Toward the genome analysis in single neurons: whole genome amplification from single cells and its application to the SNP array analysis

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Summary

Recently, genomic differences within neurons, such as aneuploidy and retrotransposition, have been proposed to contribute to neuronal complexity. Low-level of aneuploidy was reported in the normal human brain, and higher level of aneuploidy was proposed to be involved in the neuropsychiatric diseases such as schizophrenia, autism, and Alzheimer disease. Considering that almost all neurons are postmitotic, conventional cytogenetic techniques such as FISH have the limitations in the reproducibility and resolution. For example, frequency of aneuploidy in human brain was inconsistent depending on the method employed (0.4% to 4% per chromosome). On the other hand, technical difficulties have hampered genomic studies in the single cells because many analytic techniques require considerable amounts of genomic DNA (gDNA).

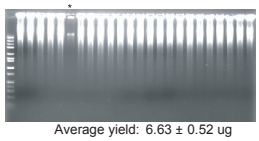
Whole-genome amplification (WGA) methods and their application to single cells have been developed to overcome this obstacle. However, single-cell WGA (S-WGA) methods are notorious for strong amplification bias such as the failure of amplification of one of the two alleles (allele dropout, AD) and excess amplification of one allele or unequal amplification of the two alleles (preferential amplification, PA). Currently, our knowledge of amplification bias, such as frequency, degree, and preference for genomic location in the S-WGA products, is very limited, and their effects on down-stream analyses have been poorly recognized.

Here we performed a high-density oligonucleotide SNP array analysis of the multiple displacement amplification (MDA)-based S-WGA products. MDA-based WGA with a phi29 DNA polymerase, which yielded the less-biased and longer (>10kb) amplified products (See Poster 4P-1024 for detail)
In addition, SNP arrays can obtain both genotype and chromosomal copy number (CCN) information. We examined the nature of the amplification bias and assessed its effect on the CCN analysis. In addition, we established an S-WGA protocol from isolated neuronal and non-neuronal nuclei from human brains, and performed CCN analysis using an optimized method.

1. Single cell whole genome amplification procedure

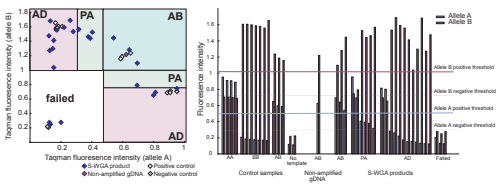
[GenomiPhi v2 kit]
Single cells were transferred into a PCR tube containing 3 ul of sample buffer.
- 1.5 ul of lysis buffer 2 (0.6M KOH, 10mM EDTA, and 100mM DTT)
- 30℃ for 10min.
- 1.5 ul of neutralizing buffer 2 (4:1 mixture of 1M Tris-HCl pH8.0, and 3M HCl)
- 4.0 ul of sample buffer,
- 10 ul of amplification mix (reaction buffer:enzyme mix = 9:1)
- 30℃ for 4 hours
- 65℃ for 10 min.





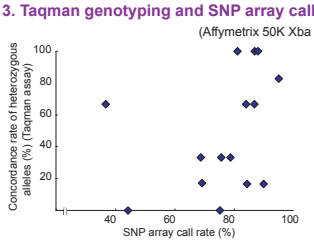
Average yield: 6.63 ± 0.52 ug

2. Taqman genotyping of the S-WGA products



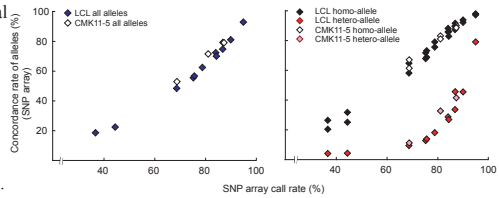
- Genotyping was performed with regard to the 6 heterozygous SNPs in the non-amplified gDNA.
- Based on the intensities of control samples, positive and negative allele thresholds were determined.
- Genotype of S-WGA product was determined by the thresholds and classified them into allele dropout (AD), preferential amplification (PA), heterozygous (AB), and failed.

3. Taqman genotyping and SNP array call rate



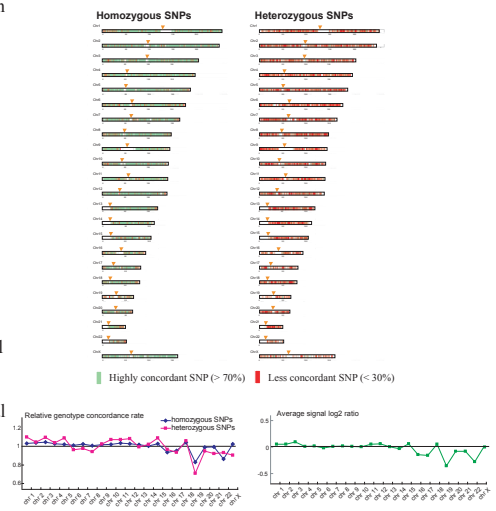
- Both PA and AB genotypes were considered to be concordant
- The results of Taqman genotyping modestyl correlated with SNP array call rate.

4. SNP array call rate and global genotype concordance



SNP array call rate strongly correlate with global genotype concordance

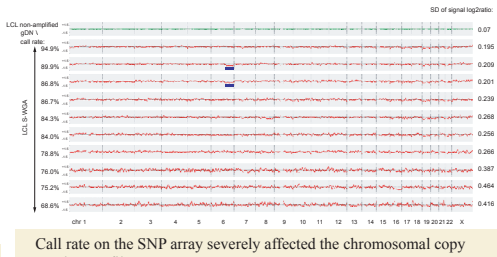
5. Genomic location of AD and insufficeint amplification



	centromere	telomere (p arm)	telomere (q arm)	other region
# of homozygous SNPs	3,678	2,202	2,581	33,665
concorance (average ± SD)	81.8± 18.1	76.1± 23.3	79.4± 20.7	82.7±16.7
P value	0.004	<0.001	<0.001	
# of SNPs	5,163	3,455	3,060	46,935
signal log2 ratio (average ± SD)	-0.004 ± 0.170	-0.054 ± 0.171	-0.124 ± 0.203	0.017 ± 0.122
P value	<0.001	<0.001	<0.001	

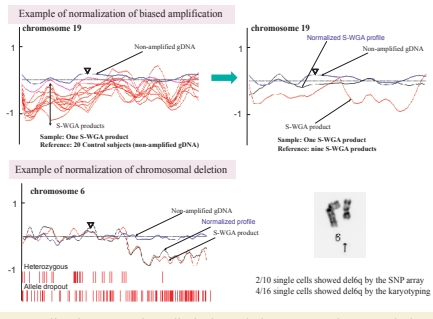
- Concordance was determined by comparing 10 S-WGA SNP array data and non-amplified gDNA SNP array data
- AD occured in a stochastic manner throughout genome.
- Regions close to telomers or centromere, and some chromosomes such as 19 and 22 tended to be insufficiently amplified.

6. Chromosomal copy number analysis in the S-WGA products



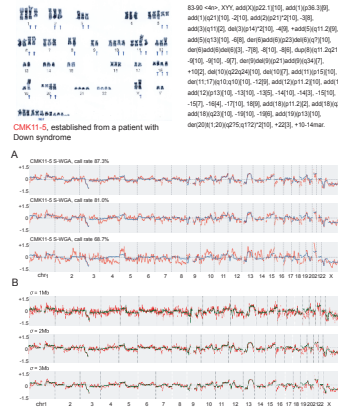
Call rate on the SNP array severely affected the chromosomal copy number profiles

7. Normalization procedure and chromosomal deletion



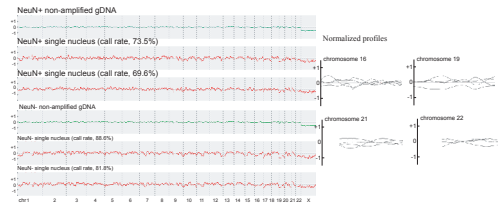
Normalization procedure disriminated chromosomal strutural alterations from biased amplification

8. CCN analysis in the single cells with complex karyotype



- S-WGA and SNP array analysis allowed precise CCN analysis in the cells with complex karyotype
- Consistent copy number profiles can be obtained in the SNP arrays with about 80% of call rate, and a genomic smoothing size of 2 to 3 Mb in the CNAT4.1 software.

9. CCN analysis of the single neuronal and non-neuronal nuclei



- Sorted nuclei were suitable for both S-WGA and FISH analysis (See Poster 4P-1023 for detail)
- Single X chromosome was clearly identified in each array data

Results and Discussion

1. The large variability in amplification bias among S-WGA products, and the severe effect of amplification bias on the CCN analysis, suggest that the quality of S-WGA products should be critically assessed before starting the down-stream analyses. Extensive Taqman genotyping of the heterozygous SNPs with consideration for PA will be effective in screening for the products with potentially high quality.

2. Insufficient genome amplification was found in the regions close to telomeres or centromeres. Certain chromosomes such as 19 and 22 showed weak signal ratio. By contrast, AD occurred throughout the genome in a stochastic manner.

3. SNP array analysis allowed us not only to carry out high-resolution CCN analysis but also to apply critical quality assessment using genotype information. Our approach of two-step examination of the candidate regions for chromosomal alterations will effectively discriminate CCN alterations from biased amplification

4. We succeeded in the S-WGA and SNP array experiments in the human neuronal and non-neuronal nuclei isolated from postmortem brain.

5. Systematic and quantitative approach with S-WGA followed by the SNP arrays will provide the entire picture of aneuploidy or other possible genomic differences in the human brain.

Acknowledgments

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Reference

Iwamoto K et al. PlosONE *in press* Detection of chromosomal structural alterations in the single cells by SNP arrays: a systematic survey of amplification bias and optimized workflow