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Note

On Macula's error-correcting pool designs<sup>☆</sup>

F.K. Hwang

*Department of Applied Mathematics, National Chiao Tung University, Hsin-chu 30050, Taiwan, ROC*

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**Abstract**

We show that Macula's claim of a Hamming distance 4 between any two candidate sets of positive clones in his pool design is incorrect. However, a previous proof of his on a weaker result (with a condition on design parameters) is correct. We also show that the condition is sharp and the distance 4 result is also sharp for arbitrary parameter values.

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**1. Introduction**

A clone library stores *clones* which are subsequence of a particular DNA sequence. Often, one needs to know which clones contain a given *probe*, a specified DNA subsequence of interest. We will call a clone *positive* if it contains the probe, and *negative* if not. It would be time-consuming and costly if we have to assay the clones one by one. Since typically the number of positive clones is small, one can pool a subset of clones together for an assay. The assay outcome is negative if all clones in the pool are negative, and is positive otherwise. A *pool design* is a 0–1 matrix where columns represent clones, rows represent pools and an 1-entry in cell  $(i, j)$  signifies that clone  $j$  is in pool  $i$ . The goal of a pool design is to identify the positive clones from the negative clones as much as possible with a minimum number of pools.

For a binary matrix with  $t$  rows, we can view each column as a subset of the set  $\{1, \dots, t\}$  in terms of the positions of the 1-entries. Such a matrix is called *d-disjunct* if no column is contained in the union of any other  $d$  columns. It is well known [1] that

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*E-mail address:* [fhwang@math.nctu.edu.tw](mailto:fhwang@math.nctu.edu.tw) (F.K. Hwang).

a  $d$ -disjunct matrix can identify all positive clones as long as the number  $p$  of positive clones satisfies  $p \leq d$ . Recently, Macula [3] introduced the notion of  $d^e$ -disjunct if any column has at least  $e + 1$  1-entries not in the union of any other  $d$  columns. Another relevant notion is the *Hamming distance*  $H(M)$  of a  $d$ -disjunct matrix  $M$  which is defined to be the minimum number of bit disagreement between a union of  $u$  columns and a union of  $v$  columns,  $u \leq v \leq d$ .

Macula [2] gave a construction of a  $d$ -disjunct matrix. Suppose there are  $z$  clones to be screened. Select  $n, k, d$  such that  $d < k$  and  $\binom{n}{k} \geq z$ . Let  $[n]$  denote the set  $\{1, \dots, n\}$  and  $\binom{[n]}{k}$  the set of all  $k$ -subsets of  $n$ . Randomly select  $z$  members of  $\binom{[n]}{k}$  to label the clones (columns), and label the rows by the set  $\binom{[n]}{d}$  (so there are  $\binom{n}{d}$  rows). The design  $\delta_z(n, d, k)$  has a 1-entry in cell  $(i, j)$  if and only if the label of row  $i$  is contained in the label of column  $j$ . Macula proved that  $\delta_z(n, d, k)$  is  $d$ -disjunct.

Macula [3] also considered the enhanced matrix  $\delta_z^*(n, d, k)$  which is obtained from  $\delta_z(n, d, k)$  by adding  $n$  additional pools labeled  $\{\bar{1}, \bar{2}, \dots, \bar{n}\}$ , where  $\bar{i}$  contains all clones whose labels do not contain  $i$ . He claimed that  $H(\delta_z^*(n, d, k)) \geq 4$  (hence 1-error-correcting) by proving

**Theorem 1.**  $\delta_z^*(n, d, k)$  is  $d^1$ -disjunct.

We will show that this claim is wrong on several counts. Nevertheless, a previous weaker claim of Macula as reported by Du and Hwang [1] remains correct:

**Theorem 2.** Suppose  $k - d \geq 3$ . Then  $H(\delta_z^*(n, d, k)) \geq 4$ .

Further, we show that both the condition  $k - d \geq 3$  and the result of distance 4 are sharp.

## 2. The main result

We first give a counter-example against Theorem 1.

**Example 1.**  $\delta_z^*(5, 2, 3)$  containing three columns  $C_0 = \{1, 2, 3\}$ ,  $C_1 = \{1, 2, 4\}$ ,  $C_2 = \{1, 3, 5\}$ . It is easily verified that the only 1-entry in  $C_0$  but not in the union of  $C_1$  and  $C_2$  is the row with label  $(2, 3)$ . Hence  $\delta_z^*(5, 2, 3)$  is not  $d^1$ -disjunct.

The problem in the proof of Theorem 1 lies in the statement that let  $C_0, C_1, \dots, C_d$  be  $d + 1$  distinct columns and  $|C_0 \setminus C_i| = 1$  for  $1 \leq i \leq d$ , then  $C_0 \setminus C_i \neq C_0 \setminus C_j$  implies  $C_i \setminus C_0 = C_j \setminus C_0$ . The above example shows that the implication is not realized since  $C_1 \setminus C_0 = 4 \neq C_3 \setminus C_0 = 5$ .

Example 1 can be extended to general  $d, k$  with  $k \geq d$ . Let

$$C_i = [k + 1] \setminus \{k + 1 - i\}, \quad 0 \leq i \leq d - 1,$$

$$C_d = [k + 2] \setminus \{k - d + 1, k + 1\}.$$

Then the only 1-entry in  $C_0$  but not in the union of  $C_1, \dots, C_d$  is the row with label  $\{k - d + 1, k - d + 2, \dots, k\}$ .

Next we argue that even though Theorem 1 were correct, it would not be enough to substantiate the claim that  $H(\delta_z^*(n, d, k)) \geq 4$ . This is because the two candidate sets of positive clones can differ only in one column  $C$ . Then the Hamming distance between those two sets is simply the number of 1-entries in  $C$  but not in the union of the other columns, which is only guaranteed to be 2 by Theorem 1. Note that  $d^1$ -disjunct would imply  $H(\delta_z^*(n, d, k)) \leq 4$  if  $d$  is the exact number of positive clones, not just an upper bound.

In a different sense, the  $d^1$ -disjunctness is too strong a property to prove a Hamming distance 4. For example, one column in one candidate set may contribute only distance 1, while the other candidate set contributes distance 3 to compensate. The two sets have Hamming distance 4, but do not satisfy  $d^1$ -disjunctness. Note that the counter-example given at the beginning of this section is not a counter-example against Theorem 2 since it is easily verified that any two candidate sets of cardinality  $\leq 2$  have Hamming distance at least 4. A formal proof of Theorem 2 can be found in [1].

Can the condition  $k - d \geq 3$  in Theorem 2 be eliminated (as in Theorem 1) or at least weakened? The following example shows that it cannot.

**Example 2.**  $\delta_z^*(7, 3, 5)$  containing columns  $C_1 = \{1, 2, 3, 4, 5\}$ ,  $C_2 = \{1, 2, 3, 4, 6\}$  and  $C_3 = \{1, 2, 3, 5, 7\}$ . Consider the two candidate sets  $\{C_1, C_2, C_3\}$  and  $\{C_2, C_3\}$ . It is easily verified that they differ only in three rows with labels  $\{1, 4, 6\}$ ,  $\{2, 4, 6\}$ ,  $\{3, 4, 6\}$ .

We now expand the example to arbitrary  $k$  with  $d = k - 2$  and  $d \geq 3$ .

Let  $n \geq k + 2$ , then  $\delta_z^*(n, k - 2, k)$  contains  $k - 2$  columns

$$C_i = [k + 1] \setminus \{k + 2 - i\}, \quad 0 \leq i \leq k - 3, \quad \text{and}$$

$$C_{k-2} = [k + 2] \setminus \{4, k + 1\}.$$

Then the two candidate sets  $\{C_0, C_1, \dots, C_{k-3}\}$  and  $\{C_1, \dots, C_{k-3}\}$  differ only in rows with labels  $\{1, 4, 5, \dots, k\}$ ,  $\{2, 4, 5, \dots, k\}$  and  $\{3, 4, 5, \dots, k\}$ .

Examples for  $k - d < 2$  are even easier to construct and omitted here.

Next we show that regardless of how large is  $k - d$ , the guaranteed Hamming distance remains at 4.

**Example 3.**  $\delta_z^*(n, 2, k)$  (where  $n \geq k + 1$ ) containing three columns  $C_1 = \{1, \dots, k\}$ ,  $C_2 = \{1, \dots, k - 1, k + 1\}$ ,  $C_3 = \{1, \dots, k - 2, k, k + 1\}$ . Consider two candidate sets  $\{C_1, C_2\}$  and  $\{C_2, C_3\}$ . It is easily verified that the only four different rows are those labeled by  $\{k - 1, k\}$ ,  $\{k, k + 1\}$ ,  $\{\overline{k - 1}\}$  and  $\{\overline{k + 1}\}$ .

Again, Example 3 can be extended to general  $d$ . Let

$$C_i = [k + 1] \setminus \{k + 2 - i\}, \quad 1 \leq i \leq d + 1.$$

Then the two candidate sets  $\{C_1, \dots, C_d\}$  and  $\{C_2, \dots, C_{d+1}\}$  differ only in the four rows with labels  $\{k - d + 1, k - d + 2, \dots, k\}$ ,  $\{k - d + 2, k - d + 3, \dots, k + 1\}$ ,  $\{\overline{k - d + 2}\}$  and  $\{\overline{k + 1}\}$ .

A referee reminds us that a  $d^e$ -disjunct matrix can correct  $e$  errors. The decoding procedure is to take a subset  $E$  of rows, and change all outcomes in these rows. Do this for all  $E$  with  $|E| \leq e$ . Let  $V$  denote the outcome vector before change, and  $V_E \equiv V \cup E$  is the outcome vector after change. Then a column  $C$  is positive if and only if there exists an  $E$  such that  $V_E$  contains  $C$ . To see this, note that when  $E$  is the set of errors, then the outcome vector is corrected back to the errorless state in which  $C$  only appears in rows with positive outcomes. On the other hand, if  $C$  is negative, then the  $d^e$ -disjunctness guarantees that  $C$  has at least  $e + 1$  rows not in  $V_E$ , and at most  $e$  of them are in  $E$ , hence  $C$  has a row not in  $V_E$ .

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