequencer-specific adapters during PCR (Figure 2). Thus, libraries emerging by combining the 48 patients' PCR product pools, comprising 48 amplicons each, can directly be used for emulsion PCR for 454 sequencing.



Figure 2: Overview of the PCR: Region-specific primers tagged with universal tags can be combined with second primers with universal tag, barcode and sequencer-specific tag.

TS = target-specific primer; CS1 = universal tag 1, forward; CS2 = universal tag 2, reverse; BC = barcode sequence; 454A = 454 sequencer-specific forward primer; 454B = 454 sequencer-specific reverse primer. (Source: Fluidigm Corporation, South San Francisco, USA)



CS2 BC 454B

In our diagnostic lab, the combination of Access Array PCR and GS Junior sequencing is being established for the parallel screening of multiple candidate genes in patients with muscle diseases. In a first step, the *DMD* gene has been re-sequenced in a total of 48 patients in whom mutations and SNPs had already been identified by Sanger sequencing. The sequence coverage was satisfying (at least 15-fold) and all known 156 variants could be reproduced (resulting in a sensitivity of 99.8%). The *DMD* primer assays can now be applied in rou-



Figure 3: Visualization of the hemizygous stop mutation c.4483C>T, p.Gln1495X in the *DMD* gene causing Duchenne muscular dystrophy.

tine diagnostics. Figure 3 and 4 show two typical *DMD*-mutations, data were analyzed with GenSearchNGS (PhenoSystems). We now are extending the system to other genes for muscular diseases, especially the genetically heterogeneous limb-girdle muscular dystrophies (LGMD).

The effectiveness of the Access Array System can be further enhanced by performing multiplex PCR as according to Fluidigm's advanced protocol for multiplex amplicon tagging for 454 sequencing.



Figure 4: Visualization of the heterozygous intronic mutation c.1332-9A>G in the *DMD* gene leading to a new splice site and causing Duchenne muscular dystrophy.



The Access Array System is predestined for the screening of several candidate genes in numerous patients in parallel. The system seems to be particularly suitable for diagnostics as it is easy-to-use and requires only small amounts of genomic DNA and PCR reagents in order to obtain a sufficient amount of sequence data

within three days. The combination of Access Array PCR and GS Junior sequencing has proven reliability and practicability as a time and cost efficient alternative for classical PCR and Sanger sequencing.