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ScienceDirect
Forensic Science International: Genetics Supplement Series 1 (2008) 7–8
www.elsevier.com/locate/FSIGSS

Research article

Reduce optimisation time and effort: Taguchi experimental design methods

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Received 14 August 2007; accepted 7 October 2007

Abstract

Development and validation of new methods and technologies frequently require long periods of time and high costs to determine the optimal system. A commonly used approach to optimisation is the factorial method, where each variable is tested at every level of the other variables. An alternative approach is to modify the experimental design using a multifactorial approach. The Taguchi design method utilises orthogonal arrays, which distribute the variables in a balanced manner, thus greatly reducing the number of experiments required. We applied the Taguchi experimental design method to PCR optimisation, and significantly reduced the number of reactions required to create highly successful reactions. We found the Taguchi design method a valuable tool for the optimisation of multifactor experiments.

Keywords: Experimental design; Optimisation; Taguchi

1. Introduction

The optimisation of new processes can be one of the most time consuming activities during product or method development. High costs for equipment, reagents and staff time can slow the process of optimisation, and may hinder laboratories developing and implementing new methodologies. For small procedures, involving a limited number of variables at few levels, the factorial strategy is commonly used. This sequential approach tests every level of every variable (referred to as factors) against every level of every other factor. This approach increases the likelihood of finding the exact optima for each factor, and is powerful at discovering interactions between factors [1]. However, complex optimisations, those involving four or more factors, each at three or more levels, require an increasing number of experiments using this approach. An alternative approach uses orthogonal arrays to reduce the number of experiments required, whilst avoiding a reduction in power that comes with using partial or fractional factorial designs [1].

A commonly used orthogonal method is the Taguchi design [1]. Extensively used for engineering process optimisation, it incorporates one primary experiment to study the main effects of each factor, and to model some of the important interactions [2]. Secondary Taguchi arrays can then be designed from the primary results, to narrow the optimal windows for each factor. The strength of Taguchi methodology lies in the orthogonal array design (Table 1). Each level (A–D) of each factor (1–4) occurs in an equal number of times across the entire array. When compared to factorial design, the possible savings are apparent—the same number of factors and levels examined with factorial design would require 256 experiments, whereas with Taguchi only 16 are needed.

An example of a complex process with a high number of variables and levels is the optimisation of PCR primers [3]. Commonly, a sequential adaptive approach is used, which can take many reactions and substantial amounts of time to find the best combination of reagents and cycling parameters. However, utilising a design strategy, such as Taguchi, can result in faster optimisation.

2. Materials and methods

Eight PCR primer pairs for STR loci required optimisation. Therefore, a $L_{16}(4^4)$ orthogonal array was designed for primer

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doi:10.1016/j.fsigss.2007.10.050

Table 1
Taguchi orthogonal array ($L_{16}(4^4)$ design)

Factor	Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	A	A	A	A	B	B	B	B	C	C	C	C	D	D	D	D
2	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
3	A	B	C	D	B	A	D	C	C	D	A	B	D	C	B	A
4	A	B	C	D	C	D	A	B	D	C	B	A	B	A	D	C

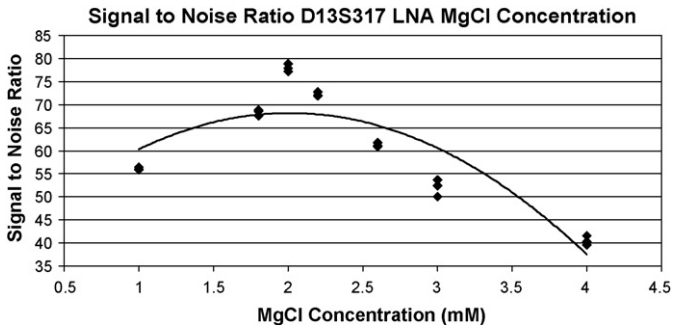

Fig. 1. Signal to noise ratios for D13S317 LNA $MgCl_2$ concentration, Taguchi array 1 and 2 (data combined). The optimal value selected from this polynomial curve was 2 mM.

concentration (0.2–0.8 μM), $MgCl_2$ concentration (1–4 mM), annealing temperature (50–65 °C), and template DNA concentration (5 pg to 1 ng). The 16 Taguchi reactions per primer pair were performed in triplicate, with peak height, as measured with an ABI 3100 Genetic Analyser and GeneMapper ID (Applied Biosystems), taken as the response variable.

Results were analysed using both level average analysis and signal to noise (SNR) calculations [3]. Level average analysis involves the calculation of the average effect of each factor (for example, the effect of factor 1, level A is calculated by averaging experiments 1–4 in Table 1). However, SNRs give more power, and were used to determine the optimal values for each factor. The SNRs are a quadratic loss function, calculated using the equation: $\mathrm{SNR} = -10 \log [(1/n)\sum(1/y^2)]$, where n = number of levels, and y = the yield of the individual reactions. The average SNR values for each level of each factor were used for polynomial regression, and optimal ranges could be selected based on the curve generated. From this preliminary array information, a smaller secondary $L_9(3^3)$ Taguchi array was designed to provide confirmatory testing of the initial Taguchi results, and to narrow the optimal windows further.

3. Results

The initial $L_{16}(4^4)$ Taguchi array identified the optimal ranges for each factor, with replicates showing low variability, and significant differences between the factor levels. The SNRs proved to be powerful for identifying the differences between the levels of each factor. In combination with polynomial regression, finding the optimal level of each factor proved simple (Fig. 1). The second Taguchi array allowed the narrowing of the optimal windows for each factor, and resulted in highly optimised, successful PCRs for each primer pair.

The Taguchi array method allowed the optimisation of eight new primer pairs with high efficiency and accuracy. In total, only 25 reactions were required for each primer pair, considerably less than usually required. Optimisation testing was completed in only 2 weeks, with significantly less expense than required for standard factorial design methods.

4. Conclusions

Taguchi orthogonal array design provides a fast and efficient method for the optimisation of PCRs. Optimum levels for many different parameters can be simultaneously discovered. A significant advantage of Taguchi methods is the reduction in time and cost—using standard factorial design, the cost for this optimisation series, at A$4 a reaction for consumables only, would be over A$26,000, whereas with Taguchi, the total cost was A$2300. Therefore, we recommend the use of Taguchi experimental design methods for multifactor process optimisation, such as PCR optimisation.

Acknowledgements

The authors are grateful to the Australian Research Council and Victoria Police Forensic Services Department for providing funding for the study.

Conflict of interest

None.

References

[1] J. Antony, Taguchi or classical design of experiment: a perspective from a practitioner, Sens. Rev. 26 (2006) 227–230.
[2] B.D. Cobb, J.M. Clarkson, A simple procedure for optimising the polymerase chain reaction (PCR) using modified Taguchi methods, Nucleic Acids Res. 22 (1994) 3801–3805.
[3] G. Caetano-Anolles, DAF optimisation using Taguchi methods and the effects of thermal cycling parameters on DNA amplification, Biotechniques 25 (1998) 472–480.