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

Supplementary methods 1

Library Preparation

Library preparation was performed using Nexetera DNA Exome kit (Illumina Inc., San Diego, CA, USA), previously known as TruSeq Rapid Exome Kit. This kit is optimized in order to provide 45 Mb coverage of exome in a uniform and specific manner enabling comprehensive exome sequencing. The workflow consisted of with the following steps:

1. Library preparation
    a. Tagmentation – DNA tagging and fragmenting by a transposome
    b. First amplification by PCR
2. Enrichment
    a. Pooling 12 libraries together
    b. First hybridization and capture – hybridization of probes to target regions
    c. Second hybridization and capture - hybridization of probes to target regions
    d. Second amplification of hybridization products by PCR
3. Sequencing elution of fragments from beads

Tagmenting

TAG58 program on the thermal cycler:

- Choose the preheat lid option and set to 100°C
- 58°C for 10 minutes
- Hold at 10°C
- Each well contains 50 µl

TAG60 program on the thermal cycler:

- Choose the preheat lid option and set to 100°C
- 60°C for 5 minutes
- Hold at 10°C
- Each well contains 65 µl.

Genomic (gDNA) is quantified using a fluorometric method and gDNA is diluted in Tris-HCl 10 mM, pH 8.5 to a final volume of 10 µl at 5 ng/µl. The following items are added in the order listed to a new Hard-Shell PCR plate:

- TD (25 µl)
- Normalized gDNA (10 µl)
- TDE2 (15 µl)

Everything is shaken at 1800 rpm for 1 minute and centrifuged at 280 × g for 1 minute then placed on the preprogrammed thermal cycler to run the tagmentation program. When the sample reaches 10°C, *immediately* proceed to step 7 because the transposome is still active. 15 µl of ST is added to each well and pipetted to mix following placement on the preprogrammed thermal cycler and run the TAG60 program.

Cleaning up of Tagmented DNA

Prior to cleaning tagemented DNA:

- Bring RSB to room temperature by holding it on the bench for 30 minutes
- Bring SPB to room temperature by holding it on the bench for 30 minutes
- Prepare fresh 80% EtOH.

Total sample volume is transferred and 52 µl SPB pipetted up and down 10 times. Everything is then incubated at room temperature for 5 minutes and afterwards placed on a magnetic stand until the liquid becomes clear. 98 µl of supernatant is transferred and 137 µl of SPB added by pipetting up and down 10 times. Again, everything is incubated at room temperature for 5 minutes and placed on a magnetic stand until the liquid is clear. All supernatant is remove and discarded. Then everything is washed 2 times with 200 µl 80% EtOH. Using a 20 µl pipette, residual 80% EtOH is removed. Air-drying on the magnetic stand for 5 minutes is performed and then the tube is removed from the magnetic stand. 22.5 µl of RSB is added and shaken at 1800 rpm for 1 minute. Then incubated at room temperature for 2 minutes and Centrifuged at 280 × g for 1 minute. Again, placed on a magnetic stand until the liquid is clear. Finally, 20 µl of supernatant is transferred for downstream applications.

Amplification of Tagmented DNA

The following LAM AMP program on the thermal cycler was used:

- Choose the preheat lid option and set to 100°C
- Set volume to 50 µl
- 72°C for 3 minutes
- 98°C for 30 seconds
- 10 cycles of:
    - 98°C for 10 seconds
    - 60°C for 30 seconds
    - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 10°C

Index Adapter Plate seal is removed. Arrange Index 1 (i7) adapters in columns 1–12. Arrange Index 2 (i5) adapters in rows A–H. Plate is placed on the TruSeq Index Plate Fixture. Using a multichannel pipette, 5 µl of each Index 1 (i7) adapter to each column is added. Using a multichannel pipette, 5 µl of each Index 2 (i5) adapter to each row is added. 20 µl of LAM is added and shaken at 1200 rpm for 1 minute. Centrifuge is done at 280 × g for 1 minute. Plate is placed on the thermal cycler and the LAM AMP program is run.

Cleaning Up of Amplified DNA

Samples are centrifuged and 50 µl of total volume is mixed with 90 µl of SPB. The mixture is shaken at 1800 rpm for 1 minute and incubated at room temperature for 5 minutes. Everything is centrifuged at 280 × g for 1 minute and place on a magnetic stand until liquid is clear. All supernatant is removed and discarded. Washing 2 times with 200 µl 80% EtOH is performed prior to using a 20 µl pipette to remove residual 80% EtOH. Samples are air-dried on the magnetic stand for 5 minutes. Following removal from the magnetic stand 17 µl of RSB is added. Shaking at 1800 rpm for 1 minute is performed and then incubation at room temperature for 2 minutes. Centrifuge is performed at 280 × g for 1 minute and samples are placed on a magnetic stand until liquid is clear. 15 µl of supernatant is transferred two times and the library is quantified using the fluorometric method.

Probe Hybridization

TRE HYB program on the thermal cycler:

- Choose the preheat lid option and set to 100°C
- 95°C for 10 minutes
- 58°C for 30 minutes
- Each well contains 10 µl

Each DNA library is combined, making sure that each library has a unique index. If the total volume is > 30 µl, the pooled sample is concentrated to 30 µl. If the total volume is < 30 µl, the volume is increased to 30 µl with RSB. 500 ng of each DNA library quantified by QuantiFluor is used and the following is added to a new plate:

- Library pool (30 µl)
- BLR (10 µl)
- CEX (10 µl)

Everything is shaken at 1200 rpm for one minute and centrifuged at 280 × g for 1 minute.125 µl of SPB is added and shaken at 1800 rpm for one minute. The mixture is incubated at room temperature for 10 minutes and centrifuged at 280 × g for one minute. Then it is placed on a magnetic stand until the liquid is clear. All supernatant is removed and discarded. Pellet is washed 2 times with 200 µl 80% EtOH and using a 20 µl pipette, residual 80% EtOH is removed. Air-

drying on the magnetic stand for 10 minutes is performed and then the sample is removed from the magnetic stand. 7.7 µl EHB1 is added and shaken at 1800 rpm for 1 minute. Everything is incubated at room temperature for two minutes and centrifuged at 280 × g for one minute. Again, placed on a magnetic stand until the liquid is clear. 7.5 µl of supernatant is transferred and 2.5 µl of EHB2 is added. after shaking at 1800 rpm for 1 minute, the mixture is centrifuge at 280 × g for one minute and placed on the thermal cycler to run the TRE HYB program.

Hybridized Probes Capture

A micro heating system with a midi plate is preheated and inserted to 50°C. Centrifugation is done and all samples are transferred. 250 µl of SMB is added and shaken at 1200 rpm for five minutes, then incubated at room temperature for 25 minutes. Centrifuge is performed at 280 × g for 1 minute. The mixture is placed on a magnetic stand until the liquid is clear. All supernatant is discarded, and the pellet is removed from the magnetic stand. 200 µl is added to each well and shaken at 1800 rpm for 4 minutes. Pipetting is done to resuspend the bead pellet further. The plate is placed on the 50°C micro heating system with the lid closed for 30 minutes and then on a magnetic stand until the liquid is clear. All supernatant is removed and discarded and then removed from the magnetic stand. The previous steps are repeated for a total of two washes. 28.5 µl EE1 and 1.5 µl HP3 are mixed, and then vortexed. 23 µl elution premix is added and everything is shaken at 1800 rpm for two minutes then incubated at room temperature for 2 minutes and centrifuged at 280 × g for 1 minute. Again, placed on a magnetic stand until the liquid is clear. 21 µl of supernatant is transferred and 4 µl of ET2 added and then shaken at 1200 rpm for 1 minute and centrifuged at 280 × g for 1 minute.

Second Hybridization

BLR (10 µl) and CEX (10 µl) are added and shaken at 1200 rpm for 1 minute then centrifuged at 280 × g for 1 minute.125 µl of SPB is added and shaken at 1800 rpm for 1 minute then incubated at room temperature for 10 minutes. Placing on a magnetic stand until the liquid is clear is done following centrifuge at 280 × g for 1 minute. All supernatant is removed and discarded. Pellet is washed 2 times with 200 µl 80% EtOH and, using a 20 µl pipette, residual 80% EtOH is removed. Air-drying is done on the magnetic stand for 10 minutes. Mixture is removed from the magnetic stand. 7.7 µl of EHB1 is added and shaken at 1800 rpm for 1 minute, then incubated at room

temperature for 2 minutes. Centrifuge is done at 280 × g for 1 minute. Mixture is placed on a magnetic stand until the liquid is clear. 7.5 µl of supernatant is transferred and 2.5 µl of EHB2 added. Shaking is done at 1800 rpm for 1 minute and centrifuge at 280 × g for 1 minute. The plate is placed on the thermal cycler to run the TRE HYB program.

Second Capture

A micro heating system with midi plate is preheated and insert to 50°C. Centrifuge is done prior to transferring 10 µl of supernatant. 250 µl of SMB is added and shaken at 1200 rpm for 5 minutes, then incubated at room temperature for 25 minutes. Centrifuge is done at 280 × g for 1 minute and the mixture is placed on a magnetic stand until the liquid is clear. All supernatant is removed and discarded and the pellet removed from the magnetic stand. 200 µl of EEW is added and shaken at 1800 rpm for 4 minutes with pipetting to resuspend the bead pellet further. Plate is placed on the 50°C micro heating system with the lid closed for 30 minutes and then placed on a magnetic stand until the liquid is clear. All supernatant is removed and discarded and then removed from the magnetic stand. The previous steps are repeated for a total of two washes. 28.5 µl EE1 and 1.5 µl HP3 are mixed, and then vortexed. 23 µl elution premix is added and everything is shaken at 1800 rpm for two minutes then incubated at room temperature for 2 minutes and centrifuged at 280 × g for 1 minute. Again, placed on a magnetic stand until the liquid is clear. 21 µl of supernatant is transferred and 4 µl of ET2 added and then shaken at 1200 rpm for 1 minute and centrifuged at 280 × g for 1 minute.

Cleaning Up of Captured Library

45 µl of SPB is added and shaken at 1800 rpm for 1 minute then incubated at room temperature for 5 minutes and then centrifuged at 280 × g for 1 minute. Everything is placed on a magnetic stand until the liquid is clear. All supernatant is removed and discarded. Pellet is washed 2 times with 200 µl 80% EtOH and, using a 20 µl pipette, residual 80% EtOH is removed. Air-drying is done on the magnetic stand. Mixture is removed from the magnetic stand. 27.5 µl of RSB is added and shaken at 1800 rpm for 1 minute, then incubated at room temperature for 2 minutes. Centrifuge is done at 280 × g for 1 minute. Mixture is placed on a magnetic stand until the liquid is clear. 25 µl of supernatant is transferred.

Amplification of Enriched Library

AMP10 program on the thermal cycler:

- Choose the preheat lid option and set to 100°C
- 98°C for 30 seconds
- 10 cycles of:
    - 98°C for 10 seconds
    - 60°C for 30 seconds
    - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 10°C
- Each well contains 50 µl.

5 µl OF PPC is added and centrifuged at 280 × g for 1 minute. Everything is placed on the thermal cycler to run the AMP10 program.

Cleaning Up of Amplified Enriched Library

Centrifuge is done at 280 × g for 1 minute and then incubated for 5 minutes before repeating the same centrifuge. Everything is placed on a magnetic stand until the liquid is clear. All supernatant is removed and discarded. Pellet is washed 2 times with 200 µl 80% EtOH and, using a 20 µl pipette, residual 80% EtOH is removed. Air-drying is done on the magnetic stand. Mixture is removed from the magnetic stand and incubated at room temperature for 2 minutes. Centrifuge is done at 280 × g for 1 minute. Mixture is placed on a magnetic stand until the liquid is clear. Supernatant is transferred.

Checking of Enriched Libraries

Quantifying Libraries

Quantify using the Qubit dsDNA Assay Kit.

Assessing Quality

If the concentration is higher than the quantitative range for the High Sensitivity DNA kit, dilute the library 1:10 with RSB. Run 1 of the pooled library.