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Exploratory genetic analysis in children with autism spectrum disorder and other developmental disorders using whole exome sequencing

Supplementary methods 2

Whole Exome Sequencing

SP SBS cartridges are thawed in a room temperature water bath for 4 hours and SP cluster cartridges are thawed in a room temperature water bath for 2 hours. Cartridge bases are dried thoroughly by blotting the foil seals dry and then the cartridge is inverted 10 times and tapped on the bench gently. Flow cell is prepared by holding it at room temperature for at least 10 minutes. ExAmp reagents (DPX1, DPX2, and DPX3) are thawed and placed on ice. Pooled library concentrations were normalized to 1.5 nM concentration by using 10 mM Tris-HCl, pH 8.5. Normalized libraries are then pooled by combining the appropriate volume of each normalized ≥ 1 nM library in a new microcentrifuge tube to result in a final volume of 18 µl. 0.2 N NaOH is prepared by diluting stock NaOH with laboratory-grade water. 4.0 µl of 0.2 N NaOH is added to the tube of 18.0 µl non-denatured library pool, caped and briefly vortexed and incubated at room temperature for 8 minutes. 5.0 µl of 400 Tris-HCl, pH 8.0 is added, capped and briefly vortexed to be placed on ice. Flow cell and dock are prepared by placing the flow cell onto the flow cell dock, placing the manifold over the flow cell and closing the clamp. ExAmp Master Mix is prepared by mixing first 126 µl of DPX1, and 18 µl of DPX 2 and then 66 µl of DPX3. The mixture is pipetted and dispensed slowly to avoid bubble formation, vortexed for 20 to 30 seconds and centrifuged at up to 280 × g for up to 1 minute. Libraries are loaded onto the flow cell. 63 µl of ExAmp master mix is added to 27 µl of denatured library pool and briefly vortexed.

Centrifuge is done at up to 280 × g for up to 1 minute. 80 µl of library/ExAmp mixture is slowly added to each manifold well taking care of the order of lanes and avoiding contact with the bottom filter of the well. Then a break of 2 minutes is done to allow the ExAmp/library mixture to reach the opposite end of each lane. Sequencing begins within 30 minutes of loading the libraries onto the flow cell. Flow cell is then loaded into the instrument and the run is set up. After the end of the run, data can be downloaded in any necessary format, including FASTQ.