Semi-automated high-throughput microbial DNA extraction protocol for population-scale mutation analysis

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At RIKEN Nishina Center for Accelerator-Based Science, one active area of research is the application of heavy-ion beams to mutation breeding, where we utilise next-generation sequencing and the massively parallel supercomputer "HOKUSAI" to characterise irradiation-induced DNA damage and subsequent genetic mutations statistically. In recent years, we have demonstrated the efficacy thereof using various model organisms, efficient identification of mutations through whole-genome and exome sequencing in *Nicotiana tabacum* L., and the applicability of the redundant sequence elimination algorithm in analysing data obtained from a complex genome such as wheat. We accordingly developed a versatile and high-accuracy mutation-detection pipeline.

Historically, only indirect measurements such as the survival rate and albino emergence rate were available to estimate the mutation-induction efficiency approximately. Whole-genome sequencing (WGS), on the other hand, enables one to count the exact number of mutations in each sample, which presents two crucial advantages: (i) The smaller error margin enables a more sensitive differentiation of the mutation-induction efficiency. (ii) Significantly fewer samples are required to estimate the optimal experimental condition compared to conventional indirect methods.

Therefore, we began testing the WGS-based mutation-induction optimisation methodology against the survival-rate-based estimates using budding yeast with a small genome size. However, investigating the multitude of irradiation conditions (ion type, linear energy transfer, and irradiation dose) and genetic backgrounds (wild type and DNA-repair deficient mutants), where all combinations thereof require several (at least 10) samples, necessitates the simultaneous cultivation of cultures on a considerable scale whilst maintaining the overall pace of execution to control for their time-sensitive nature properly.

To overcome this challenge, we devised a series of techniques to achieve efficiency based on the knowledge that 500 μL of culture per sample is sufficient for DNA extraction and sequencing. This small volume requirement permits us to use 96 deep-well plates with significantly high density (a desktop incubator can cultivate up to 384 samples) and higher ease of manipulation than conventional test tubes. Moreover, after centrifugation and a brief lysis procedure, we can directly transfer them onto the automated liquid-handling robot (OT-2, Opentrons) in a smooth production-line-like manner. Eliminating the necessity to pipette the liquid culture from one tube to another ensures that the entire volume of the obtained culture proceeds for DNA extraction, reducing human labour and risks of cross-contamination. The 96 deep-well plates we tested can also be used for cryogenic storage of the liquid culture.

Hence, we tested the yeast DNA extraction through the automated procedure. Following trial and error, we verified the robot's reliability to automatically execute a protocol consisting of (i) cell-wall digestion with Zymolyase, (ii) extraction of DNA using magnetic beads, and (iii) elution of the extracted DNA. Although the reagents must be carefully prepared in advance, we estimate that the combined use of 96 deep-well plate cultivation and robot DNA extraction would require as little human labour as approximately half an hour in all to prepare up to 192 DNA samples per day, in comparison with the 4.5 hours including active and standby time required for the manual alternative. Figure 1 presents an example of concentrations in ng/μL of the DNA thereby extracted across the well plate. From past trials, we know that 700 ng is adequate for sequencing. Thus, when the final elution volume is specified as 100 μL, the minimum concentration of 7 ng/μL suffices.

	1	2	3	4	5	6	7	8	9	10	11	12
A	15.5	20.3	22.9	12.3	24.1	17.0	19.4	20.5	15.8	15.5	15.5	21.9
B	15.6	17.7	21.9	16.1	21.2	21.4	21.8	23.0	20.6	15.5	18.3	22.9
C	16.8	19.5	21.1	13.6	21.5	19.7	16.5	15.0	16.4	14.4	18.5	21.2
D	9.9	19.6	23.3	14.2	19.5	21.2	19.0	20.1	19.5	14.6	18.5	24.1
E	16.5	19.4	25.3	7.4	24.3	17.5	14.1	17.1	15.0	13.5	18.1	22.8
F	17.0	19.9	24.9	20.6	28.4	7.3	19.6	18.2	14.4	14.5	15.5	24.0
G	15.2	16.5	22.8	13.3	16.1	19.8	18.2	20.6	16.2	16.1	13.0	21.0
H	17.5	16.1	25.5	14.7	19.2	22.8	20.9	22.4	22.1	17.8	17.6	20.7

Fig. 1. Example distribution of extracted DNA concentration in ng/μL on a grid corresponding to the 96 deep-well plate. The blue-red bi-colour scale represents the increasing yield.

At the time of writing this manuscript, we had acquired approximately 700 distinct yeast DNA samples following irradiation with carbon, argon, and iron beams, the WGS data of which are presently being analysed. Our current objectives are to establish the population-scale WGS-based mutation analysis protocol as a more efficient and accurate method to advance our understanding of heavy-ion beam-induced mutations, DNA repair mechanisms, and their applications to mutation breeding technology, and to develop statistical models to efficiently learn about and infer from the newly acquired rich source of genomic data.

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