Available online at www.isas.org.in/jisas
JOURNAL OF THE INDIAN SOCIETY OF AGRICULTURAL STATISTICS 64(1) 2010 89-117

Efficient Row-Column Designs for Microarray Experiments

Ananta Sarkar[1], Rajender Parsad[1], V.K. Gupta*[1], Kashinath Chatterjee[2] and Abhishek Rathore[3]
[1] *Indian Agricultural Statistics Research Institute, New Delhi*
[2] *Visva Bharati University, Santiniketan, West Bengal*
[3] *International Crops Research Institute for the Semi-Arid Tropics, Hyderabad*

SUMMARY

This article deals with the problem of obtaining efficient designs for 2-colour microarray experiments where same set of genes are spotted on each array. In the literature, optimality aspects of designs for microarray experiments have been investigated under a restricted model involving array and variety effects. The dye effects have been ignored from the model. If dye effects are also included in the model, then the structure of the design becomes that of a row-column design where arrays represent columns, dyes represent rows and varieties represent treatments. Further, the array effects in microarray experiments may be taken as random {see e.g. Kerr and Churchill (2001a), Lee (2004)}. For obtaining efficient row-column designs under fixed/mixed effects model, exchange and interchange algorithms of Eccleston and Jones (1980) and Rathore *et al*. (2006) have been modified. The algorithm has been translated into a computer program using Microsoft Visual C++. The algorithm is general in nature and can be used for generating efficient row-column designs for any $2 \le k < v$, where v is the number of treatments (varieties) and k is number of rows (dyes). Here, the algorithm has been exploited for computer aided search of efficient row-column designs for making all possible pairwise treatment comparisons for $k = 2$ (2-colour microarray experiments) in the parametric range $3 \le v \le 10$, $v \le b \le v(v-1)/2$; $11 \le v \le 25$, $b = v$ and $(v, b) = (11, 13), (12, 14), (13, 14)$ and $(13, 15)$, where b is the number of arrays (columns). Efficient row-column designs obtained under fixed effects model have been compared with the best available designs and best even designs. 45 designs have been obtained with higher efficiencies than the best available designs and even designs. The robustness aspect of efficient row-column designs obtained under a fixed effects model and best available designs were investigated under a mixed effects model. Strength of the algorithm for obtaining row-column designs for 3-colour microarray experiments has been demonstrated with the help of examples.

Keywords: Microarray experiments, Fixed/Mixed effects model, Row-column designs, A-efficiency, D-efficiency.

1. INTRODUCTION

In microarray experiments there are four experimental factors viz. array (A), dye (D), variety (V) and gene (G). These four experimental factors give rise to $2^4 = 16$ possible experimental effects. Out of these 16 possible experimental effects, seven effects viz. array effects (A), dye effects (D), variety effects (V), gene effects (G), array-gene interaction (AG), dye-gene interaction (DG), variety-gene interaction (VG) are of main interest to the experimenter. In the present investigation, we consider a situation where same set of genes is spotted on each array in microarray experiments. Therefore, genes/gene specific effects (G, AG, DG, VG) are orthogonal to global effects (A, D, V). Optimality aspects of designs for microarray experiments can be studied leaving gene specific effects from the model, *i.e*., by taking only array, dye and variety effects in the model. Designs that are efficient under the model containing global (A, D, V) effects are also efficient under the model containing both global and gene specific effects.

Corresponding author : V.K. Gupta
E-mail address : vkgupta_1751@yahoo.co.in

In 2-colour microarray experiments only two varieties labelled with two different dyes can be accommodated on a single array. Therefore, arrays may be considered as blocks of size 2 each. In the literature, attempts have been made to obtain optimal/efficient block designs of block size 2 for microarray experiments. Optimal/efficient block designs for 2-colour microarray experiments available in the literature have been obtained ignoring the dye effects from the model. If dye effects are included in the model, then the design becomes a row-column design with arrays representing columns, dyes representing rows and varieties representing the treatments. A design which is efficient under a restricted model involving array and variety effects may not be efficient under a 3-way classified model involving array, dye and variety effects. To make the exposition clear, consider the following example. For $v = 6$ and $b = 8$, the most efficient design obtained by taking array and variety effects in the model (Nguyen and Williams 2005) is

	Array							
D1	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dye 1	3	1	1	6	4	5	2	2
Dye 2	5	6	3	2	1	4	4	3

In design D1, the dye effects are not orthogonal with respect to varieties because all the varieties are not labelled with both the dyes in same proportion. For example, varieties 1 and 2 are labelled twice with Dye 1 and labelled once with Dye 2, whereas varieties 3 and 4 are labelled once with Dye 1 and labelled twice with Dye 2. Varieties 5 and 6 are labelled once with both dyes 1 and 2. D1 can be assumed as row-column design with 6 treatments arranged in 2 rows (dyes) and 8 columns (arrays). The average variance of the best linear unbiased estimator (BLUE) of all the possible elementary contrasts of varieties under a 2-way classified model involving only array and variety effects in the model (ignoring the row classification) is $3.7500\sigma^2$. On the other hand, the average variance under a 3-way classified model involving array, dye and variety effects in the model is $3.8571\sigma^2$. A corresponding design (D2) for this situation is

	Array							
D2	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dye 1	1	5	2	5	3	4	6	4
Dye 2	4	1	4	2	5	3	5	6

Design D2 gives average variance of the BLUE of all the possible elementary contrasts of varieties under both 2-way and 3-way classified model as $3.8333\sigma^2$. This happens because dye versus variety classification is orthogonal and, therefore, the information matrix for inferring on variety effects is same for both 2-way and 3-way classified data. The average variance for 2-way classified data using D2 is larger than that obtained from D1, but the average variance for 3-way classified data using D2 is smaller than that obtained using D1. Hence, D1 is more efficient than D2 under block design set up but D2 is more efficient than D1 under a row-column set up.

From the above examples, it is clear that obtaining efficient designs by considering array, dye and variety effects in the model *i.e.* under a row-column set up is important and needs attention. In this article the focus of the study is on optimality aspects of designs for microarray experiments under fixed effects model containing array, dye and variety effects *i.e.* under a row-column design set up. Further, in a 2-colour microarray experiment only two varieties can be accommodated on a single array. In that sense the arrays are incomplete blocks and varieties versus arrays classification is non-orthogonal. In view of this, Kerr and Churchill (2001a), Wolfinger *et al.* (2001) and Lee (2004) emphasized that array effects should be taken as random, and then the fixed effects model becomes a mixed effects model. It is indeed possible that a design optimal/efficient under fixed effects model may not be optimal/efficient under a mixed effects model. Therefore, there is a need to generate optimal/efficient row-column designs under a mixed effects model by taking array effects as random.

We begin by giving some preliminaries in Section 2. In Section 3 we give lower bounds to A- and D-efficiency of row-column designs both under fixed effects and a mixed effects model. This is used to obtain robust row-column designs optimal/efficient under fixed effects model as well as under a mixed effects model. In Section 4, we modify the exchange and interchange algorithm of Jones and Eccleston (1980) and Sarkar *et al*. (2007) to obtain efficient row-column designs. The proposed algorithm generates efficient row-column designs for any v, $b \geq v$ and $2 \leq k < v$. But in this article we have used this algorithm to generate efficient/optimal row-column designs for any v, $b \geq v$ and $k = 2$. The algorithm has been developed in Visual C++ code and computer aided search has been made for obtaining efficient row-column designs in the parametric range $3 \leq v \leq 10$; $v \leq b \leq v(v-1)/2$; and (v, b) = (11, 13), (12, 14), (13, 14) and (13, 15). Section 5 is devoted to a discussion of the efficient row-column designs generated.

In the literature, three catalogues of block designs for microarray experiments are available. Using these catalogues, Sarkar and Parsad (2006) gave a comprehensive review of designs for 2-colour microarray experiments and prepared a catalogue of 562 most A-efficient designs available in the literature. Sarkar *et al*. (2007) developed an algorithm for obtaining efficient block designs for 2-colour microarray experiments. Wit *et al*. (2005) also studied near-optimal designs as well as interwoven loop designs for dual channel microarray experiments using simulated annealing for which no catalogue is available. But it appears that perhaps no serious effort has been made to obtain optimal/efficient row-column designs for 2-colour microarray experiments. Bailey (2007) obtained efficient designs for 2-colour microarray experiments under a block design set up and suggested that there is no need to use a design in which each dye appears equally often. Instead, an efficient row-column design should be used. In the present investigation, to study the performance of best available block designs in the literature in a row-column set up, the block contents were rearranged in a row-column set up such that varieties are most balanced with respect to dyes and their lower bounds to A-efficiencies were obtained in a row-column set up. These designs are then compared with the efficient designs obtained through the proposed algorithm. The results are given in Section 5.1.

Using the proposed algorithm optimal/efficient row-column designs with two rows have been obtained under a fixed effects model. Since array effects in microarray experiments need to be taken as random, it is desirable to obtain optimal/efficient designs under a mixed effects model. It may not be possible always to obtain a design for a mixed effects model because it would require the knowledge of ρ, a function of error variance and inter column variance, which is generally unknown. An alternative is to use an optimal/efficient design obtained under a fixed effects model in the mixed effects model. But it is indeed possible that a design which is optimal/ efficient under fixed effects model may not remain optimal/efficient under a mixed effects model. Therefore, there is a need to investigate if the designs optimal/efficient under fixed effects model remain optimal/efficient under a mixed effects model when array effects are considered as random. Further, if one looks at expression (6) in Section 3, it becomes clear that lower bounds to A- and D-efficiencies of row-column designs under a mixed effects model depend upon $\rho = \sigma^2/(\sigma^2 + k\sigma_\beta^2)$, a function of error variance and inter column variance, $0 < \rho < 1$. $\rho = 0$ corresponds to the fixed effects model. In the present investigation, the algorithm generates an efficient row-column design under a fixed effects model ($\rho = 0$). Then to study the behavior of the A- and D-efficiencies of the efficient design obtained under fixed effects model, the lower bounds to A- [D-] efficiencies were obtained under a mixed effects model for different values of $0 \leq \rho \leq 0.9$. The per cent coefficient of variation (CV) of these values are then computed. If CV is small, then we say that the design is robust against different values of ρ and can be used for any value of ρ otherwise not. The robustness aspects of the designs obtained are then compared with the robustness of best available designs in Section 6.

The above discussion has been restricted to 2-colour microarray experiments. Recently microarray experiments for 3 and 4 dyes have also been proposed in the literature (Woo *et al*. 2005). The strength of the algorithm to obtain efficient row-column designs for 3-colour microarray experiments under a fixed effects model has been demonstrated with the help of some examples in Section 7. We begin with some preliminaries in Section 2.

2. PRELIMINARIES OF ROW-COLUMN DESIGNS

In this section, we describe some preliminaries of row-column designs under fixed/mixed effects model, which would be useful for a 2-colour microarray experiment run in a row-column design set up involving arrays, dyes and varieties effects, with arrays as columns, dyes as rows and varieties as treatments..

Consider a row-column design d with v treatments, k rows and b columns, vector of row sizes $\mathbf{k}' = (k_1, \ldots, k_b)$, vector of column sizes $\mathbf{b}' = (b_1, \ldots, b_k)$ and vector of replication numbers $\mathbf{r}' = (r_1, \ldots, r_v)$. We denote by $\mathbf{R} = \text{diag}(r_1, \ldots r_v)$, $\mathbf{K} = \text{diag}(k_1, \ldots, k_b)$ and $\mathbf{B} = \text{diag}(b_1, \ldots, b_k)$ diagonal matrices with diagonal elements as elements of vectors **r**, **k** and **b** respectively. With each row-column design are associated block designs that are obtainable by considering rows (columns) as blocks ignoring columns (rows). Let $\mathbf{M} = ((m_{hi}))_{v \times k}$, $\mathbf{N} = ((n_{hj}))_{v \times b}$, and $\mathbf{W} = ((w_{ji}))_{b \times k}$ denote treatments versus rows, treatments versus columns and rows versus columns incidence matrices, respectively. For $h = 1, \ldots, v$; $i = 1, \ldots, k$ and $j = 1, \ldots, b$, the nonnegative numbers $m_{hi}(n_{hj})$ denote the number of times treatment h appears in the i^{th} row (j^{th} column). $w_{ji} = 0$ or 1 indicates empty or nonempty nodes of $b \times k$ lattice according to assignment or non-assignment of a treatment to the cell (j, i). Consider the additive homoscedastic 3-way classified linear model,

$$\mathbf{y} = \mu\mathbf{1} + \Delta'\boldsymbol{\tau} + \mathbf{D}_1'\boldsymbol{\beta} + \mathbf{D}_2'\boldsymbol{\gamma} + \boldsymbol{\varepsilon} \qquad (1)$$

where n be the total number of observations, **y** is $n \times 1$ vector of observations, μ is general mean effect, $\boldsymbol{\tau}$ is $v \times 1$ vector of treatment effects, $\boldsymbol{\beta}$ is $b \times 1$ vector of column effects, $\boldsymbol{\gamma}$ is $k \times 1$ vector of row effects, $\boldsymbol{\varepsilon}$ is the $n \times 1$ vector of error components, $\boldsymbol{\varepsilon} \sim \text{IID}(0, \sigma^2\mathbf{I}_n)$, where σ^2 unknown, **1** is $n \times 1$ unit vector, Δ' is $n \times v$ design matrix for treatment effects, $\mathbf{D}_1'$ is $n \times b$ design matrix for column effects and $\mathbf{D}_2'$ is $n \times k$ design matrix for row effects.

Using the principle of ordinary least squares, the coefficient matrix of reduced normal equations for estimating the linear functions of treatment effects for general row-column design under fixed effects model (1) is

$$\mathbf{C} = \mathbf{R} - \mathbf{N}\mathbf{K}^{-1}\mathbf{N}' - (\mathbf{M} - \mathbf{N}\mathbf{K}^{-1}\mathbf{W})(\mathbf{B} - \mathbf{W}'\mathbf{K}^{-1}\mathbf{W})^{-}(\mathbf{M}' - \mathbf{W}'\mathbf{K}^{-1}\mathbf{N}') \qquad (2a)$$

C-matrix in (2a) is the general form of **C**-matrix when rows versus column classification is non orthogonal *i.e.* some of the cells of $b \times k$ lattice are empty. But when rows are orthogonal to columns and the treatments versus rows and treatments versus columns classifications are binary [(0, 1) type], **C**-matrix in (2a) under fixed effects model reduces to

$$\mathbf{C} = \mathbf{R} - \frac{1}{k}\mathbf{N}\mathbf{N}' - \frac{1}{b}\mathbf{M}\mathbf{M}' + \frac{1}{bk}\mathbf{r}\mathbf{r}' \qquad (2b)$$

where,

$\mathbf{N}\mathbf{N}' = (\lambda_{hh'})_{v \times v}$, treatment-column concurrence matrix,

$\lambda_{hh'} = \sum_{j=1}^{b} n_{hj} n_{h'j}$, inner product of h and h'^{th} row of **N**,

$\mathbf{M}\mathbf{M}' = (\mu_{hh'})_{v \times v}$, treatment-row concurrence matrix,

$\mu_{hh'} = \sum_{i=1}^{k} m_{hi} m_{h'i}$, inner product of h and h'^{th} row of **M**.

In microarray experiments, array effects may be taken as random. Therefore, the model for arrays effects as random is same as (1) with an additional assumption that $\boldsymbol{\beta}$ and $\boldsymbol{\varepsilon}$ are independently and identically distributed with $\boldsymbol{\beta} \sim \text{IID}\left(0,\ \sigma_\beta^2 \mathbf{I}_b\right)$ and $\boldsymbol{\varepsilon} \sim \text{IID}(0, \sigma^2\mathbf{I}_n)$, where σ_β^2 and σ^2 are unknown variance parameters. Following Shah and Sinha (1989), the **C**-matrix for row-column designs under mixed effects model when rows are orthogonal to columns and column effects are considered as random is

$$\mathbf{C} = \mathbf{R} - \frac{1}{k}\mathbf{N}\mathbf{N}' - \frac{1}{b}\mathbf{M}\mathbf{M}' + \frac{1}{bk}\mathbf{r}\mathbf{r}' + \rho\left(\frac{1}{k}\mathbf{N}\mathbf{N}' - \frac{1}{bk}\mathbf{r}\mathbf{r}'\right) \qquad (2c)$$

where $\rho = \dfrac{\sigma^2}{\sigma^2 + k\sigma_\beta^2}$. ρ can be estimated from the data or can be assumed to be known. But ρ is generally unknown.

The $v \times v$ matrix **C** in (2a), (2b) or (2c) is symmetric, non-negative definite and has row sums zero. For a connected design, Rank (**C**) = $v - 1$. Henceforth, we shall be concerned with connected designs only. Let $\mathbf{C}^-$ be a generalized inverse of **C** *i.e.* $\mathbf{C}\mathbf{C}^-\mathbf{C} = \mathbf{C}$. A linear function of treatment effects $\mathbf{p}'\boldsymbol{\tau}$, where $\mathbf{p}$ is a $v \times 1$ vector, is called a treatment contrast if $\mathbf{p}'\mathbf{1} = 0$. For a connected design, all treatment contrasts are estimable. The best linear unbiased estimator (BLUE) of $\mathbf{p}'\boldsymbol{\tau}$ is $\mathbf{p}'\hat{\boldsymbol{\tau}}$, where $\hat{\boldsymbol{\tau}}$ is a solution of the normal equations with var($\mathbf{p}'\hat{\boldsymbol{\tau}}$) = $\mathbf{p}'\mathbf{C}^{-}\mathbf{p}\sigma^2$ (which is invariant with respect to choice of $\mathbf{C}^{-}$). Suppose that the experimenter is interested in obtaining BLUEs of s treatment contrasts $\mathbf{p}_1'\boldsymbol{\tau}$, $\mathbf{p}_2'\boldsymbol{\tau}$, ..., $\mathbf{p}_s'\boldsymbol{\tau}$ with differential precision depending upon their respective importance. Accordingly, different weights are attached with the precision of estimation of the contrasts depending upon their importance. To deal with such situations, Freeman (1976) introduced a weighted A-optimality criterion for choice of designs in which weights are used to represent the relative importance of the 's' contrasts of interest. To be specific, a design d is said to be weighted A-optimal if it minimizes the following function over a class of designs $\mathbf{D}$ with specified parameters:

$$T = \sum_{t=1}^{s} \omega_t \,\text{var}(\mathbf{p}_t'\hat{\boldsymbol{\tau}}) = \sigma^2 \sum_{t=1}^{s} \omega_t \mathbf{p}_t'\mathbf{C}^{-}\mathbf{p}_t = \sigma^2 \text{trace}(\mathbf{W}_T\mathbf{P}'\mathbf{C}^{-}\mathbf{P}) \quad (3)$$

where $\mathbf{W}_T = \text{diag}(\omega_1, \omega_2,...,\omega_s)$ is the weight matrix (to be set by the experimenter) and $\mathbf{P}' = (\mathbf{p}_1\ \mathbf{p}_2\ ...\ \mathbf{p}_s)'$ is an $s \times v$ matrix of coefficients of the s treatment contrasts. In the context of microarray experiments our interest is in all the possible pair wise comparisons of varieties. Therefore, $\mathbf{P}'$ (matrix of coefficients of ${}^vC_2$ treatment contrasts) will be a matrix of order ${}^vC_2 \times v$. It may be noted that for $s = {}^vC_2$, $\mathbf{PP}' = v\mathbf{I} - \mathbf{11}'$. Further if comparisons among treatments are made with the same precision, then $\mathbf{W}_T = \mathbf{I}_s$ and then (3) can be rewritten as

$$\begin{aligned}\sigma^{-2}T &= \text{trace}(\mathbf{W}_T\mathbf{P}'\mathbf{C}^{-}\mathbf{P}) \\ &= \text{trace}(\mathbf{P}'\mathbf{C}^{-}\mathbf{P}) \\ &= \text{trace}(\mathbf{C}^{+}\mathbf{PP}') \\ &= \text{trace}(v\mathbf{C}^{+}) \quad (4)\end{aligned}$$

where $\mathbf{C}^{+}$ is the Moore-Penrose inverse of $\mathbf{C}$ and satisfies $\mathbf{C}^{+}\mathbf{1} = \mathbf{0}$.

Let $\mathbf{D} = \mathbf{D}(v, b, k, \rho)$ denote the class of connected row-column designs with v treatments, k rows and b columns such that rows versus column classification is orthogonal, column effects are random and $\rho = \sigma^2/(\sigma^2 + k\sigma_\beta^2)$. A design $d^* \in \mathbf{D}(v, b, k, \rho)$ is said to be A-optimal if it has minimum value of criterion T over all possible combinatorial solutions of designs in $\mathbf{D}(v, b, k, \rho)$ Similarly a design $d^* \in \mathbf{D}(v, b, k, \rho)$ is said to be D-optimal if it minimizes the determinant of the variance-covariance matrix $\mathbf{P}'\mathbf{C}^{-}\mathbf{P}$ over D(v, b, k, ρ).

For $\rho = 0$, $\mathbf{C}$-matrix in (2c) reduces to $\mathbf{C}$-matrix in (2b). Therefore, in the following section we obtain lower bounds to A- [D-] efficiencies under a mixed effects model. The lower bounds to A- [D-] efficiencies under a fixed effects model fall out as a particular case for $\rho = 0$.

3. LOWER BOUNDS TO A- AND D- EFFICIENCY OF ROW-COLUMN DESIGNS

In this section, we obtain the expressions for lower bounds to A- [D-] efficiencies of row-column designs under a mixed effects model. It can be shown easily that the problem of obtaining an A- [D-] optimal design for all possible pair wise treatment comparisons is equivalent to the problem of obtaining an A- [D-] optimal design for a complete set of orthonormal treatment contrasts $\mathbf{P}'\tau$; $\mathbf{P}'\mathbf{P} = \mathbf{I}_{v-1}$, $\mathbf{PP}' = \mathbf{I}_v - \mathbf{11}'/v$. For a row-column design d, let $\theta_1, \theta_2, ...\ \theta_{v-1}$ be the non-zero eigen values of $\mathbf{C}$. Define $\phi_A(d) = \sum_{i=1}^{v-1} \theta_i^{-1}$ and $\phi_D(d) = \prod_{i=1}^{v-1} \theta_i^{-1}$. For inferring on a complete set of orthonormal treatment contrasts, a design is said to be A- [D-] optimal if it minimizes $\phi_A(d)[\phi_D(d)]$ over $\mathbf{D}(v, b, k, \rho)$.

The A-efficiency $\{e_A(d)\}$ and D-efficiency $\{e_D(d)\}$ of any design d over $\mathbf{D}(v, b, k, \rho)$ is defined as

$$e_A(d) = \frac{\phi_A(d_A^*)}{\phi_A(d)} \text{ and } e_D(d) = \left[\frac{\phi_D(d_D^*)}{\phi_D(d)}\right]^{1/(v-1)}$$

where, d_A^* and d_D^* are the hypothetical A-optimal and D-optimal designs over $\mathbf{D}(v, b, k, \rho)$ respectively.

Let $\mathbf{C} = (c_{hh})$, $h = 1, 2, ..., v$. The inequality given by Shah and Sinha (1989) is

$$\sum_{i=1}^{v-1} f(\theta_i) \geq \frac{v-1}{v} \sum_{h=1}^{v} f\left(\frac{v}{v-1} c_{hh}\right) \quad (5)$$

where f is convex and assumed to be non-increasing over $[0, \infty)$.

Following Shah and Sinha's (1989) inequality given in (5) above, the lower bounds to A-efficiency and D-efficiency for a connected row-column design under mixed effects model with column effects as random are computed as

$$e_A(d) = \frac{(v-1)^2}{\{b(k-1)+\rho b(1-k/v)\}\phi_A(d)} \quad \text{and}$$

$$e_D(d) = \frac{(v-1)}{\{b(k-1)+\rho b(1-k/v)\}\{\phi_D(d)\}^{\frac{1}{v-1}}} \qquad (6)$$

The lower bounds to A-efficiency and D-efficiency of a row-column design under fixed effects model obtained by substituting $\rho = 0$ in (6) are

$$e_A(d) = \frac{(v-1)^2}{b(k-1)\phi_A(d)}$$

and $$e_D(d) = \frac{(v-1)}{b(k-1)\{\phi_D(d)\}^{1/(v-1)}} \qquad (7)$$

Remark 1. The lower bounds in (7) have similar expressions as in case of block designs obtained by Rathore *et al*. (2006). More efforts need to be made to obtain sharper lower bounds for row-column designs.

4. ALGORITHM BASED ON EXCHANGE AND INTERCHANGE OF TREATMENTS

In this Section we describe the algorithm for obtaining efficient row-column designs based on exchange and interchange of treatments. The exchange steps are same as those of Eccleston and Jones (1980) and Jones and Eccleston (1980). The interchange steps are on the similar lines of Rathore *et al*. (2006) and modifications to suit the requirements of a row-column setting. The broad outline of the algorithm is described below:

1. Input v (number of treatments), b (number of columns) and k (number of rows) and generate randomly a column-wise binary, treatment connected row-column design for given parameters. A design is treatment connected if $\mathbf{C} + (1/v)\mathbf{11}'$ is non-singular. The procedure of random selection of design is described in the sequel.
2. Employ exchange procedure as explained by Jones and Eccleston (1980). In this step weakest observation is replaced by the strongest observation. The exchange procedure is continued until no further improvement is made in the design in terms of the criterion used.
3. After the termination of exchange steps, apply the interchange procedure. In this step the algorithm differs from that of Eccleston and Jones (1980). Here we follow the steps similar to that of Rathore *et al*. (2006). In this procedure a pair of treatments is swapped in their position of occurrence in the design (both within and between columns) taking care that the design remains binary in columns. Changes in the criterion value are recorded for all possible swapping of the positions. The interchange that yields maximum improvement with respect to optimality criterion is implemented. This is called as strongest treatment interchange. The interchange procedure is continued until no further improvement is possible in the design in terms of the criterion used.
4. After the termination of Step 3, the lower bounds to A- and D- efficiencies of the final design obtained are computed using expression (7).
5. If a design with more A- and D-efficiencies needs to be obtained, then steps 1 to 4 are repeated again by selecting a new starting design randomly. Let A_i and D_i denote the lower bounds to A- and D-efficiencies, respectively of the design generated at i^{th} iteration and $A_{(i-1)}$ and $D_{(i-1)}$ the corresponding lower bounds at $(i-1)^{th}$ iteration. If $|A_i - A_{(i-1)}| < \in$ and $|D_i - D_{(i-1)}| < \in$, the algorithm goes to step 6. Here $\in > 0$ is a very small real number. Otherwise, the whole procedure is continued till a design with desired efficiency is obtained. In the present investigation, all the designs are obtained with maximum of 3 to 4 random starts.
6. The A- and D-efficiencies of the final design obtained in Step 5 are computed for different values of ρ viz. $\rho = 0.1, 0.2, \ldots, 0.9$ using **C**-matrix in (2c) and the expression (6). The per cent coefficient of variation (CV) in the efficiencies for different values of $0 \le \rho \le 0.9$ is computed.

Remark 2. Following modification is also made in this algorithm which was not in the algorithm of Eccleston and Jones (1980) or Rathore *et al*. (2006).

If the CV is small, then we can say that the design is robust under a mixed effects model and can be used for any value of ρ. The algorithm is general in nature and can be used for obtaining efficient row-column designs for any number of rows $2 \le k < v$. However, this algorithm has been exploited in detail here for $k = 2$.

We now describe various terms used in the broad outline of the algorithm.

4.1 Random Selection of Design

The random selection of the initial design is an important step to begin with. Choice of different initial designs may result in different designs with different lower bounds to A- and D-efficiencies. Once v, b, k are entered by the user, the routine computes the total number of experimental units, $n = bk$. If $n < v + b + k - 2$, then the routine gives the message that the generation of connected row-column design is not possible for this parametric combination and the program terminates. When $n \ge v + b + k - 2$, we generate the starting design using the following two approaches:

First Approach

In this approach, the selection of the design is completely random. We start the routine by considering only number of experimental units available and restricting to column wise binary property of design. We select a set of three random numbers (h, j, i); $h = 1, 2, ..., v$; $j = 1, 2, ..., b$; $i = 1, 2, \dots, k$. Now allocate treatment h to the i^{th} position of the j^{th} column. Select another set of random numbers (h', j', i'); $h' = 1, 2, ..., v$, $j' = 1, 2, ..., b$ and $i' = 1, 2, \dots, k$. Allocate the treatment h' to the i'^{th} position in j'^{th} column if it is empty. Otherwise reject the random number generated and generate a fresh set of random number. A set of fresh random numbers is also selected if the column j' already contains treatment h'. Repeat this process till all the $n = bk$ experimental units have been allotted the v treatments and also ensuring that the columns are binary. Further, check the connectedness of the design generated. If the design is disconnected, repeat the whole procedure again until a connected design is obtained. The replication of treatments is arbitrary in this approach.

Second Approach

In this approach we fix the replication number of treatments and then allocate the treatments randomly to available rows and columns. The approach of generating random design then is the same as in first approach. The replication of treatments for a column-wise binary row-column design is computed as,

(i) If bk is divisible by v, then the replication of treatments is taken as bk/v. This reduces the number of exchange steps.

(ii) If bk is not divisible by v, then $v - t$ treatments are replicated int$[bk/v]$ times and t randomly selected treatments are replicated int$[bk/v] + 1$ times, where $t = bk - v\{\text{int}[bk/v]\}$. Here int[.] denotes the greatest integer function.

It can be seen easily that the scheme (ii) is feasible only for those situations for which $\{\text{int}[bk/v] + t\} \le b$. When $\{\text{int}[bk/v] + t\} > b$, the generation of a column-wise binary design is not possible.

Remark 3. We have used the first approach mostly for generation of efficient designs; the second approach may yield efficient designs in fewer trials, though. Therefore, we have used the second approach whenever the resulting design from first approach is not found efficient.

4.2 Exchange and Interchange Steps

Once a random, treatment connected initial design is obtained, we apply *exchange* and *interchange* steps as discussed in the sequel. Let $\mathbf{N} = (n_{hj})_{v\times b}$ and $M = (m_{hi})_{v\times k}$, where n_{hj} denotes the number of times h^{th} treatment appears in j^{th} column and m_{hi} denotes the number of times h^{th} treatment appears in i^{th} row, respectively, $j = 1, 2, ..., b$; $i = 1, 2, \dots, k$.

Exchange Step refers to exchange of a treatment within a column by any one of the remaining $v - k$ treatments not contained in the column. This will change replication vector but vector of row sizes and column sizes will not change.

Interchange Step refers to interchange of a pair of treatments belonging to two different positions in the row-column setup. This step leaves replication vector unchanged. At every exchange and interchange steps, the algorithm tests for connectedness of the resulting design.

In the Exchange step, the weakest observation is replaced with strongest observation. Exchange step and weakest and strongest observations used here are same as discussed by Jones and Eccleston (1980), Eccleston and Jones (1980) and Rathore *et al*. (2006). Let $\mathbf{C}_{n(o)}$, $\mathbf{C}_{n-1}$ and $\mathbf{C}_{n(n)}$ be, respectively the information matrices of the design with original n observations, design with $n-1$ observations obtained on deleting one observation and new design with new n observations obtained on addition of a new observation.

Weakest Observation: An observation for which trace ($\mathbf{C}_{n-1}^{-}\mathbf{PP}'$) – trace($\mathbf{C}_{n(o)}^{-}\mathbf{PP}'$) is minimum for all possible $\mathbf{C}_{n-1}^{-}$ obtained on deletion of an observation; in other words trace($\mathbf{C}_{n-1}^{-}\mathbf{PP}'$) is minimum.

Strongest Observation: The observation for which trace($\mathbf{C}_{n-1}^{-}\mathbf{PP}'$) – trace($\mathbf{C}_{n(n)}^{-}\mathbf{PP}'$) is maximum for all possible $\mathbf{C}_{n(n)}^{-}$ obtained on addition of a new observation; in other words trace($\mathbf{C}_{n(n)}^{-}\mathbf{PP}'$) is minimum.

For *exchange* process, we start from an observation in first row and first column and search for weakest observation in the whole design. In other words, we delete each observation one by one from the design and compute trace($\mathbf{C}_{n-1}^{-}\mathbf{PP}'$) at each step. Once we delete one observation, the row-column classification becomes non-orthogonal, therefore, $\mathbf{C}_{n-1}^{-}$ is computed using (2a). We delete the observation for which trace($\mathbf{C}_{n-1}^{-}\mathbf{PP}'$) – trace($\mathbf{C}_{n(o)}^{-}\mathbf{PP}'$) is minimum. It is followed by the search of the strongest observation as a replacement of the deleted observation. For identifying the strongest observation, we replace the deleted observation by each of the remaining $v-k$ treatments not present in the column so as to be able to have a column-wise binary design and compute trace ($\mathbf{C}_{n(n)}^{-}\mathbf{PP}'$) at each step ($\mathbf{C}_{n(n)}^{-}$ is computed using (2b)). The deleted observation is replaced by the observation for which trace($\mathbf{C}_{n(n)}^{-}\mathbf{PP}'$) is minimum. Since in the replacement scheme the original treatment is also included, therefore, minimum of trace($\mathbf{C}_{n(n)}^{-}\mathbf{PP}'$) is always less than or equal to trace($\mathbf{C}_{n(o)}^{-}\mathbf{PP}'$). If minimum of trace($\mathbf{C}_{n(n)}^{-}\mathbf{PP}'$) is less than trace($\mathbf{C}_{n(o)}^{-}\mathbf{PP}'$), then we say that there is an improvement in the design. The exchange step is implemented when there is an improvement in the design. Using the improved design, we go on to find weakest observation and replace it with strongest observation until the strongest substitute for the weakest observation is same as the weakest observation. The exchange procedure fixes the replication vector of treatments.

Now after termination of exchange process a different process named ***strongest** treatment interchange* is implemented to achieve an optimal assignment of treatments to rows and columns.

Strongest Treatment Interchange is an interchange process where we substitute the h^{th} treatment in i^{th} position of j^{th} column with h'^{th} ($\neq h$) treatment appearing in any position of any column in the design including the column under consideration, which favours the criterion most, *i.e.*, for which the trace($\mathbf{C}_{n(o)}^{-}\mathbf{PP}'$) – trace($\mathbf{C}_{n(I)}^{-}\mathbf{PP}'$) is maximum. Here, $\mathbf{C}_{n(o)}$ and $\mathbf{C}_{n(I)}$ are the information matrices of the starting design at the interchange step and new design obtained after interchange, respectively. If there is an improvement in the design, then this interchange of a pair of treatments is implemented and the old design is replaced by the new design. If no improvement is achieved, then the original design is retained as new design. This interchange will be attempted for all positions in the design one by one. The process stops when all required treatments get interchanged with the appropriate ones. At this stage the algorithm possesses a design, which it could find in this try.

The proposed strongest treatment interchange is a modification of the interchange process of Eccleston and Jones (1980), where they search for any pair of treatments h, h' between the two cells (i, j), (i', j') for all $h \neq h'$, (=1, ..., v), i, i'(= 1, ..., k) j, j'(=1, ..., b) and which favours the criterion most. In the proposed interchange procedure, we search for a strongest interchange for a given treatment in a given cell with all other treatments in all other cells. This given treatment is selected from each cell one by one.

The **C**-matrix of the final design is computed under fixed effects model using (2b) and the lower bounds to A- and D-efficiencies are calculated using (7). If the efficiencies are satisfactory, we stop making the search; otherwise one can make a retry and may get a better design next time, better in terms of A- and D-efficiency. However, there is no guarantee that the new search will yield a better design.

To this, we add the process of computing the lower bounds to A- and D-efficiencies of the final design under a mixed effects model. A- and D-efficiencies of the final design are computed for different values of ρ = 0.1, 0.2, ..., 0.9 and the per cent coefficient of variation (CV) of the efficiencies of the generated designs are computed and in Sraker *et al*. (2007). If the CV is low then the design which is efficient under fixed effects model can also be used when array effects are random. In the present investigation, we term a design as robust (strongly robust) if the coefficient of variation (CV) in the efficiencies of the design for different values of ρ's (ρ = 0.1, 0.2, . . . , 0.9) is less than 5% (1%).

5. EFFICIENT ROW-COLUMN DESIGNS FOR 2-COLOUR MICROARRAY EXPERIMENTS

The algorithm developed in Section 4 has been converted into a Visual C++ code. Although the algorithm and the Visual C++ code are general in nature and can be used for generation of efficient row-column designs for any v, b and k, in this section we exploit the algorithm to obtain efficient row-column designs with two rows (k = 2) and $b \geq v$.

The computer aided search is made for A- and D-efficient designs in the parametric range $3 \leq v \leq 10$, $v \leq b \leq v(v-1)/2$; $11 \leq v \leq 25$, $b = v$ and (v, b) = (11, 13), (12, 14), (13, 14) and (13, 15). A total of 139 row-column designs with k = 2 have been obtained under fixed effects model. Among the 139 designs generated, designs for 132 parametric combinations are available for restricted model ignoring dye effects in the literature along with their lower bounds to A- and D- efficiencies. For the 7 parametric combinations no designs (even under restricted model) are available in the literature. These designs are given in Table 7 in the Appendix.

In Table 7, the design marked with asterisk (*) is row-regular Generalized Youden Design (GYD). We know that for odd v, $b = v(v-1)/2$ and k = 2 the A-optimal design is row-regular Generalized Youden Design (GYD) under row-column set up. In our case we find row-regular GYD for (v, b) = (3, 3), (5, 10), (7, 21) and (9, 36).

5.1 Comparisons with Best Available Designs

In the present investigation we consider the 3-way classified model containing array, dye and variety effects in the model. In the literature, optimal/efficient designs for 2-colour microarray experiments are studied under linear fixed effects model containing array and variety effects. Very little seems to have been done to study optimal/efficient designs under the model containing array, dye and variety effects in the model. Therefore, we found best available designs and best even designs available under the restricted model. The block contents of these designs are rearranged in such a fashion that the varieties are most balanced with respect to the two positions in blocks. We say that varieties at position 1 are labelled with one dye and at position 2 are labelled with other dye. The rearranged designs are then compared with the designs obtained under 3-way classified model.

Best Available Designs

In the literature there exist three catalogues of efficient designs under a restricted model for microarray experiments (see e.g. Kerr and Churchill 2001a, Yang 2003 and Nguyen and Williams 2005). Among the designs available, the designs of Nguyen and Williams (2005) are most balanced with respect to dyes effects and are available at http://mcs.une.edu.au/~nkn/mad/. For the remaining catalogues (Kerr and Churchill 2001a, Yang 2003) the designs are not in the most balanced form with respect to dyes. These designs are catalogued in Sarkar and Parsad (2006). Therefore, we tried to arrange the contents of these designs such that varieties are most balanced with respect to dyes. The lower bounds to A-efficiencies are then computed under row-column design set up for all the designs available in the literature in the parametric range under investigation. These lower bounds are compared with lower bounds to A-efficiencies of the row-column designs generated through the proposed algorithm to identify the best available row-column design. It is observed that designs of Yang (2003) have maximum lower bound to A-efficiencies for $3 \leq v \leq 25$ and $b = v$. For (v, b) = (13, 14), designs given by Kerr and Churchill (2001a) have maximum lower bound to

A-efficiency and for the remaining parametric combinations Nguyen and Williams (2005) designs has maximum lower bound to A-efficiency. Most of the designs of Kerr and Churchill (2001a) have same lower bound to A-efficiency as that of the designs given by Nguyen and Williams (2005). For those parametric combinations where the efficiencies of designs given by Kerr and Churchill (2001a) and Nguyen and Williams (2005) are same, we take the designs of Nguyen and Williams (2005) as these are available in the form where varieties are most balanced with respect to dyes. Design layout of 132 best available designs and designs obtained for these parametric combinations along with their lower bounds to A- and D-efficiencies are also available with the first author and can be obtained by sending an E-mail to ananta8976@gmail.com.

Best Even/Row-Orthogonal Designs

Even/row-orthogonal designs are also catalogued in literature by Kerr and Churchill (2001a) and Nguyen and Williams (2005). The lower bound to A-efficiencies of even designs is found to be the same for both the catalogues. Therefore, out of 132 parametric combinations we find 108 even designs of Nguyen and Williams (2005) as best even designs. In the parametric range, no even design is catalogued for the following 24 parametric combinations

Table 1. Parameters for which no even design is Catalogued in Literature

v	3	4	5	6	6	6	7	7	8	8	8	8
b	3	4	5	13	14	15	19	20	25	26	27	28
v	9	9	10	10	10	10	10	21	22	23	24	25
b	34	35	41	42	43	44	45	21	22	23	24	25

Out of these 24 parametric combinations, it is possible to get an even design for the 8 parametric combinations for $b = v$. Clearly a loop design is the only even design for any $b = v$. Therefore, loop designs are considered as best available even design for the following 8 parametric combinations (v, b) = (3, 3), (4, 4), (5, 5), (21, 21), (22, 22), (23, 23), (24, 24) and (25, 25).

The even designs are row orthogonal, therefore, the lower bounds to A-efficiencies under fixed effects model containing array and variety effects in the model (restricted model) remain same with the lower bounds to A-efficiencies under fixed effects model containing array, dye and variety effects in the model (full model).

We have compared the designs obtained with the best available designs as well as with the best even designs. The number of designs in the parametric range having higher efficiencies, same efficiencies and lower efficiencies than that of the best available designs after rearranging the block contents such that varieties are most balanced with respect to dyes and best even designs are summarized in Table 2.

Table 2. Comparison of the Designs obtained with Best Available/Even Designs

	Best Available Designs	Best Even Designs
Higher efficiency	45	90
Same efficiency	64	16 (11* + 5)
Lower efficiency	23	10 (6* + 4)
Total	**132**	**116**

* Lower bound to A-efficiencies is same as that of best available designs under block design setup

45 designs (obtained) have been found to have higher efficiencies, 64 designs to have same efficiencies and 23 designs to have lower efficiencies than the best available designs. Out of these 45 designs, 40 designs obtained have higher efficiencies than those of the best even designs; for 3 parametric combinations (v, b) = (6, 8), (9, 9) and (10, 12) the design obtained have same lower bound to A-efficiency as that of the best even design and for remaining 2 parametric combinations (v, b) = (7, 19) and (9, 34) no even design is given in the literature. 45 designs with higher efficiencies are given in Table 6 in APPENDIX.

A comparison of best even designs among the 116 parametric combinations with best available design under block design set up revealed that only 17 even designs have same lower bound to A-efficiencies as that of the best available designs under block design set up. The remaining 99 best even designs have smaller lower bound to A-efficiencies than those of the best available designs. In even designs, dye effects are always orthogonal to variety effects. Therefore, one can assume that an even design may have higher efficiency than the best available design under row-column design set up. We have compared optimality aspects of row-column designs generated through the proposed algorithm with those of best even designs under row-column design set up. We find that out of 99 cases (where best available designs have higher efficiency than best even designs) 90 row-column designs have higher efficiency, 5 designs have same efficiency and remaining 4 designs have lower efficiency than that of best even designs under row-column design set up. Again, among the 17 cases (where best even designs are best available designs) 11 row-column designs have same lower bound to A-efficiencies and 6 designs have smaller lower bound to A-efficiencies than that of best even designs.

Again among the 90 parametric combinations for which the designs obtained have higher lower bound to A-efficiency than best even designs, for 38 parametric combinations the designs obtained have higher lower bound to A-efficiency; for 40 parametric combinations the designs obtained have same lower bound to A-efficiency and for remaining 12 parametric combinations the designs obtained have smaller lower bound to A-efficiency than best available row-column designs.

We now consider two row-column designs obtained by the proposed method which have higher lower bound to A-efficiencies than those of the best available ones. For $v = 6$, $b = 9$, the best available design and the row-column design obtained are given in Table A.

For $v = 7$, $b = 19$, the best available design and the row-column design obtained are given in Table B.

Remark 4. Even designs are row-orthogonal with respect to treatments when block size $k = 2$. Therefore, in the literature even designs are catalogued as A-optimal designs under the restricted class that treatments are row-orthogonal. Therefore, one advantage of searching row-column design is one can obtain an optimal/efficient design under both the restricted class (when treatments are row-orthogonal) and the non-restricted class (when treatments are not row-orthogonal) *i.e.*, under the model containing all three array, dye and variety effects in the model. The example for $v = 7$ and $b = 19$ shows that a non-even design is better that an even design.

6. ROBUSTNESS OF EFFICIENT DESIGNS UNDER MIXED EFFECTS MODEL

In Section 5, we have obtained efficient row-column designs under a fixed effects model. Kerr and Churchill (2001a), Wolfinger *et al*. (2001) and Lee (2004) have advocated that the array effects may be taken as random in microarray experiments. If we consider array effects as random, the fixed effects

Table (A)

D1: Best Available Row-Column Design									
Dye 1	5	5	3	1	1	4	6	2	2
Dye 2	4	6	4	6	2	1	3	5	3
Lower Bound to A-eff = 0.8758 Lower Bound to D-eff = 0.9132									

D2: Row-Column Design Obtained									
Dye 1	5	1	6	3	1	2	4	2	4
Dye 2	1	3	2	4	6	5	6	3	5
Lower Bound to A-eff = 0.9132 Lower Bound to D-eff = 0.9350									

Table (B)

D1: Best Available Row-Column Design																			
Dye 1	3	2	1	1	7	3	7	3	6	4	2	1	5	7	5	2	4	4	6
Dye 2	5	3	6	7	5	4	3	1	2	1	4	2	1	2	6	5	6	7	3
Lower Bound to A-eff = 0.9665; Lower Bound to D-eff = 0.9809																			

D2: Row-Column Design Obtained																			
Dye 1	5	7	5	7	2	6	1	3	4	2	7	4	2	1	4	6	1	3	5
Dye 2	1	2	6	3	6	7	4	4	5	4	5	6	3	7	7	1	3	5	2
Lower Bound to A-eff = 0.9708; Lower Bound to D-eff = 0.9830																			

Table 3. Robustness of Designs Obtained and Best Available Designs

Row-Column Design Obtained					Best Available Row-Column Design				
Efficiency					Efficiency				
	Higher	Same	Lower	Total		Higher	Same	Lower	Total
Strongly Robust	9	22	5	36	Strongly Robust	7	22	7	36
Robust	22	32	15	69	Robust	23	32	11	66
Non-robust	14	10	3	27	Non-robust	15	10	5	30
Total	45	64	23	132	Total	45	64	23	132

model then becomes a mixed effects model. Designs optimal/efficient under fixed effects model may not be optimal/efficient under a mixed effects model. There is need to study optimality aspects of designs for microarray experiments under a mixed effects model considering array effects random. The lower bounds to A- and D-efficiencies in a mixed effects model are dependent on ρ. The value of ρ is unknown. Therefore, we need to search designs which are efficient for any value of ρ. To meet this objective we study the robustness property of the row-column designs under fixed effects model ($\rho = 0$) against different values of ρ in the range $0 \leq \rho \leq 0.9$ under a mixed effects model. For this purpose, lower bounds to A- and D-efficiencies of the efficient designs under fixed effects model are studied for $0 \leq \rho \leq 0.9$. The percent coefficient of variation (CV) of lower bounds to A-efficiencies for $0 \leq \rho \leq 0.9$ is also computed.

A design is said to be robust (strongly robust) if the coefficient of variation (CV) in the A-efficiencies of the designs is less than 5% (1%). Lower bounds to the A- and D-efficiency have been obtained in Section 5. The robustness of all the efficient row-column designs generated under fixed effects model in Section 5 is investigated against the value of ρ. The lower bounds to A- and D-efficiencies for $0 \leq \rho \leq 0.9$ and their CV are computed. The number of designs which are strongly robust, robust or non-robust are summarized in Table 3.

Designs Obtained: Among the 132 designs obtained, 36 designs are found to be strongly robust and 69 designs are found to be robust under a mixed effects model. The remaining 27 designs which are not robust in the range $\rho = 0.0$ to 0.9 are found to be robust in the range $\rho = 0.3$ to 0.9.

Best Available Designs: Among the 132 designs obtained, 36 designs are found to be strongly robust and 66 designs are found to be robust under a mixed effects model. The remaining 30 designs which are not robust in the range $0 \leq \rho \leq 0.9$ are found to be robust in the range $0.3 \leq \rho \leq 0.9$.

The robustness aspects of the designs are studied separately for designs obtained with higher efficiency, same efficiency and lower efficiency than the best available designs and are summarized in Table 3. Among the 45 designs obtained with higher efficiency, 9 designs are found to be strongly robust, 22 designs are found to be robust and remaining 14 designs are not robust under a mixed effects model. Out of these 45 parametric combinations for which a best available design is less efficient than the design obtained, 7 designs are strongly robust, 23 are robust and 15 are not robust.

The designs with higher A- and D-efficiencies and A- and D-efficiencies of corresponding best available designs are separately catalogued in Table 6 in APPENDIX. To study the designs with higher efficiencies than that of the best available designs we made a comparison of both robustness and the CV (A-efficiencies) of both the designs. The results are summarized in Table 4.

Table 4. Designs with Higher Efficiency: Robustness versus CV (A-Efficiencies) Designs with Higher Efficiencies: Robustness vs CV of lower bound to A-efficiencies

	Strongly Robust	Robust	Non-robust	Total
lower CV (A-eff)	9	21	13	43
same CV (A-eff)	0	0	0	0
higher CV(A-eff)	0	1	1	2
Total	9	22	14	45

Out of 45 designs with higher efficiencies, 43 designs have lower CV of A-efficiencies and 2 designs have higher CV of A-efficiencies than the corresponding best available designs. From Table 6 in APPENDIX, it was also observed that out of 43 designs (with lower CV of A-efficiencies) 26 designs have higher A-efficiencies for $0 \leq \rho \leq 0.9$ and for the remaining 17 designs (with lower CV of A-efficiencies) have higher A-efficiencies at smaller values of ρ's and lower A-efficiencies at higher values of ρ's. The number of designs and the corresponding ρ-value at which lower bound to A-efficiency changes from higher to lower are summarized as

Efficiencies changes from higher to lower for ρ	(v, b)	No. of Designs
0.1	(6, 8), (7, 9), (8, 22), (9, 24), (10, 12), (11, 13), (12, 14), (13, 15)	8
0.2	(7, 12)	1
0.3	(10, 13)	1
0.4	(6, 10), (9, 16), (10, 17)	3
0.5	(8, 13), (9, 15)	2
0.7	(7, 11), (9, 14)	2
Total		**17**

Among 64 designs with same efficiency 22 designs are found to be strongly robust, 32 designs are found to be robust and remaining 10 designs are non-robust for both our designs and the best available designs under mixed effects model.

Among our 23 designs with lower efficiency 5 designs are found to be strongly robust, 15 designs are found to be robust and remaining 3 designs are non-robust under mixed effects model whereas for these 23 parametric combinations among the best available designs 7 designs are strongly robust, 11 are robust and 5 are non-robust among the best available designs.

Mix designs have been studied by Yang (2003) under block design setup for the situation where number of blocks is equal to number of treatments in the experiment *i.e.*, $v = b$ and $3 \leq v \leq 25$. We have studied Mix designs under row-column design setup. We have computed lower bounds to A-efficiencies for Mix(2), Mix(3), Mix(4), Mix(5) and loop designs [Mix(v)] in the same parametric range (*i.e.* for all $v = b$ and $3 \leq v \leq 25$) and find 5 designs with parameters (v, b) = (13, 13), (14, 14), (15, 15), (16, 16) and (17, 17) to have higher lower bound to A-efficiencies than the best available design (Yang 2003) or the row-column designs obtained.

7. ROW-COLUMN DESIGNS FOR 3-COLOUR MICROARRAYS

This paper describes an algorithm for generating efficient/optimal row-column designs for microarray experiments for given v, b and k under a fixed effects model. The robustness aspects of efficient designs under a mixed effects model are investigated under a mixed effects model. The case $k = 2$, i.e. 2-color microarray experiments, has been studied in detail. 3- and 4-colour microarrays have also been proposed in the literature where more than two (*i.e.* three or four or more) dyes may be used in a single microarray experiment (see, e.g., Woo *et al*. 2005). The proposed algorithm and Visual C++ code developed are capable of generating efficient row-column designs for any v, b, k such that $k < v$. Therefore, it is possible to obtain optimal/efficient row-column designs for any $k < v$. Some efficient row-column designs for $k = 3$ are obtained (see Table 5).

The design for $v = b = 7$ and $k = 3$ is a Youden Square Design. It demonstrates the usefulness of the algorithm in generation of Youden Square Designs as well.

8. DISCUSSION

Efficient row-column designs have been obtained for two-colour microarray experiments under fixed / mixed effects model. The methodology described in this paper may be expressed in more general framework with applications to a broader collection of problems rather than limiting to two-color microarray experiments. For example, a common problem encountered in microarray experiments is the batch effects, especially in large cell cycle experiments. A batch is defined as a set of microarrays that are

Table 5. Row-column Designs for 3-Colour Microarray Experiments $k = 3$ (> 2)

v	*b*	*k*	A-Efficiency	D- Efficiency	CV (A-Eff)	CV (D-Eff)			
6	4	3	0.8082	0.8423	2.7023	1.7194			
Dye 1		2	5	6	1				
Dye 2		4	2	3	5				
Dye 3		1	6	4	3				
6	6	3	0.9804	0.9903	0.6358	0.3124			
Dye 1		3	1	5	6	4	2		
Dye 2		5	2	1	3	6	4		
Dye 3		1	4	6	2	3	5		
6	8	3	0.9573	0.9630	0.5490	0.3681			
Dye 1		2	2	5	6	3	4	5	1
Dye 2		5	4	1	3	2	6	3	4
Dye 3		1	1	3	2	4	5	6	6
7	7	3	1.0000	1.0000	0.0000	0.0000			
Dye 1		5	7	6	2	1	4	3	
Dye 2		1	5	7	4	2	3	6	
Dye 3		4	2	1	6	3	7	5	

processed together within a single experiment. Different batches of the same microarray experiment are processed at different times, in different laboratories, by different operators, and so on. Batch effects may be caused by many factors such as the methods for RNA isolation, amplification and target labeling, and array processing and scanning. There is a need to relook at the methodology presented in this paper and explore the possibility of its application to the problem of batch effects in microarray experiments by taking batch effects in the model as random effects. There is also a need to investigate whether or not the designs efficient under a two tailed alternative remain efficient for order-restricted inference, i.e. when "treatments" are ordered (such as doses, time, tumor stage, etc.).

ACKNOWLEDGEMENTS

Authors are grateful to the reviewer for his valuable suggestions that have led to considerable improvement in presentation of the results. Infact, the Section on "Discussion" was added on the suggestion of the reviewer. Senior Research Fellowship of IASRI and Junior Research Fellowship of CSIR received by the first author is gratefully acknowledged.

REFERENCES

Bailey, R.A. (2007). Designs for 2-colour microarray experiments. *J. Roy. Statist. Soc.*, **C56(4)**, 365-394.

Datta, S. (2003). Statistical techniques for microarray data: A partial overview. *Comm. Statist.–Theory Methods,* **32**, 263-280.

Eccleston, J.A. and Jones, B. (1980). Exchange and interchange procedures to search for optimal row-and-column designs. *J. Roy. Statist. Soc.*, **B42**, 372-376.

Freeman, G.H. (1976). On the selection of designs for comparative experiments. *Biometrics,* **32**, 195-199.

Gupta, P.K., Roy, J.K. and Prasad, M. (1999). DNA chips, microarray and genomics. *Current Sci.,* **77(7)**, 875-884.

Jones, B. and Eccleston, J.A. (1980). Exchange and interchange procedure to search for optimal design. *J. Roy. Statist. Soc.*, **B42**, 291-297.

Kerr, M.K. (2003). Design considerations for efficient and effective microarray studies. *Biometrics,* **59**, 822-828.

Kerr, M.K. and Churchill, G.A. (2001a). Experimental design for gene expression microarrays. *Biostatistics,* **2(2)**, 183-201.

Kerr, M.K. and Churchill, G.A. (2001b). Statistical design and the analysis of gene expression. *Genet. Res.,* **77**, 123-128.

Lee, M.-L.T. (2004). *Analysis of Microarray Gene Expression Data*. Kluwer Academic Publishers, United States of America.

Nguyen, N.K. and Williams, E.R. (2005). Experimental designs for 2-colour cDNA microarray experiments. (Invited paper), *Proc. of the 4th International Symposium on Business and Industrial Statistics, Palm Cove Resort (Tropical North Queensland).*

Rathore, A., Parsad, R. and Gupta, V.K. (2006). Computer aided search of efficient block designs for making all possible pairwise treatment comparisons. *J. Statist. Appl.*: *The Journal Forum Interdis. Math.*, **1**, 15-33.

Sarkar, A. and Parsad, R. (2006). Block designs for 2-colour micro-array experiments: An overview. *Pusa AgriScience*, **29**, 38-54.

Sarkar, A., Parsad, R., Rathore, A. and Gupta, V.K. (2007). Efficient block designs for microarray experiments. *J. Ind. Soc. Agril. Statist.*, **61(2)**, 227-248.

Sebastiani, P., Gussoni, E., Kohane, I.S. and Ramoni, M.F. (2003). Statistical challenges in functional genomics. *Statist. Sc.,* **18**(**1**), 33-70.

Shah, K.R. and Sinha, B.K. (1989). *Theory of optimal designs*. Lecture notes in Statistics, Springer-Verlag, New York, **54**, 70.

Tjur, T. (1993). An algorithm for optimization of block designs. *J. Statist. Plann. Inf*., **36**, 277-282.

Wit, E., Nobile, A. and Kahanin, R. (2005). Near optimal designs for dual channel microarray studies. *J. Roy. Statist. Soc*., **C54(5)**, 817-830.

Wolfinger, R.D., Gibson, G., Wolfinger, E.D., Bennet, L., Hamadeh, H., Busherl, P., Afshari, C. and Paules, R. (2001). Assessing gene significance from cDNA microarray expression data via mixed models. *J. Comput. Biol*., **8**, 625-637.

Woo, Y., Krueger, W., Kaur, A. and Churchill, G. (2005). Experimental design for three-color and four-color gene expression microarrays. *Bioinformatics*, **21**, 459-467.

Yang, X. (2003). *Optimal design of single factor cDNA microarray experiments and mixed models for gene expression data*. Ph. D. Thesis, Virginia Polytechnic Institute and State University.

Yang, Y.H. and Speed, T. (2002). Design issues for cDNA microarray experiments. *Nature Rev. Genet.*, **3**, 579-588.

http://mcs.une.edu.au/~nkn/mad/

http://www.jax.org/staff/churchill/labsite/pubs/index.html

APPENDIX

Table 6. Row-Column Designs Obtained which are More Efficient than Best Available Row-Column Designs for 2-Colour Microarray Experiments in Parametric Range $3 \le v \le 10$, $v \le b \le v(v-1)/2$, $11 \le v \le 25$, $b = v$ and $(v, b) = (11, 13), (12, 14), (13, 14)$ and $(13, 15)$

Sl.No.	*v*	*b*	Eff	$\rho = 0.0$	$\rho = 0.1$	$\rho = 0.2$	$\rho = 0.3$	$\rho = 0.4$	$\rho = 0.5$	$\rho = 0.6$	$\rho = 0.7$	$\rho = 0.8$	$\rho = 0.9$	CV(Eff)	Robustness	CV(Eff)
1	**6**	**8**	**AEff**	**0.8152**	**0.8334**	**0.8484**	**0.8607**	**0.8707**	**0.8789**	**0.8855**	**0.8907**	**0.8948**	**0.8978**	**3.0421**	**Robust**	**lessCV**
	6	8	AEff	0.8102	0.8499	0.8789	0.9004	0.9163	0.9280	0.9364	0.9424	0.9463	0.9485	4.8577	Robust	
	6	**8**	**DEff**	**0.8944**	**0.9049**	**0.9131**	**0.9194**	**0.9242**	**0.9278**	**0.9304**	**0.9323**	**0.9335**	**0.9341**	**1.3865**		
	6	8	DEff	0.8915	0.9115	0.9262	0.9371	0.9452	0.9512	0.9556	0.9586	0.9607	0.9619	2.3799		
	Dye1	**5**	**6**	**3**	**5**	**6**	**2**	**1**	**4**							
	Dye2	**2**	**1**	**5**	**4**	**3**	**6**	**5**	**6**							
	Dye1	3	1	1	6	4	5	2	2							
	Dye2	5	6	3	2	1	4	4	3							
2	**6**	**9**	**AEff**	**0.9132**	**0.9303**	**0.9437**	**0.9541**	**0.9619**	**0.9677**	**0.9718**	**0.9744**	**0.9758**	**0.9762**	**2.1410**	**Robust**	**lessCV**
	6	9	AEff	0.8758	0.8988	0.9174	0.9323	0.9443	0.9538	0.9612	0.9668	0.9709	0.9738	3.3436	Robust	
	6	**9**	**DEff**	**0.9350**	**0.9460**	**0.9545**	**0.9611**	**0.9661**	**0.9699**	**0.9727**	**0.9746**	**0.9758**	**0.9765**	**1.3865**		
	6	9	DEff	0.9132	0.9281	0.9399	0.9493	0.9567	0.9626	0.9672	0.9707	0.9734	0.9754	2.0769		
	Dye1	**5**	**1**	**6**	**3**	**1**	**2**	**4**	**2**	**4**						
	Dye2	**1**	**3**	**2**	**4**	**6**	**5**	**6**	**3**	**5**						
	Dye1	5	5	3	1	1	4	6	2	2						
	Dye2	4	6	4	6	2	1	3	5	3						
3	**6**	**10**	**AEff**	**0.8958**	**0.9122**	**0.9249**	**0.9346**	**0.9421**	**0.9476**	**0.9516**	**0.9544**	**0.9562**	**0.9571**	**2.1022**	**Robust**	**lessCV**
	6	10	AEff	0.8859	0.9057	0.9212	0.9334	0.9430	0.9504	0.9561	0.9603	0.9634	0.9655	2.7091	Robust	
	6	**10**	**DEff**	**0.9258**	**0.9357**	**0.9433**	**0.9492**	**0.9537**	**0.9572**	**0.9598**	**0.9617**	**0.9631**	**0.9639**	**1.2777**		
	6	10	DEff	0.9296	0.9410	0.9498	0.9567	0.9620	0.9660	0.9692	0.9715	0.9732	0.9744	1.4853		
	Dye1	**4**	**6**	**3**	**6**	**4**	**4**	**1**	**5**	**2**	**2**					
	Dye2	**1**	**1**	**4**	**3**	**6**	**5**	**2**	**6**	**3**	**5**					
	Dye1	3	1	6	1	5	5	2	4	2	6					
	Dye2	5	4	2	6	1	2	4	3	1	3					
4	**7**	**9**	**AEff**	**0.7659**	**0.8127**	**0.8462**	**0.8708**	**0.8889**	**0.9023**	**0.9120**	**0.9190**	**0.9238**	**0.9269**	**5.8071**	**Non-robust**	**lessCV**
	7	9	AEff	0.7652	0.8180	0.8565	0.8850	0.9059	0.9213	0.9324	0.9403	0.9455	0.9487	6.5352	Non-robust	

	7	**9**	**DEff**	**0.8677**	**0.8929**	**0.9109**	**0.9239**	**0.9334**	**0.9402**	**0.9451**	**0.9484**	**0.9506**	**0.9518**	**2.8809**		
	7	9	DEff	0.8644	0.8936	0.9147	0.9300	0.9412	0.9494	0.9553	0.9595	0.9623	0.9640	3.3807		
	Dye1	**1**	**4**	**7**	**6**	**4**	**3**	**5**	**6**	**2**						
	Dye2	**6**	**1**	**2**	**5**	**7**	**7**	**4**	**3**	**6**						
	Dye1	3	1	2	4	7	5	6	1	4						
	Dye2	6	7	4	5	2	3	2	3	1						
5	**7**	**10**	**AEff**	**0.8327**	**0.8708**	**0.8988**	**0.9195**	**0.9347**	**0.9456**	**0.9533**	**0.9585**	**0.9616**	**0.9631**	**4.5151**	**Robust**	**lessCV**
	7	10	AEff	0.8081	0.8512	0.8831	0.9069	0.9247	0.9379	0.9475	0.9544	0.9590	0.9619	5.3561	Non-robust	
	7	**10**	**DEff**	**0.9004**	**0.9212**	**0.9364**	**0.9476**	**0.9558**	**0.9618**	**0.9660**	**0.9689**	**0.9708**	**0.9718**	**2.3945**		
	7	10	DEff	0.8860	0.9098	0.9274	0.9405	0.9502	0.9575	0.9628	0.9667	0.9694	0.9712	2.8601		
	Dye1	**7**	**3**	**1**	**5**	**1**	**6**	**3**	**2**	**5**	**4**					
	Dye2	**1**	**4**	**2**	**6**	**6**	**3**	**7**	**4**	**7**	**5**					
	Dye1	6	3	4	2	3	5	7	2	4	1					
	Dye2	4	1	5	5	2	1	6	6	3	7					
6	**7**	**11**	**AEff**	**0.8605**	**0.8916**	**0.9144**	**0.9312**	**0.9435**	**0.9523**	**0.9584**	**0.9624**	**0.9648**	**0.9658**	**3.6075**	**Robust**	**lessCV**
	7	11	AEff	0.8354	0.8733	0.9014	0.9224	0.9381	0.9498	0.9584	0.9646	0.9689	0.9716	4.6593	Robust	
	7	**11**	**DEff**	**0.9083**	**0.9258**	**0.9386**	**0.9480**	**0.9551**	**0.9602**	**0.9639**	**0.9665**	**0.9682**	**0.9692**	**2.0384**		
	7	11	DEff	0.9020	0.9228	0.9382	0.9497	0.9583	0.9647	0.9695	0.9729	0.9754	0.9770	2.4939		
	Dye1	**2**	**4**	**7**	**7**	**6**	**3**	**1**	**1**	**5**	**6**	**6**				
	Dye2	**1**	**2**	**4**	**5**	**3**	**7**	**5**	**3**	**6**	**4**	**2**				
	Dye1	7	5	2	1	4	5	3	2	4	6	1				
	Dye2	1	2	4	3	6	3	7	1	7	5	6				
7	**7**	**12**	**AEff**	**0.8936**	**0.9096**	**0.9223**	**0.9322**	**0.9399**	**0.9457**	**0.9500**	**0.9530**	**0.9548**	**0.9558**	**2.1465**	**Robust**	**lessCV**
	7	12	AEff	0.8905	0.9088	0.9235	0.9354	0.9448	0.9523	0.9581	0.9625	0.9658	0.9680	2.6402	Robust	
	7	**12**	**DEff**	**0.9222**	**0.9328**	**0.9411**	**0.9475**	**0.9524**	**0.9562**	**0.9590**	**0.9611**	**0.9625**	**0.9634**	**1.3857**		
	7	12	DEff	0.9322	0.9438	0.9528	0.9598	0.9653	0.9696	0.9728	0.9752	0.9769	0.9781	1.5218		
	Dye1	**3**	**1**	**4**	**7**	**7**	**6**	**2**	**5**	**5**	**1**	**2**	**6**			
	Dye2	**1**	**5**	**2**	**3**	**4**	**3**	**5**	**6**	**7**	**4**	**3**	**4**			
	Dye1	2	1	3	6	4	3	7	2	6	4	5	1			
	Dye2	4	5	5	2	1	7	1	7	3	3	2	6			

8	**7**	**17**	**AEff**	**0.9531**	**0.9628**	**0.9700**	**0.9754**	**0.9794**	**0.9824**	**0.9846**	**0.9861**	**0.9871**	**0.9878**	**1.1286**	**Robust**	**lessCV**				
	7	17	AEff	0.9502	0.9606	0.9684	0.9742	0.9785	0.9817	0.9841	0.9858	0.9869	0.9877	1.2196	Robust					
	7	**17**	**DEff**	**0.9715**	**0.9767**	**0.9805**	**0.9834**	**0.9856**	**0.9872**	**0.9885**	**0.9894**	**0.9900**	**0.9904**	**0.6101**						
	7	17	DEff	0.9701	0.9756	0.9797	0.9828	0.9851	0.9869	0.9882	0.9892	0.9899	0.9904	0.6534						
	Dye1	**6**	**7**	**2**	**5**	**3**	**5**	**5**	**4**	**6**	**6**	**3**	**7**	**4**	**1**	**2**	**1**	**7**		
	Dye2	**1**	**1**	**3**	**3**	**4**	**1**	**4**	**7**	**5**	**2**	**6**	**3**	**6**	**4**	**7**	**2**	**5**		
	Dye1	3	5	2	1	3	7	4	2	4	6	1	6	7	5	2	4	6		
	Dye2	1	7	1	4	2	3	2	6	6	3	7	5	4	3	5	5	1		
9	**7**	**19**	**AEff**	**0.9708**	**0.9755**	**0.9790**	**0.9817**	**0.9836**	**0.9851**	**0.9862**	**0.9870**	**0.9875**	**0.9879**	**0.5515**	**S-robust**	**lessCV**				
	7	19	AEff	0.9665	0.9721	0.9764	0.9796	0.9820	0.9839	0.9853	0.9864	0.9871	0.9877	0.6853	S-robust					
	7	**19**	**DEff**	**0.9830**	**0.9853**	**0.9870**	**0.9884**	**0.9894**	**0.9901**	**0.9907**	**0.9911**	**0.9914**	**0.9916**	**0.2752**						
	7	19	DEff	0.9809	0.9837	0.9858	0.9874	0.9886	0.9895	0.9902	0.9908	0.9912	0.9915	0.3379						
	Dye1	**5**	**7**	**5**	**7**	**2**	**6**	**1**	**3**	**4**	**2**	**7**	**4**	**2**	**1**	**4**	**6**	**1**	**3**	**5**
	Dye2	**1**	**2**	**6**	**3**	**6**	**7**	**4**	**4**	**5**	**4**	**5**	**6**	**3**	**7**	**7**	**1**	**3**	**5**	**2**
	Dye1	3	2	1	1	7	3	7	3	6	4	2	1	5	7	5	2	4	4	6
	Dye2	5	3	6	7	5	4	3	1	2	1	4	2	1	2	6	5	6	7	3
10	**8**	**10**	**AEff**	**0.7364**	**0.8007**	**0.8467**	**0.8800**	**0.9041**	**0.9214**	**0.9337**	**0.9422**	**0.9476**	**0.9508**	**7.6723**	**Non-robust**	**lessCV**				
	8	10	AEff	0.7259	0.7915	0.8390	0.8737	0.8991	0.9176	0.9309	0.9402	0.9464	0.9503	8.0702	Non-robust					
	8	**10**	**DEff**	**0.8502**	**0.8861**	**0.9113**	**0.9293**	**0.9421**	**0.9513**	**0.9577**	**0.9621**	**0.9650**	**0.9666**	**3.9501**						
	8	10	DEff	0.8406	0.8786	0.9055	0.9248	0.9387	0.9487	0.9559	0.9609	0.9642	0.9663	4.2783						
	Dye1	**4**	**8**	**6**	**1**	**4**	**3**	**7**	**2**	**6**	**5**									
	Dye2	**5**	**2**	**2**	**7**	**7**	**4**	**8**	**3**	**1**	**6**									
	Dye1	3	5	1	1	4	2	7	8	6	2									
	Dye2	7	4	8	6	3	1	2	3	4	5									
11	**8**	**11**	**AEff**	**0.7831**	**0.8352**	**0.8727**	**0.9000**	**0.9198**	**0.9339**	**0.9438**	**0.9505**	**0.9547**	**0.9569**	**6.1118**	**Non-robust**	**lessCV**				
	8	11	AEff	0.7641	0.8212	0.8623	0.8921	0.9138	0.9295	0.9407	0.9483	0.9534	0.9563	6.7900	Non-robust					
	8	**11**	**DEff**	**0.8766**	**0.9049**	**0.9251**	**0.9396**	**0.9501**	**0.9576**	**0.9628**	**0.9664**	**0.9687**	**0.9699**	**3.1447**						
	8	11	DEff	0.8665	0.8974	0.9194	0.9353	0.9468	0.9551	0.9611	0.9652	0.9679	0.9696	3.4758						
	Dye1	**8**	**7**	**2**	**1**	**7**	**3**	**6**	**2**	**5**	**4**	**6**								
	Dye2	**1**	**5**	**3**	**2**	**8**	**6**	**8**	**4**	**3**	**7**	**4**								
	Dye1	3	2	7	1	6	5	8	4	4	6	2								
	Dye2	5	6	4	8	3	2	3	5	1	7	1								

12	**8**	**12**	**AEff**	**0.8393**	**0.8812**	**0.9118**	**0.9344**	**0.9509**	**0.9628**	**0.9711**	**0.9768**	**0.9802**	**0.9820**	**4.8512**	**Robust**	**lessCV**				
	8	12	AEff	0.8358	0.8772	0.9078	0.9306	0.9474	0.9598	0.9687	0.9749	0.9790	0.9814	4.9533	Robust					
	8	**12**	**DEff**	**0.9012**	**0.9251**	**0.9424**	**0.9551**	**0.9643**	**0.9711**	**0.9759**	**0.9792**	**0.9814**	**0.9827**	**2.7041**						
	8	12	DEff	0.8975	0.9217	0.9395	0.9525	0.9622	0.9693	0.9745	0.9783	0.9808	0.9824	2.8158						
	Dye1	**2**	**4**	**8**	**5**	**1**	**4**	**8**	**6**	**7**	**7**	**3**	**6**							
	Dye2	**1**	**1**	**5**	**7**	**8**	**5**	**6**	**2**	**3**	**2**	**4**	**3**							
	Dye1	7	7	8	3	5	5	4	6	1	2	4	6							
	Dye2	1	2	4	6	2	8	3	5	8	3	7	1							
13	**8**	**13**	**AEff**	**0.8482**	**0.8823**	**0.9074**	**0.9260**	**0.9397**	**0.9497**	**0.9569**	**0.9618**	**0.9649**	**0.9665**	**4.0570**	**Robust**	**lessCV**				
	8	13	AEff	0.8416	0.8775	0.9042	0.9242	0.9392	0.9504	0.9586	0.9645	0.9685	0.9710	4.4240	Robust					
	8	**13**	**DEff**	**0.9057**	**0.9249**	**0.9391**	**0.9495**	**0.9573**	**0.9629**	**0.9670**	**0.9699**	**0.9718**	**0.9730**	**2.2480**						
	8	13	DEff	0.9064	0.9268	0.9417	0.9529	0.9612	0.9673	0.9718	0.9750	0.9773	0.9787	2.3995						
	Dye1	**6**	**8**	**4**	**3**	**2**	**5**	**4**	**7**	**6**	**2**	**1**	**3**	**8**						
	Dye2	**1**	**2**	**5**	**8**	**5**	**6**	**1**	**3**	**8**	**7**	**7**	**5**	**4**						
	Dye1	8	7	1	1	5	2	3	4	4	2	8	6	6						
	Dye2	7	2	6	7	1	4	5	8	1	5	3	3	2						
14	**8**	**14**	**AEff**	**0.8667**	**0.8963**	**0.9181**	**0.9342**	**0.9462**	**0.9551**	**0.9615**	**0.9660**	**0.9691**	**0.9709**	**3.5302**	**Robust**	**lessCV**				
	8	14	AEff	0.8622	0.8924	0.9149	0.9318	0.9444	0.9537	0.9605	0.9654	0.9687	0.9707	3.6883	Robust					
	8	**14**	**DEff**	**0.9252**	**0.9411**	**0.9528**	**0.9614**	**0.9678**	**0.9726**	**0.9760**	**0.9784**	**0.9800**	**0.9810**	**1.8396**						
	8	14	DEff	0.9220	0.9388	0.9511	0.9602	0.9670	0.9720	0.9756	0.9781	0.9799	0.9809	1.9450						
	Dye1	**6**	**8**	**2**	**4**	**5**	**3**	**1**	**4**	**2**	**7**	**1**	**5**	**3**	**8**					
	Dye2	**1**	**2**	**6**	**8**	**2**	**7**	**5**	**6**	**3**	**8**	**3**	**7**	**4**	**1**					
	Dye1	5	3	4	6	1	4	7	8	6	2	8	1	3	2					
	Dye2	3	7	5	4	8	1	4	2	2	1	7	3	6	5					
15	**8**	**19**	**AEff**	**0.9316**	**0.9469**	**0.9583**	**0.9668**	**0.9731**	**0.9778**	**0.9811**	**0.9835**	**0.9850**	**0.9860**	**1.7886**	**Robust**	**moreCV**				
	8	19	AEff	0.9315	0.9469	0.9583	0.9667	0.9730	0.9776	0.9810	0.9834	0.9850	0.9859	1.7866	Robust					
	8	**19**	**DEff**	**0.9607**	**0.9690**	**0.9751**	**0.9797**	**0.9830**	**0.9855**	**0.9874**	**0.9886**	**0.9895**	**0.9900**	**0.9518**						
	8	19	DEff	0.9605	0.9688	0.9750	0.9796	0.9830	0.9855	0.9873	0.9886	0.9895	0.9900	0.9570						
	Dye1	**5**	**6**	**2**	**2**	**3**	**7**	**4**	**8**	**6**	**1**	**7**	**4**	**1**	**6**	**1**	**3**	**8**	**8**	**5**
	Dye2	**2**	**2**	**1**	**3**	**4**	**5**	**8**	**5**	**1**	**7**	**6**	**5**	**8**	**4**	**3**	**7**	**7**	**2**	**6**
	Dye1	7	5	8	2	6	5	8	1	1	7	1	5	3	4	3	6	2	4	4
	Dye2	5	6	4	8	2	2	5	6	3	2	4	1	7	6	8	3	1	7	3

16	**8**	**21**	**AEff**	**0.9553**	**0.9647**	**0.9718**	**0.9772**	**0.9812**	**0.9842**	**0.9863**	**0.9878**	**0.9887**	**0.9893**	**1.1115**	**Robust**	**lessCV**						
	8	21	AEff	0.9501	0.9605	0.9684	0.9744	0.9790	0.9825	0.9851	0.9869	0.9882	0.9890	1.2713	Robust							
	8	**21**	**DEff**	**0.9732**	**0.9785**	**0.9824**	**0.9854**	**0.9876**	**0.9892**	**0.9904**	**0.9912**	**0.9917**	**0.9921**	**0.6104**								
	8	21	DEff	0.9704	0.9762	0.9806	0.9840	0.9865	0.9884	0.9898	0.9908	0.9915	0.9919	0.6953								
	Dye1	**6**	**5**	**7**	**6**	**3**	**8**	**2**	**4**	**7**	**2**	**5**	**3**	**8**	**5**	**4**	**1**	**1**	**8**	**4**	**6**	**3**
	Dye2	**1**	**1**	**5**	**3**	**5**	**7**	**3**	**5**	**6**	**4**	**2**	**1**	**2**	**8**	**6**	**4**	**8**	**6**	**7**	**2**	**7**
	Dye1	3	7	7	5	2	1	5	4	1	8	1	3	2	5	4	2	3	6	8	6	4
	Dye2	1	6	2	8	6	6	4	7	7	7	4	2	5	1	2	8	8	5	1	3	3
17	**8**	**22**	**AEff**	**0.9551**	**0.9639**	**0.9705**	**0.9754**	**0.9791**	**0.9818**	**0.9837**	**0.9851**	**0.9860**	**0.9866**	**1.0264**	**Robust**	**lessCV**						
	8	22	AEff	0.9549	0.9641	0.9711	0.9764	0.9803	0.9832	0.9853	0.9869	0.9879	0.9885	1.0973	Robust							
	8	**22**	**DEff**	**0.9730**	**0.9778**	**0.9814**	**0.9840**	**0.9860**	**0.9875**	**0.9886**	**0.9894**	**0.9899**	**0.9903**	**0.5557**								
	8	22	DEff	0.9749	0.9798	0.9835	0.9862	0.9882	0.9897	0.9908	0.9916	0.9921	0.9925	0.5657								
	Dye1	**2**	**4**	**6**	**2**	**6**	**6**	**2**	**5**	**8**	**4**	**3**	**1**	**7**	**5**	**5**	**4**	**8**	**7**	**3**	**7**	**1**	**8**
	Dye2	**1**	**1**	**1**	**3**	**2**	**4**	**4**	**6**	**5**	**5**	**8**	**5**	**3**	**3**	**7**	**7**	**4**	**1**	**6**	**2**	**8**	**2**
	Dye1	3	1	8	3	4	7	3	1	2	5	1	4	5	7	4	5	6	6	2	8	6	2
	Dye2	7	5	2	2	8	6	4	2	4	3	8	1	4	1	7	6	1	8	7	3	3	5
18	**9**	**9**	**AEff**	**0.5333**	**0.7088**	**0.8134**	**0.8803**	**0.9247**	**0.9545**	**0.9743**	**0.9872**	**0.9949**	**0.9988**	**16.4878**	**Non-robust**	**moreCV**						
	9	9	AEff	0.5120	0.5520	0.5770	0.5941	0.6065	0.6157	0.6227	0.6280	0.6322	0.6353	6.3538	Non-robust							
	9	**9**	**DEff**	**0.7698**	**0.8575**	**0.9085**	**0.9411**	**0.9628**	**0.9775**	**0.9873**	**0.9936**	**0.9974**	**0.9994**	**7.5740**								
	9	9	DEff	0.6285	0.6508	0.6655	0.6757	0.6831	0.6885	0.6924	0.6952	0.6972	0.6986	3.2255								
	Dye1	**4**	**8**	**5**	**7**	**3**	**1**	**9**	**2**	**6**												
	Dye2	**6**	**3**	**7**	**9**	**1**	**5**	**4**	**8**	**2**												
	Dye1	1	2	3	4	1	1	1	1	1												
	Dye2	2	3	4	1	5	6	7	8	9												
19	**9**	**12**	**AEff**	**0.7500**	**0.8118**	**0.8561**	**0.8881**	**0.9111**	**0.9275**	**0.9389**	**0.9466**	**0.9514**	**0.9541**	**7.2594**	**Non-robust**	**lessCV**						
	9	12	AEff	0.7331	0.7992	0.8467	0.8810	0.9059	0.9237	0.9363	0.9448	0.9504	0.9536	7.8846	Non-robust							
	9	**12**	**DEff**	**0.8589**	**0.8934**	**0.9175**	**0.9346**	**0.9468**	**0.9554**	**0.9614**	**0.9655**	**0.9681**	**0.9695**	**3.7346**								
	9	12	DEff	0.8493	0.8863	0.9122	0.9307	0.9439	0.9533	0.9599	0.9645	0.9675	0.9692	4.0595								
	Dye1	**9**	**6**	**4**	**3**	**1**	**1**	**2**	**6**	**5**	**7**	**8**	**8**									
	Dye2	**1**	**3**	**2**	**9**	**5**	**2**	**6**	**7**	**7**	**8**	**9**	**4**									
	Dye1	6	7	1	3	8	9	5	4	3	2	6	2									
	Dye2	9	4	7	1	5	2	4	6	8	1	3	5									

20	**9**	**14**	**AEff**	**0.8212**	**0.8659**	**0.8986**	**0.9226**	**0.9401**	**0.9527**	**0.9617**	**0.9677**	**0.9715**	**0.9736**	**5.2356**	**Non-robust**	**lessCV**									
	9	14	AEff	0.7996	0.8509	0.8883	0.9158	0.9360	0.9509	0.9615	0.9690	0.9740	0.9771	6.1103	Non-robust										
	9	**14**	**DEff**	**0.8898**	**0.9154**	**0.9340**	**0.9475**	**0.9573**	**0.9645**	**0.9696**	**0.9732**	**0.9756**	**0.9770**	**2.9112**											
	9	14	DEff	0.8838	0.9125	0.9332	0.9482	0.9592	0.9672	0.9730	0.9771	0.9799	0.9817	3.2552											
	Dye1	**9**	**5**	**6**	**1**	**3**	**4**	**2**	**7**	**2**	**8**	**1**	**7**	**7**	**8**										
	Dye2	**2**	**1**	**8**	**3**	**6**	**9**	**3**	**4**	**7**	**9**	**4**	**6**	**5**	**5**										
	Dye1	8	8	1	4	3	1	4	7	2	9	6	5	6	2										
	Dye2	1	5	9	5	7	2	6	8	4	3	3	9	1	7										
21	**9**	**15**	**AEff**	**0.8389**	**0.8772**	**0.9049**	**0.9250**	**0.9397**	**0.9503**	**0.9578**	**0.9629**	**0.9661**	**0.9678**	**4.4145**	**Robust**	**lessCV**									
	9	15	AEff	0.8331	0.8728	0.9017	0.9231	0.9389	0.9505	0.9589	0.9648	0.9688	0.9712	4.7244	Robust										
	9	**15**	**DEff**	**0.9040**	**0.9254**	**0.9407**	**0.9519**	**0.9600**	**0.9659**	**0.9701**	**0.9730**	**0.9749**	**0.9760**	**2.3926**											
	9	15	DEff	0.9039	0.9262	0.9423	0.9541	0.9628	0.9691	0.9736	0.9768	0.9789	0.9802	2.5286											
	Dye1	**7**	**8**	**2**	**3**	**4**	**6**	**4**	**1**	**5**	**5**	**2**	**9**	**9**	**3**	**6**									
	Dye2	**2**	**4**	**1**	**4**	**9**	**5**	**6**	**5**	**3**	**8**	**8**	**7**	**1**	**7**	**7**									
	Dye1	3	7	9	3	8	5	2	6	4	6	9	1	2	1	8									
	Dye2	6	4	2	5	2	1	5	7	8	1	3	4	7	9	3									
22	**9**	**16**	**AEff**	**0.8532**	**0.8866**	**0.9113**	**0.9295**	**0.9429**	**0.9527**	**0.9596**	**0.9645**	**0.9676**	**0.9693**	**3.9638**	**Robust**	**lessCV**									
	9	16	AEff	0.8451	0.8825	0.9094	0.9290	0.9434	0.9539	0.9615	0.9669	0.9705	0.9727	4.3297	Robust										
	9	**16**	**DEff**	**0.9144**	**0.9333**	**0.9470**	**0.9570**	**0.9644**	**0.9697**	**0.9735**	**0.9762**	**0.9779**	**0.9789**	**2.1373**											
	9	16	DEff	0.9157	0.9355	0.9498	0.9602	0.9678	0.9733	0.9773	0.9800	0.9819	0.9830	2.2158											
	Dye1	**9**	**1**	**7**	**9**	**3**	**2**	**4**	**7**	**3**	**6**	**1**	**8**	**5**	**2**	**1**	**6**								
	Dye2	**4**	**4**	**3**	**3**	**1**	**8**	**6**	**9**	**2**	**7**	**8**	**9**	**7**	**5**	**5**	**2**								
	Dye1	4	3	9	2	1	5	8	9	7	2	6	3	4	1	7	5								
	Dye2	2	6	1	1	3	8	2	4	5	6	5	8	3	7	4	9								
23	**9**	**24**	**AEff**	**0.9403**	**0.9520**	**0.9609**	**0.9676**	**0.9726**	**0.9763**	**0.9790**	**0.9809**	**0.9821**	**0.9827**	**1.4009**	**Robust**	**lessCV**									
	9	24	AEff	0.9400	0.9535	0.9636	0.9712	0.9769	0.9811	0.9842	0.9864	0.9879	0.9888	1.5976	Robust										
	9	**24**	**DEff**	**0.9647**	**0.9713**	**0.9762**	**0.9799**	**0.9826**	**0.9846**	**0.9860**	**0.9870**	**0.9877**	**0.9881**	**0.7600**											
	9	24	DEff	0.9657	0.9731	0.9787	0.9829	0.9859	0.9882	0.9899	0.9911	0.9919	0.9924	0.8633											
	Dye1	**9**	**7**	**9**	**9**	**3**	**8**	**4**	**5**	**7**	**3**	**8**	**4**	**1**	**5**	**8**	**5**	**2**	**1**	**1**	**6**	**3**	**2**	**6**	**6**
	Dye2	**2**	**5**	**4**	**1**	**4**	**1**	**6**	**9**	**3**	**9**	**9**	**5**	**7**	**2**	**7**	**8**	**3**	**4**	**2**	**9**	**8**	**6**	**8**	**7**
	Dye1	9	2	3	2	3	4	5	5	7	9	8	6	3	5	1	8	4	7	9	6	1	4	1	2
	Dye2	6	4	9	8	2	7	2	7	1	4	3	1	6	3	2	9	1	8	5	7	8	3	5	6

24	**9**	**25**	**AEff**	**0.9450**	**0.9578**	**0.9673**	**0.9742**	**0.9793**	**0.9831**	**0.9857**	**0.9875**	**0.9887**	**0.9894**	**0.9894**	**S-robust**	**lessCV**															
	9	25	AEff	0.9437	0.9567	0.9663	0.9735	0.9787	0.9826	0.9853	0.9873	0.9885	0.9893	1.4875	Robust																
	9	**25**	**DEff**	**0.9701**	**0.9769**	**0.9818**	**0.9855**	**0.9881**	**0.9900**	**0.9914**	**0.9923**	**0.9929**	**0.9933**	**0.9933**																	
	9	25	DEff	0.9694	0.9763	0.9814	0.9851	0.9878	0.9898	0.9912	0.9922	0.9929	0.9932	0.7680																	
	Dye1	**6**	**8**	**5**	**9**	**5**	**2**	**8**	**6**	**5**	**7**	**8**	**2**	**9**	**4**	**1**	**1**	**7**	**2**	**6**	**3**	**4**	**4**	**9**	**1**	**3**					
	Dye2	**1**	**1**	**1**	**5**	**3**	**8**	**5**	**8**	**7**	**6**	**4**	**7**	**3**	**5**	**9**	**4**	**8**	**9**	**3**	**2**	**7**	**9**	**6**	**2**	**4**					
	Dye1	7	6	2	4	1	7	8	3	9	2	8	9	5	6	2	8	3	1	9	4	4	1	5	5	3					
	Dye2	1	3	1	8	4	2	5	5	7	6	9	6	6	1	3	2	9	8	4	7	5	3	7	2	4					
25	**9**	**31**	**AEff**	**0.9764**	**0.9819**	**0.9859**	**0.9888**	**0.9910**	**0.9925**	**0.9936**	**0.9944**	**0.9949**	**0.9952**	**0.6034**	**S-robust**	**lessCV**															
	9	31	AEff	0.9750	0.9808	0.9850	0.9881	0.9904	0.9921	0.9933	0.9942	0.9948	0.9952	0.6462	S-robust																
	9	**31**	**DEff**	**0.9862**	**0.9891**	**0.9912**	**0.9927**	**0.9939**	**0.9947**	**0.9953**	**0.9958**	**0.9961**	**0.9963**	**0.3223**																	
	9	31	DEff	0.9854	0.9885	0.9907	0.9924	0.9936	0.9945	0.9952	0.9957	0.9960	0.9963	0.3450																	
	Dye1	**6**	**3**	**8**	**5**	**7**	**7**	**8**	**4**	**8**	**5**	**4**	**6**	**9**	**5**	**4**	**6**	**3**	**6**	**2**	**2**	**1**	**1**	**8**	**2**	**1**	**7**	**3**	**3**	**9**	**9**
	Dye2	**1**	**1**	**1**	**4**	**1**	**8**	**4**	**2**	**6**	**8**	**7**	**5**	**7**	**9**	**6**	**2**	**7**	**7**	**3**	**5**	**4**	**5**	**2**	**9**	**9**	**2**	**8**	**5**	**6**	**3**
	Dye1	**4**																													
	Dye2	**3**																													
	Dye1	6	3	1	7	4	7	5	2	6	1	4	2	8	8	3	3	7	4	8	8	6	2	1	5	9	5	4	2	7	9
	Dye2	1	4	7	9	6	8	6	6	3	3	8	5	3	2	7	5	2	1	9	5	8	4	9	4	6	1	7	1	5	3
	Dye1	9																													
	Dye2	2																													
26	**9**	**32**	**AEff**	**0.9813**	**0.9855**	**0.9886**	**0.9908**	**0.9925**	**0.9937**	**0.9946**	**0.9952**	**0.9956**	**0.9958**	**0.4648**	**S-robust**	**lessCV**															
	9	32	AEff	0.9803	0.9847	0.9880	0.9904	0.9921	0.9934	0.9944	0.9950	0.9955	0.9958	0.4958	S-robust																
	9	**32**	**DEff**	**0.9887**	**0.9909**	**0.9925**	**0.9938**	**0.9947**	**0.9953**	**0.9958**	**0.9962**	**0.9964**	**0.9966**	**0.2533**																	
	9	32	DEff	0.9882	0.9905	0.9922	0.9935	0.9945	0.9952	0.9957	0.9961	0.9964	0.9966	0.2687																	
	Dye1	**2**	**4**	**8**	**3**	**9**	**7**	**3**	**2**	**5**	**4**	**8**	**6**	**9**	**9**	**2**	**6**	**9**	**5**	**6**	**8**	**3**	**4**	**5**	**2**	**3**	**8**	**7**	**1**	**7**	**1**
	Dye2	**1**	**1**	**1**	**8**	**6**	**6**	**7**	**4**	**3**	**7**	**4**	**3**	**2**	**8**	**7**	**1**	**4**	**6**	**2**	**5**	**4**	**5**	**9**	**5**	**2**	**6**	**9**	**5**	**8**	**7**
	Dye1	**1**	**1**																												
	Dye2	**3**	**9**																												
	Dye1	6	3	9	5	2	3	5	5	9	2	4	9	6	2	8	1	7	4	8	4	7	1	1	7	8	7	6	3	4	6
	Dye2	3	4	1	1	5	7	8	7	3	1	5	5	9	9	2	6	9	6	3	2	6	8	4	2	4	8	8	2	9	5
	Dye1	3	1																												
	Dye2	1	7																												

27	**9**	**34**	**AEff**	**0.9867**	**0.9889**	**0.9905**	**0.9917**	**0.9926**	**0.9932**	**0.9937**	**0.9940**	**0.9942**	**0.9944**	**0.2452**	**S-robust**	**lessCV**
	9	34	AEff	0.9857	0.9881	0.9899	0.9912	0.9922	0.9929	0.9935	0.9939	0.9942	0.9944	0.2758	S-robust	
	9	**34**	**DEff**	**0.9927**	**0.9938**	**0.9945**	**0.9951**	**0.9955**	**0.9958**	**0.9961**	**0.9962**	**0.9964**	**0.9964**	**0.1185**		
	9	34	DEff	0.9922	0.9934	0.9943	0.9949	0.9954	0.9957	0.9960	0.9962	0.9963	0.9964	0.1329		

Dye1	**2**	**3**	**2**	**3**	**7**	**2**	**2**	**9**	**9**	**8**	**6**	**6**	**4**	**3**	**1**	**9**	**3**	**1**	**7**	**4**	**9**	**6**	**5**	**5**	**1**	**4**	**7**	**8**	**1**	**7**
Dye2	**1**	**1**	**6**	**6**	**1**	**9**	**5**	**6**	**3**	**1**	**4**	**5**	**9**	**8**	**5**	**8**	**2**	**9**	**3**	**2**	**7**	**8**	**4**	**3**	**6**	**3**	**4**	**7**	**4**	**2**
Dye1	**5**	**6**	**5**	**8**																										
Dye2	**7**	**7**	**8**	**2**																										

Dye1	2	2	7	8	9	8	4	5	1	7	8	6	1	2	5	7	9	9	3	4	4	6	1	3	2	3	3	6	4	5
Dye2	6	3	1	5	8	4	1	6	5	2	1	9	2	8	2	3	3	2	5	7	6	7	9	8	4	4	1	3	9	9
Dye1	8	7	1	5																										
Dye2	7	5	6	4																										

28	**10**	**12**	**AEff**	**0.6650**	**0.7458**	**0.8026**	**0.8429**	**0.8715**	**0.8917**	**0.9057**	**0.9151**	**0.9211**	**0.9245**	**9.6821**	**Non-robust**	**lessCV**
	10	12	AEff	0.6618	0.7599	0.8248	0.8690	0.8996	0.9209	0.9356	0.9454	0.9516	0.9552	10.5587	Non-robust	
	10	**12**	**DEff**	**0.8149**	**0.8603**	**0.8908**	**0.9119**	**0.9265**	**0.9366**	**0.9435**	**0.9480**	**0.9508**	**0.9522**	**4.7419**		
	10	12	DEff	0.8137	0.8671	0.9019	0.9254	0.9416	0.9529	0.9606	0.9658	0.9691	0.9709	5.3153		

Dye1	**3**	**10**	**9**	**1**	**10**	**5**	**2**	**7**	**8**	**4**	**9**	**6**
Dye2	**4**	**2**	**7**	**9**	**5**	**1**	**8**	**6**	**9**	**10**	**3**	**10**

Dye1	1	9	7	10	2	2	6	8	4	3	5	1
Dye2	8	2	3	4	7	6	1	3	9	5	4	10

29	**10**	**13**	**AEff**	**0.7101**	**0.7819**	**0.8313**	**0.8658**	**0.8903**	**0.9076**	**0.9196**	**0.9278**	**0.9330**	**0.9360**	**8.1899**	**Non-robust**	**lessCV**
	10	13	AEff	0.6922	0.7742	0.8309	0.8708	0.8992	0.9192	0.9332	0.9428	0.9489	0.9525	9.3757	Non-robust	
	10	**13**	**DEff**	**0.8379**	**0.8778**	**0.9047**	**0.9234**	**0.9365**	**0.9456**	**0.9519**	**0.9561**	**0.9587**	**0.9601**	**4.1630**		
	10	13	DEff	0.8281	0.8737	0.9045	0.9259	0.9410	0.9517	0.9591	0.9642	0.9675	0.9694	4.7886		

Dye1	**7**	**5**	**10**	**8**	**4**	**1**	**6**	**6**	**3**	**2**	**9**	**9**	**3**
Dye2	**9**	**3**	**6**	**1**	**10**	**5**	**7**	**1**	**10**	**9**	**4**	**8**	**2**

Dye1	5	10	4	5	9	2	2	7	3	6	8	1	4
Dye2	9	1	10	8	2	7	4	6	6	1	3	5	3

30	**10**	**14**	**AEff**	**0.7418**	**0.8122**	**0.8605**	**0.8944**	**0.9185**	**0.9355**	**0.9473**	**0.9552**	**0.9601**	**0.9628**	**7.7695**	**Non-robust**	**lessCV**
	10	14	AEff	0.7336	0.8066	0.8564	0.8914	0.9163	0.9339	0.9462	0.9544	0.9597	0.9626	8.0655	Non-robust	
	10	**14**	**DEff**	**0.8577**	**0.8950**	**0.9206**	**0.9386**	**0.9513**	**0.9603**	**0.9666**	**0.9708**	**0.9735**	**0.9751**	**3.9362**		
	10	14	DEff	0.8536	0.8922	0.9186	0.9371	0.9502	0.9595	0.9660	0.9704	0.9733	0.9750	4.0708		

	Dye1	**8**	**1**	**6**	**10**	**3**	**4**	**2**	**9**	**5**	**7**	**3**	**4**	**7**	**10**			
	Dye2	**2**	**4**	**1**	**1**	**5**	**9**	**10**	**7**	**8**	**6**	**9**	**8**	**2**	**3**			
	Dye1	1	3	2	7	3	1	4	8	9	6	10	5	8	2			
	Dye2	7	6	4	8	2	10	5	6	4	5	3	1	9	7			
31	**10**	**15**	**AEff**	**0.8021**	**0.8563**	**0.8959**	**0.9248**	**0.9458**	**0.9609**	**0.9714**	**0.9786**	**0.9830**	**0.9855**	**6.2807**	**Non-robust**	**lessCV**		
	10	15	AEff	0.7759	0.8367	0.8810	0.9135	0.9373	0.9546	0.9669	0.9755	0.9812	0.9846	7.2011	Non-robust			
	10	**15**	**DEff**	**0.8827**	**0.9141**	**0.9364**	**0.9524**	**0.9640**	**0.9723**	**0.9781**	**0.9821**	**0.9847**	**0.9863**	**3.4340**				
	10	15	DEff	0.8693	0.9042	0.9290	0.9469	0.9598	0.9692	0.9759	0.9807	0.9839	0.9859	3.8768				
	Dye1	**6**	**8**	**2**	**9**	**4**	**10**	**5**	**1**	**4**	**3**	**7**	**9**	**2**	**3**	**1**		
	Dye2	**2**	**3**	**5**	**1**	**10**	**8**	**7**	**5**	**6**	**9**	**4**	**6**	**8**	**7**	**10**		
	Dye1	5	3	1	7	6	4	7	8	9	2	2	4	10	8	10		
	Dye2	3	9	6	8	5	6	4	1	2	1	10	9	7	3	5		
32	**10**	**16**	**AEff**	**0.8106**	**0.8616**	**0.8978**	**0.9237**	**0.9422**	**0.9553**	**0.9644**	**0.9704**	**0.9741**	**0.9760**	**5.6659**	**Non-robust**	**lessCV**		
	10	16	AEff	0.7937	0.8479	0.8866	0.9146	0.9350	0.9497	0.9602	0.9675	0.9723	0.9751	6.2476	Non-robust			
	10	**16**	**DEff**	**0.8933**	**0.9209**	**0.9403**	**0.9541**	**0.9639**	**0.9709**	**0.9758**	**0.9791**	**0.9811**	**0.9823**	**2.9493**				
	10	16	DEff	0.8833	0.9131	0.9342	0.9494	0.9603	0.9682	0.9738	0.9777	0.9803	0.9819	3.2731				
	Dye1	**2**	**5**	**5**	**3**	**10**	**7**	**8**	**8**	**9**	**4**	**7**	**9**	**2**	**6**	**1**	**4**	
	Dye2	**1**	**1**	**6**	**2**	**8**	**3**	**3**	**5**	**8**	**9**	**6**	**7**	**10**	**4**	**9**	**10**	
	Dye1	7	1	3	9	2	4	6	8	10	1	7	4	8	2	6	5	
	Dye2	3	6	10	1	4	3	5	2	5	8	2	1	10	9	7	9	
33	**10**	**17**	**AEff**	**0.8334**	**0.8732**	**0.9023**	**0.9236**	**0.9392**	**0.9504**	**0.9584**	**0.9638**	**0.9672**	**0.9691**	**4.6581**	**Robust**	**lessCV**		
	10	17	AEff	0.8290	0.8704	0.9007	0.9230	0.9394	0.9513	0.9599	0.9658	0.9697	0.9719	4.8995	Robust			
	10	**17**	**DEff**	**0.9021**	**0.9248**	**0.9412**	**0.9531**	**0.9616**	**0.9678**	**0.9722**	**0.9752**	**0.9771**	**0.9783**	**2.5307**				
	10	17	DEff	0.9026	0.9262	0.9431	0.9554	0.9643	0.9707	0.9753	0.9784	0.9805	0.9817	2.6181				
	Dye1	**6**	**4**	**10**	**9**	**2**	**10**	**5**	**8**	**8**	**4**	**6**	**1**	**1**	**2**	**3**	**5**	**7**
	Dye2	**3**	**2**	**1**	**5**	**3**	**6**	**7**	**9**	**10**	**9**	**4**	**9**	**2**	**7**	**8**	**6**	**10**
	Dye1	1	2	5	1	2	8	10	3	7	8	3	9	4	6	4	6	10
	Dye2	9	10	1	7	8	1	7	9	4	4	5	2	3	2	6	5	3
34	**10**	**18**	**AEff**	**0.8444**	**0.8823**	**0.9101**	**0.9305**	**0.9455**	**0.9564**	**0.9641**	**0.9695**	**0.9729**	**0.9748**	**4.4414**	**Robust**	**lessCV**		
	10	18	AEff	0.8416	0.8804	0.9086	0.9293	0.9444	0.9555	0.9634	0.9688	0.9724	0.9745	4.5245	Robust			

	10	**18**	**DEff**	**0.9147**	**0.9356**	**0.9507**	**0.9616**	**0.9695**	**0.9752**	**0.9792**	**0.9820**	**0.9837**	**0.9847**	**2.3095**														
	10	18	DEff	0.9129	0.9344	0.9498	0.9609	0.9690	0.9748	0.9789	0.9818	0.9836	0.9847	2.3625														
	Dye1	**9**	**10**	**10**	**4**	**7**	**9**	**8**	**3**	**1**	**7**	**8**	**1**	**5**	**6**	**2**	**2**	**3**	**5**									
	Dye2	**1**	**8**	**7**	**10**	**5**	**7**	**5**	**8**	**6**	**3**	**9**	**10**	**2**	**3**	**9**	**4**	**4**	**6**									
	Dye1	9	7	3	1	3	7	5	5	2	10	4	6	9	4	6	8	2	1									
	Dye2	3	4	10	2	1	2	4	3	5	6	1	7	6	9	8	5	10	8									
35	**10**	**24**	**AEff**	**0.9308**	**0.9464**	**0.9582**	**0.9673**	**0.9741**	**0.9793**	**0.9830**	**0.9856**	**0.9873**	**0.9882**	**1.8986**	**Robust**	**lessCV**												
	10	24	AEff	0.9269	0.9430	0.9553	0.9648	0.9721	0.9776	0.9817	0.9847	0.9867	0.9879	2.0148	Robust													
	10	**24**	**DEff**	**0.9590**	**0.9682**	**0.9750**	**0.9802**	**0.9841**	**0.9869**	**0.9890**	**0.9904**	**0.9913**	**0.9919**	**1.0692**														
	10	24	DEff	0.9562	0.9658	0.9731	0.9786	0.9828	0.9859	0.9882	0.9899	0.9910	0.9917	1.1547														
	Dye1	**6**	**9**	**8**	**2**	**4**	**10**	**6**	**4**	**10**	**1**	**10**	**1**	**7**	**2**	**5**	**5**	**7**	**1**	**3**	**8**	**3**	**9**	**5**	**3**			
	Dye2	**1**	**7**	**1**	**5**	**5**	**6**	**7**	**10**	**2**	**2**	**8**	**4**	**8**	**3**	**8**	**6**	**4**	**9**	**9**	**3**	**4**	**10**	**9**	**6**			
	Dye1	3	9	3	6	7	8	9	5	7	1	1	8	2	1	6	4	6	2	10	5	2	10	7	4			
	Dye2	8	6	4	3	3	2	4	8	2	7	8	9	10	10	1	1	5	4	5	7	6	3	9	5			
36	**10**	**26**	**AEff**	**0.9414**	**0.9544**	**0.9645**	**0.9723**	**0.9783**	**0.9829**	**0.9862**	**0.9886**	**0.9901**	**0.9909**	**1.6332**	**Robust**	**lessCV**												
	10	26	AEff	0.9409	0.9537	0.9636	0.9714	0.9774	0.9820	0.9854	0.9879	0.9896	0.9906	1.6345	Robust													
	10	**26**	**DEff**	**0.9632**	**0.9714**	**0.9777**	**0.9824**	**0.9860**	**0.9887**	**0.9906**	**0.9920**	**0.9928**	**0.9933**	**0.9801**														
	10	26	DEff	0.9636	0.9715	0.9776	0.9822	0.9857	0.9883	0.9903	0.9917	0.9926	0.9932	0.9598														
	Dye1	**3**	**8**	**6**	**10**	**7**	**8**	**9**	**7**	**2**	**7**	**2**	**1**	**1**	**6**	**5**	**4**	**4**	**4**	**10**	**10**	**5**	**2**	**6**	**3**	**1**	**9**	
	Dye2	**1**	**1**	**3**	**3**	**10**	**2**	**4**	**6**	**3**	**2**	**5**	**7**	**9**	**9**	**10**	**5**	**7**	**8**	**8**	**7**	**6**	**9**	**8**	**4**	**5**	**10**	
	Dye1	3	2	5	5	1	9	6	9	2	9	3	3	7	7	8	8	6	2	1	1	10	4	4	10	4	6	
	Dye2	7	8	8	7	2	7	1	8	10	6	6	10	2	1	1	3	2	4	10	4	5	9	5	9	3	5	
37	**10**	**27**	**AEff**	**0.9429**	**0.9554**	**0.9649**	**0.9722**	**0.9777**	**0.9819**	**0.9849**	**0.9870**	**0.9884**	**0.9891**	**1.5196**	**Robust**	**lessCV**												
	10	27	AEff	0.9407	0.9535	0.9633	0.9709	0.9766	0.9810	0.9842	0.9865	0.9881	0.9890	1.5876	Robust													
	10	**27**	**DEff**	**0.9665**	**0.9739**	**0.9794**	**0.9836**	**0.9867**	**0.9889**	**0.9906**	**0.9917**	**0.9925**	**0.9929**	**0.8552**														
	10	27	DEff	0.9652	0.9728	0.9785	0.9828	0.9861	0.9885	0.9902	0.9915	0.9923	0.9928	0.8952														
	Dye1	**10**	**1**	**3**	**10**	**5**	**8**	**6**	**6**	**1**	**3**	**7**	**4**	**4**	**2**	**5**	**9**	**8**	**9**	**1**	**4**	**2**	**6**	**2**	**10**	**7**	**9**	**5**
	Dye2	**1**	**3**	**4**	**4**	**1**	**2**	**8**	**7**	**9**	**6**	**9**	**5**	**7**	**7**	**2**	**5**	**9**	**10**	**8**	**8**	**10**	**10**	**3**	**5**	**1**	**3**	**6**
	Dye1	3	1	4	4	5	5	9	1	3	3	7	6	5	7	9	8	2	10	6	2	7	8	1	4	8	10	2
	Dye2	1	8	2	9	2	8	6	6	4	10	6	3	4	4	10	3	3	7	5	9	2	9	10	1	7	5	1

38	**10**	**32**	**AEff**	**0.9597**	**0.9691**	**0.9762**	**0.9815**	**0.9854**	**0.9883**	**0.9903**	**0.9918**	**0.9927**	**0.9932**	**1.0901**																**Robust**	**lessCV**
	10	32	AEff	0.9591	0.9686	0.9758	0.9812	0.9851	0.9881	0.9902	0.9917	0.9926	0.9932	1.1083																Robust	
	10	**32**	**DEff**	**0.9777**	**0.9829**	**0.9868**	**0.9896**	**0.9917**	**0.9932**	**0.9942**	**0.9950**	**0.9954**	**0.9957**	**0.5785**																	
	10	32	DEff	0.9774	0.9826	0.9865	0.9894	0.9915	0.9931	0.9942	0.9949	0.9954	0.9957	0.5900																	
	Dye1	**5**	**9**	**10**	**4**	**4**	**8**	**1**	**6**	**10**	**4**	**1**	**6**	**8**	**5**	**7**	**2**	**7**	**5**	**1**	**6**	**9**	**3**	**10**	**2**	**10**	**8**	**3**	**7**	**9**	**9**
	Dye2	**1**	**3**	**5**	**3**	**6**	**1**	**3**	**8**	**7**	**7**	**6**	**5**	**9**	**2**	**5**	**4**	**9**	**9**	**7**	**10**	**4**	**10**	**4**	**1**	**2**	**10**	**5**	**8**	**6**	**2**
	Dye1	**2**	**3**																												
	Dye2	**8**	**8**																												
	Dye1	1	10	7	9	5	3	1	4	9	4	10	10	9	6	5	3	4	2	7	8	1	1	2	7	6	3	4	5	2	8
	Dye2	6	8	1	1	4	10	4	10	3	6	2	5	4	8	2	2	3	6	2	3	8	10	1	5	5	7	7	9	9	7
	Dye1	8	6																												
	Dye2	9	3																												
39	**10**	**33**	**AEff**	**0.9639**	**0.9719**	**0.9779**	**0.9825**	**0.9859**	**0.9884**	**0.9902**	**0.9915**	**0.9923**	**0.9928**	**0.9394**																**S-robust**	**lessCV**
	10	33	AEff	0.9597	0.9691	0.9761	0.9813	0.9851	0.9879	0.9899	0.9913	0.9922	0.9927	1.0727																Robust	
	10	**33**	**DEff**	**0.9795**	**0.9840**	**0.9874**	**0.9899**	**0.9917**	**0.9930**	**0.9940**	**0.9946**	**0.9951**	**0.9953**	**0.5084**																	
	10	33	DEff	0.9777	0.9828	0.9865	0.9893	0.9913	0.9928	0.9938	0.9945	0.9950	0.9953	0.5637																	
	Dye1	**6**	**9**	**6**	**3**	**4**	**9**	**6**	**5**	**3**	**10**	**2**	**2**	**7**	**7**	**5**	**2**	**8**	**5**	**1**	**1**	**9**	**8**	**3**	**4**	**1**	**10**	**5**	**4**	**1**	**7**
	Dye2	**1**	**1**	**9**	**8**	**1**	**5**	**8**	**6**	**2**	**6**	**10**	**6**	**6**	**5**	**4**	**7**	**5**	**3**	**8**	**3**	**3**	**2**	**7**	**2**	**7**	**3**	**10**	**9**	**10**	**4**
	Dye1	**2**	**10**	**8**																											
	Dye2	**9**	**4**	**4**																											
	Dye1	9	4	7	9	7	1	10	3	1	3	8	5	7	2	1	2	4	9	3	10	2	2	6	6	10	5	8	1	5	6
	Dye2	2	10	5	8	10	5	9	4	6	6	7	3	4	8	10	1	9	1	8	3	3	7	2	5	6	4	6	7	9	4
	Dye1	4	8	3																											
	Dye2	2	5	1																											
40	**10**	**35**	**AEff**	**0.9709**	**0.9787**	**0.9844**	**0.9886**	**0.9917**	**0.9939**	**0.9954**	**0.9965**	**0.9972**	**0.9975**	**0.8577**																**S-robust**	**lessCV**
	10	35	AEff	0.9696	0.9777	0.9836	0.9880	0.9912	0.9935	0.9951	0.9963	0.9970	0.9975	0.8952																S-robust	
	10	**35**	**DEff**	**0.9830**	**0.9872**	**0.9903**	**0.9926**	**0.9942**	**0.9955**	**0.9963**	**0.9969**	**0.9973**	**0.9976**	**0.4661**																	
	10	35	DEff	0.9823	0.9867	0.9899	0.9922	0.9940	0.9953	0.9962	0.9968	0.9973	0.9976	0.4871																	
	Dye1	**2**	**8**	**3**	**3**	**5**	**9**	**8**	**6**	**3**	**9**	**10**	**5**	**5**	**1**	**7**	**10**	**6**	**7**	**2**	**10**	**7**	**5**	**6**	**2**	**7**	**9**	**4**	**3**	**4**	**6**
	Dye2	**1**	**1**	**1**	**5**	**9**	**10**	**2**	**8**	**6**	**7**	**2**	**7**	**8**	**7**	**8**	**1**	**2**	**4**	**5**	**8**	**2**	**10**	**4**	**3**	**6**	**4**	**10**	**9**	**5**	**9**
	Dye1	**10**	**1**	**1**	**4**	**8**																									
	Dye2	**6**	**9**	**4**	**3**	**3**																									
	Dye1	9	10	9	7	1	9	1	2	7	7	5	7	10	4	5	10	1	8	4	5	5	8	2	6	8	3	2	10	6	6
	Dye2	7	4	3	5	9	10	4	8	8	4	10	2	8	9	1	6	2	1	2	4	6	9	3	9	5	8	10	1	1	7
	Dye1	6	4	3	3	2																									
	Dye2	3	3	5	7	6																									

41	**10**	**36**	**AEff**	**0.9720**	**0.9787**	**0.9836**	**0.9873**	**0.9900**	**0.9919**	**0.9933**	**0.9942**	**0.9948**	**0.9952**	**0.7473**	**S-robust**	**lessCV**															
	10	36	AEff	0.9706	0.9776	0.9828	0.9866	0.9894	0.9915	0.9930	0.9940	0.9947	0.9951	0.7886	S-robust																
	10	**36**	**DEff**	**0.9841**	**0.9877**	**0.9903**	**0.9922**	**0.9937**	**0.9947**	**0.9954**	**0.9959**	**0.9963**	**0.9965**	**0.3981**																	
	10	36	DEff	0.9833	0.9871	0.9899	0.9919	0.9934	0.9945	0.9953	0.9958	0.9962	0.9965	0.4198																	
	Dye1	**6**	**4**	**9**	**8**	**9**	**7**	**3**	**10**	**8**	**10**	**2**	**8**	**7**	**3**	**5**	**7**	**4**	**5**	**9**	**10**	**8**	**6**	**6**	**1**	**5**	**5**	**9**	**2**	**7**	**4**
	Dye2	**1**	**1**	**10**	**1**	**6**	**6**	**10**	**6**	**4**	**8**	**4**	**7**	**4**	**1**	**9**	**9**	**9**	**7**	**3**	**2**	**3**	**5**	**8**	**10**	**3**	**8**	**2**	**8**	**2**	**6**
	Dye1	**2**	**3**	**1**	**4**	**1**	**10**																								
	Dye2	**5**	**7**	**2**	**3**	**5**	**7**																								
	Dye1	3	4	1	5	7	1	9	3	9	7	3	8	7	6	2	1	2	1	4	10	7	5	5	4	10	6	8	8	2	9
	Dye2	1	10	8	1	3	10	3	10	2	4	6	3	2	7	4	7	8	9	9	7	9	10	3	5	2	4	6	9	5	5
	Dye1	10	4	5	2	6	6																								
	Dye2	8	8	6	1	2	1																								
42	**10**	**37**	**AEff**	**0.9746**	**0.9803**	**0.9845**	**0.9876**	**0.9898**	**0.9915**	**0.9926**	**0.9934**	**0.9940**	**0.9942**	**0.6324**	**S-robust**	**lessCV**															
	10	37	AEff	0.9733	0.9792	0.9836	0.9869	0.9893	0.9911	0.9923	0.9932	0.9938	0.9942	0.6733	S-robust																
	10	**37**	**DEff**	**0.9859**	**0.9889**	**0.9911**	**0.9927**	**0.9939**	**0.9948**	**0.9954**	**0.9958**	**0.9961**	**0.9962**	**0.3306**																	
	10	37	DEff	0.9852	0.9884	0.9907	0.9924	0.9937	0.9946	0.9952	0.9957	0.9960	0.9962	0.3515																	
	Dye1	**4**	**5**	**7**	**9**	**2**	**6**	**8**	**4**	**7**	**2**	**9**	**4**	**1**	**3**	**10**	**8**	**7**	**2**	**5**	**5**	**10**	**6**	**9**	**6**	**5**	**10**	**3**	**10**	**2**	**3**
	Dye2	**1**	**1**	**1**	**4**	**4**	**4**	**1**	**3**	**10**	**9**	**10**	**8**	**10**	**5**	**8**	**9**	**5**	**7**	**9**	**6**	**6**	**8**	**7**	**7**	**8**	**3**	**2**	**2**	**5**	**6**
	Dye1	**9**	**1**	**1**	**1**	**4**	**6**	**8**																							
	Dye2	**3**	**9**	**3**	**6**	**7**	**2**	**2**																							
	Dye1	1	2	3	5	7	2	5	7	1	5	3	3	7	4	9	5	1	4	1	9	6	7	2	8	6	4	2	3	9	10
	Dye2	5	6	7	2	1	8	4	6	6	8	4	2	2	6	3	10	2	8	4	8	5	10	10	7	9	7	9	5	1	9
	Dye1	10	10	4	8	10	6	8																							
	Dye2	4	3	9	3	1	3	1																							
43	**11**	**13**	**AEff**	**0.6271**	**0.7279**	**0.7948**	**0.8407**	**0.8725**	**0.8946**	**0.9096**	**0.9197**	**0.9259**	**0.9294**	**11.2607**	**Non-robust**	**lessCV**															
	11	13	AEff	0.6242	0.7415	0.8157	0.8650	0.8985	0.9214	0.9370	0.9474	0.9539	0.9575	12.0172	Non-robust																
	11	**13**	**DEff**	**0.7984**	**0.8527**	**0.8878**	**0.9115**	**0.9276**	**0.9386**	**0.9461**	**0.9510**	**0.9539**	**0.9554**	**5.4021**																	
	11	13	DEff	0.7986	0.8602	0.8989	0.9245	0.9420	0.9540	0.9621	0.9675	0.9709	0.9728	5.8780																	
	Dye1	**1**	**6**	**4**	**3**	**8**	**11**	**2**	**9**	**5**	**9**	**10**	**7**	**3**																	
	Dye2	**2**	**5**	**8**	**1**	**3**	**3**	**9**	**7**	**9**	**4**	**11**	**10**	**6**																	
	Dye1	4	3	7	2	2	6	3	8	5	10	9	11	1																	
	Dye2	9	7	1	8	5	4	10	11	1	4	2	3	6																	

44	**12**	**14**	**AEff**	**0.5951**	**0.7228**	**0.8045**	**0.8590**	**0.8960**	**0.9212**	**0.9382**	**0.9493**	**0.9561**	**0.9598**	**13.2763**	**Non-robust**	**lessCV**
	12	14	AEff	0.5920	0.7245	0.8067	0.8606	0.8969	0.9216	0.9382	0.9491	0.9559	0.9596	13.3198	Non-robust	
	12	**14**	**DEff**	**0.7860**	**0.8537**	**0.8958**	**0.9235**	**0.9422**	**0.9550**	**0.9636**	**0.9692**	**0.9727**	**0.9745**	**6.3685**		
	12	14	DEff	0.7836	0.8530	0.8955	0.9233	0.9420	0.9548	0.9634	0.9690	0.9725	0.9744	6.4357		
	Dye1	**8**	**9**	**10**	**2**	**10**	**7**	**4**	**11**	**1**	**9**	**5**	**6**	**3**	**12**	
	Dye2	**2**	**7**	**8**	**11**	**12**	**6**	**5**	**1**	**3**	**11**	**9**	**10**	**12**	**4**	
	Dye1	7	3	1	11	5	3	8	4	6	10	12	9	2	4	
	Dye2	2	8	12	10	2	6	7	11	4	1	3	1	9	5	
45	**13**	**15**	**AEff**	**0.5685**	**0.7073**	**0.7917**	**0.8466**	**0.8835**	**0.9085**	**0.9254**	**0.9364**	**0.9433**	**0.9471**	**13.9229**	**Non-robust**	**lessCV**
	13	15	AEff	0.5647	0.7123	0.8011	0.8584	0.8965	0.9223	0.9396	0.9509	0.9578	0.9616	14.3983	Non-robust	
	13	**15**	**DEff**	**0.7634**	**0.8366**	**0.8808**	**0.9097**	**0.9292**	**0.9425**	**0.9515**	**0.9575**	**0.9613**	**0.9635**	**6.8333**		
	13	15	DEff	0.7721	0.8483	0.8936	0.9229	0.9425	0.9557	0.9646	0.9704	0.9740	0.9759	6.8623		
	Dye1	**10**	**7**	**3**	**9**	**11**	**1**	**12**	**13**	**4**	**5**	**7**	**2**	**8**	**6**	**4**
	Dye2	**11**	**9**	**1**	**12**	**5**	**6**	**2**	**3**	**2**	**8**	**1**	**13**	**9**	**10**	**11**
	Dye1	4	4	11	5	10	2	1	13	2	3	6	7	9	12	8
	Dye2	10	12	4	2	5	8	6	1	9	13	11	3	1	3	7

- Bold faced indicates for the designs obtained in the present investigation; Contents of best available designs are given below the contents of design obtained for each parametric combination
- S-robust indicates that percentage CV(A-efficiency) of the design is less than 1%; Robust indicates that percentage CV(A-efficiency) of the design is less than 5%
- Non-robust indicates that percentage CV(A-efficiency) of the design is more than 5%; moreCV (lessCV) indicates that percentage CV(A-efficiency) of the design obtained in the present investigation is more (less) than the best available design

Table 7. Seven New Row-Column Designs for 2-Colour Microarray Experiments not Catalogued in Literature of Microarray Experiments

Sl.No.	v	b	Eff	$\rho = 0.0$	$\rho = 0.1$	$\rho = 0.2$	$\rho = 0.3$	$\rho = 0.4$	$\rho = 0.5$	$\rho = 0.6$	$\rho = 0.7$	$\rho = 0.8$	$\rho = 0.9$	CV(Eff)	Robustness
1	4	5	AEff	0.8757	0.8903	0.9020	0.9115	0.9192	0.9255	0.9305	0.9345	0.9377	0.9401	2.2359	Robust
			DEff	0.9196	0.9266	0.9322	0.9367	0.9404	0.9433	0.9457	0.9475	0.9490	0.9502	1.0348	
	Dye1	3	3	2	4	1									
	Dye2	2	4	4	1	3									
2	4	6	AEff	0.9375	0.9408	0.9438	0.9465	0.9490	0.9512	0.9533	0.9552	0.9569	0.9585	0.7028	S-robust
			DEff	0.9410	0.9440	0.9467	0.9491	0.9514	0.9534	0.9553	0.9570	0.9586	0.9601	0.6370	
	Dye1	4	2	3	1	3	1								
	Dye2	1	4	4	3	2	2								
3	5	6	AEff	0.8466	0.8698	0.8888	0.9042	0.9168	0.9268	0.9347	0.9408	0.9454	0.9487	3.5876	Robust
			DEff	0.9008	0.9154	0.9269	0.9360	0.9432	0.9488	0.9531	0.9564	0.9587	0.9603	2.0250	
	Dye1	5	3	4	5	1	2								
	Dye2	1	4	5	2	3	3								
4	5	7	AEff	0.8571	0.8817	0.9005	0.9150	0.9260	0.9343	0.9405	0.9451	0.9482	0.9502	3.2281	Robust
			DEff	0.9099	0.9228	0.9327	0.9403	0.9462	0.9507	0.9541	0.9567	0.9585	0.9598	1.6833	
	Dye1	3	3	1	5	4	1	2							
	Dye2	1	4	4	3	2	5	5							
5	5	8	AEff	0.9184	0.9304	0.9397	0.9468	0.9522	0.9562	0.9592	0.9613	0.9626	0.9634	1.5212	Robust
			DEff	0.9431	0.9498	0.9550	0.9590	0.9621	0.9645	0.9664	0.9678	0.9688	0.9695	0.8749	
	Dye1	3	2	4	4	5	3	1	1						
	Dye2	5	4	5	3	1	2	2	3						
6	5	9	AEff	0.9259	0.9361	0.9440	0.9503	0.9552	0.9591	0.9623	0.9647	0.9667	0.9682	1.4038	Robust
			DEff	0.9593	0.9638	0.9673	0.9701	0.9723	0.9741	0.9754	0.9766	0.9774	0.9781	0.6140	
	Dye1	5	5	4	4	1	3	3	1	2					
	Dye2	1	2	1	5	2	5	4	3	4					
*7	5	10	AEff	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	S-robust
			DEff	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	
	Dye1	3	2	5	3	2	4	5	1	4	1				
	Dye2	1	4	3	4	3	5	2	5	1	2				

- S-robust indicates that percentage CV(A-efficiency) of the design is less than 1%
- Robust indicates that percentage CV(A-efficiency) of the design is less than 5%
- Non-robust indicates that percentage CV(A-efficiency) of the design is more than 5%