

The University of Hong Kong Genome Research Centre Affymetrix Human Mapping GeneChip® Service

The GRC offers convenient and subsidized services and expertise to HKU investigators to facilitate genomics research and discovery. Basic pricing for our subsidized services is adjusted periodically to reflect operational and material cost from our suppliers. The GRC welcomes large projects and will be happy to discuss experimental design and cost management with investigators. Non-HKU investigators are also welcomed; please enquire.

General Conditions and Policy

1. The customer will be charged for all GeneChips® ordered as indicated at the bottom of this form.
2. Unless other arrangements are made, charges will be invoiced monthly for GeneChip® data delivered.
3. As with any technology, microarray experiments carry an inherent failure rate. While GRC will employ all necessary quality control and quality assurance steps that are in accordance with established in-house and supplier protocols (Affymetrix) and will make the best faith effort to produce quality results, the GRC cannot be held liable for any failure due to sub-optimal sample quality or any unforeseeable reason.
4. In the case of lower call rates (<92.5% as for the Human Mapping 500K GeneChip®), Affymetrix had previously replaced the product. However, under their current policy, Affymetrix will replace GeneChips® only AFTER we have demonstrated that the problem was solely due to poor manufacturing quality. Please refer to **Appendix A** for details of replacement policy. Affymetrix may change their replacement policy in future and the GRC will advise our clients accordingly.
5. To enable clients to make an assessment of GeneChip® results from our Centre, GRC has provided performance data and error estimation based on data processed from over 800 representative GeneChips®. We also demonstrate that call rates of 90% or lower are still highly informative. Please refer to **Appendix B** for details.
6. The above conditions and policy are subject to internal review from time to time and may be subject to change.
7. Please note clients will be invoiced for 50% of the total charges at the beginning of the project to cover part of the chip and reagent costs from suppliers. The remaining 50% balance will be invoiced once the data is available.

Service Agreement

By signing below, I agree to the above policy, and the financial commitment of a service order consisting of _____ Affymetrix _____ GeneChips® at current pricing of HK\$_____.

Name:

Position:

Department:

Institute: The University of Hong Kong

Billing or Cost-Center Number

Date: _____ Signature: _____

APPENDIX A

Investigation of manufacturing quality of GeneChip®

Based on past experience at GRC, a small proportion of GeneChips® do come with sub-optimal manufacturing quality, and quality seems to be lot-dependent. Affymetrix has agreed to a GeneChip® replacement cut-off of <92.5% for the Human Mapping 500K GeneChip® (consisting of 2x250K chips). The new 5.0 and 6.0 Gene Chips® are subject to a similar replacement procedure. The GRC will advise our clients of the exact details once it is established.

If you suspect you have come across a poor quality GeneChip® leading to a call rate lower than the designated cut-off (according to type of chip), GRC will kindly offer assistance to investigate the possibility of a replacement GeneChip®.

The procedures, as requested by Affymetrix, are as follows:

1. The customer should put in a written request to GRC and identify the GeneChip(s) to be investigated with the sample ID(s) and the job order number. The request should be made within one month from data delivery.
2. GRC will then obtain new GeneChip(s), either from our inventory, or purchase from Affymetrix.
3. GRC will retrieve the hybridization mix used for the GeneChip(s) in question from our archive and re-hybridize onto the new GeneChip(s).
 - a) if the new call rate upon re-hybridization to a new GeneChip® is above 92.5%, Affymetrix will provide a replacement GeneChip® to GRC at no cost. No additional cost will be charged to the customer including personnel cost for this re-hybridization.
 - b) if the call rate is still below 92.5%, the customer is required to pay for the additional GeneChip® cost.

Please be reminded that poor GeneChip® manufacturing quality is not the sole reason for lower call rates. Sub-optimal DNA sample quality can also lead to lower call rates.

Before requesting a re-hybridization, we strongly recommend you to consult **Appendix B** to assess your data quality and re-evaluate your quality requirement.

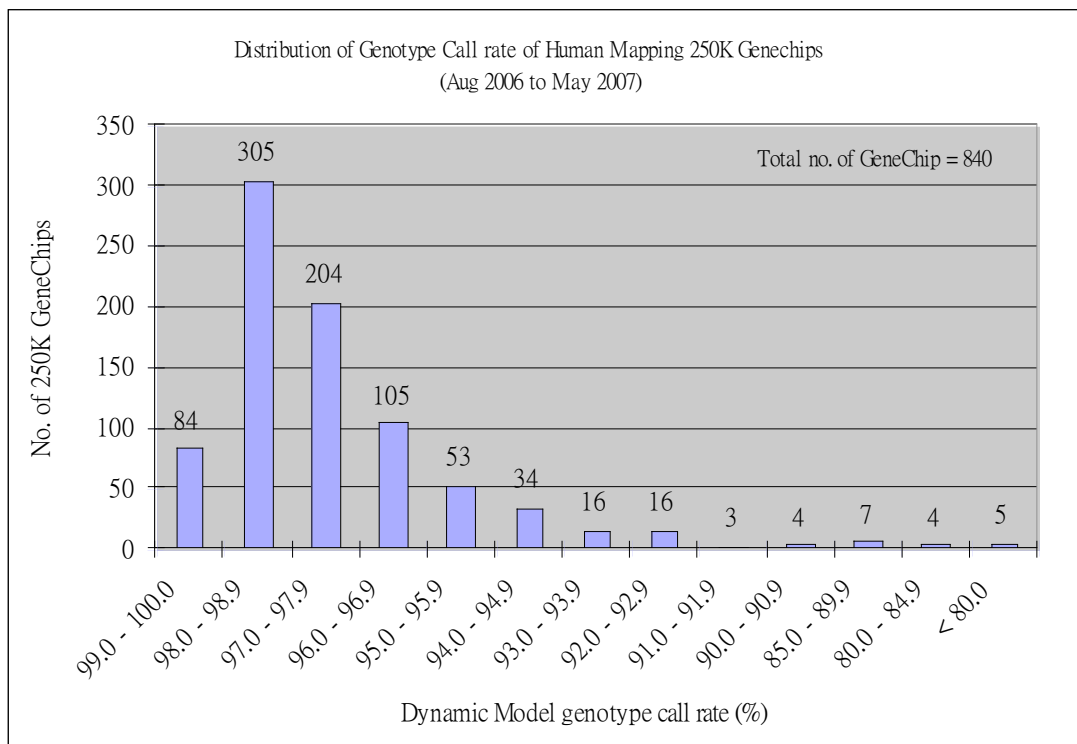
If you still have concern with data quality, you may also contact us directly for further discussion.

APPENDIX B

Affymetrix 500K GeneChips Data Quality Issues

In an ideal world each GeneChip® should achieve 100% genotype call rate and have no genotyping errors. In practice, experimental data are always imperfect to some degree, and it is important for researchers to be able to assess the quality of the genotype data of each GeneChip®, and to have an appreciation of the impact of genotype data quality on study design and data analysis. That said, GRC will make every effort to achieve the highest data quality for our customers.

Overview of Human Mapping 500K GeneChip® (2X250K) call rate at GRC

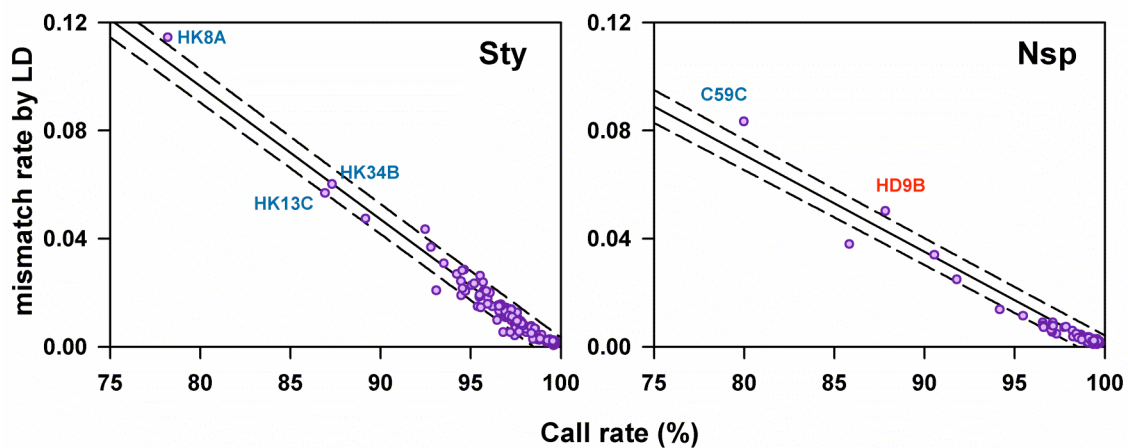


Evaluation of genotype data quality

The GRC has developed an online system, called ADAM, for the management of Affymetrix 500K genotype data. ADAM incorporates two genotype-calling algorithms, DM and BRLMM. Comparative studies of the two algorithms have shown BRLMM to be the superior algorithm, with generally higher genotype call rates and lower genotyping error rates. ADAM generates a BRLMM genotype call-rate for each GeneChip® as one index of genotype data quality.

Another index of genotype data quality is the rate of genotyping errors. ADAM generates an estimate of genotyping error rate by considering the pairs of linked SNPs which are in perfect linkage disequilibrium ($R^2=1$) in all four HapMap samples. Since these pairs of SNPs are also likely to be in perfect (or at least very strong) linkage disequilibrium in any population, “mismatches” between the two members of a pair are very likely to represent a genotyping error of either SNP. An estimate of the genotyping error rate of a GeneChip® is therefore given by the proportion of “mismatches” among these SNP pairs. This is a second index of the genotype data quality of a GeneChip®, and can be generated by ADAM.

Analyses of GeneChip® data generated in the GRC indicate a nearly perfect linear relationship between the two indices of genotype data quality; the higher the BRLMM genotype call rate, the lower the estimated genotyping error rate.



From the above data, it is clear that the estimated genotyping error rate is under 6% even when the genotype call rate is as low as 75%. When the genotype call rate is 90%, the estimated genotyping error rate is only around 2%.

Effects of genotype data quality on association analysis

One potential consequence of poor data quality is false positive association. However, this would only arise if case and control samples have very different levels of data quality, leading to artificial differences in allele frequencies. This can be avoided in the design stage by “balancing” the ratio of cases and controls in each batch of samples subjected to genotyping, or in the analysis stage by matching or adjusting for data quality.

Another potential consequence of poor data quality is reduction in statistical power. Since information for case-control association is linearly related to sample size, the expected contribution of a GeneChip® to the information should be proportional to the genotype call rate. However, the situation is likely to be more favorable because information on missing genotypes is often available from neighboring SNPs, unless the SNP with missing genotype is a “loner” with has no appreciable linkage disequilibrium relationships. Therefore, when the genotype call rate is 90%, the actual proportion of information retained is certainly more than 90%, probably at least 92.5%, provided that the analysis takes advantage of linkage disequilibrium information to “impute” missing genotypes.

The impact of genotyping errors is also less than might be anticipated. In general, genotyping errors tend to bias the sample allele frequency to 0.5, and reduce the allele frequency difference between cases and controls. In analytic studies by the GRC, we have shown that when the genotyping error rate is 20%, this results in loss of approximately half of the information for association. For a genotyping error rate of 2%, the loss of information is only around 7.5%.

The implication of these considerations is that, for a GeneChip® with a low genotype call rate of 90% and an estimated genotyping error rate of 2%, at least 85% of the information for association is retained. This means that, given the choice of using limited resources to repeat GeneChips® of this level of quality, or to genotype new samples, it is usually more cost-effective to genotype new samples to increase the overall sample size.