SEQMAK Users' Manual

Version 0.14

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Contents

Chapter 1

Prerequisites

SEQMAK is written in Python 2.7. In order to run the program you must have Python installed on your computer (preferably version 2.7x but version 2.4x should also work). This software has not been tested with Python 3.0 or higher, so I cannot guarantee it will work under those environments. If you do not have Python installed on your computer, you may download the latest version at http://www.python.org/download/. Python is GNU/Linux, Windows, and OS X compatible.

Chapter 2

Introduction

This is documentation for the program `SEQMAK` (pronounced SEC-mac), which stands for *sequence maker*. The inspiration for `SEQMAK` comes from SEQUIN which was written by N. C. Seeman[1, 2]. The main differences are

- much of the repetitive nature of SEQUIN has been automated with some added functionality (*e.g.* checking dyad symmetric segments),

- exception handling has been much improved, and

- all features have been modularized, making the code much easier to read, learn, and maintain.

As is the case with SEQUIN, kinetic and thermodynamic factors are not taken into account when designing structures with `SEQMAK` . Only different combinations of DNA bases are considered. If you would like to consider other factors when designing your DNA structure, you may want to use a more complex program such as DNAdesign.[1]

The first step in designing any DNA structure is to draw up a blueprint of the structure itself. Once that has been done, the sequence of each strand must be determined. This is where `SEQMAK` comes in. By dividing the structure into different (topological) domains called arms and subarms, `SEQMAK` produces segments (called "critons") of random or user determined bases. Concatenating these critons produces the final strands which (with some luck) creates the intended structure. Since bases are produced at the criton level and then concatenated to form strands, strands of any significant length will inevitably have repeating sequence segments. Heuristically, this is thought to be undesirable as too many repeating segments may lead to crosstalk, *i.e.* hybridization between unintended domains of same/different strands. `SEQMAK` can minimize repeating sequence segments and hence crosstalk by calculating the frequency a certain criton occurs in all the strands and selecting critons which appear with the least frequency. After the sequence of all the critons have been determined, `SEQMAK` produces a text file with the sequence of the final strands.

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[1]`http://dna.caltech.edu/DNAdesign/`

`SEQMAK` is licensed under the GNU GPL so you are free to read, modify, and run the code as you see fit. If you would like to distribute any modified versions of the code, please attribute credit to the original author. If you happen to you use it in your research, please give it the following citation :

Kim, J. (2016, August 7). SEQMAK. Zenodo. http://doi.org/10.5281/zenodo.59874

Chapter 3

Running the Program

3.1   A Single Tile

Below, two existing DNA structures are provided as pedagogical examples, the double-crossover (DX) tile[3] and 3-point star[4] (since the structural design has already been done, the focus henceforth is put on the use of SEQMAK). By design, both structures include Holliday junctions, which serve as fixtures, giving rigidity to the structures. Topologically, these junctions serve as indicators where the structure should be initially dissected into arms (see examples below and Fig. 3.1).

Among other things, DX tiles have been used to create DNA crystals[5], *i.e.* periodic arrays of DNA molecules, and molecular analogs of Wang tiles[6] to perform computations[7]. The DX tiles shown in Fig. 3.1 are of the **d**ouble-crossover **a**ntiparallel **e**ven (DAE) type. "Double-crossover" refers to the two junctions present in each DX tile, "antiparallel" is the relative orientation of the helical domains, and "even" refers to the number of half-turns between the two crossover points of a tile (sometimes you'll see DX tiles such as DAE-O where the extra "-O" stands for an odd number of half-turns in between crossover points of adjacent tiles). Once the structural design has been done, the following steps need to be carried out by hand before using SEQMAK :

1. index the strands

2. divide and index the strands into arms

3. divide and index the arms into subarms

First, the strands must be indexed. For the DAE DX tile shown in Fig. 3.1(b), they are s1, s2, . . . , s5. The order of the strands do not matter, *e.g.* s1 may be interchanged with s5. Next, divide the strands into arm domains. This is done by identifying the junctions and partitioning the structure along the junctions. The left and right dashed red lines of Fig. 3.1(b) illustrate these divisions. In addition, if you would like to further partition the strands for any structure with more than two or more junctions, bisecting lines in-between every junction (which is the dashed red line down the center of the
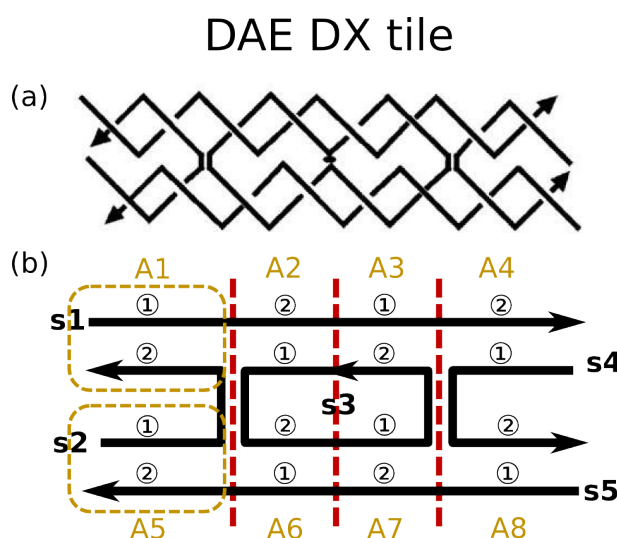
Figure 3.1: A double-crossover antiparallel even (DAE) type DX tile. (a) A schematic diagram of the DAE DX tile (taken from Ref. 3). (b) A DAE DX tile divided into strands (*e.g.* s1), arms (*e.g.* A1), and subarms (*e.g.* ①). The dashed red lines indicate the division of the structure into arm domains and should be made at every junction and in-between every junction. Each arm consists of two subarms (① and ②) encompassed by the dashed gold squares.

structure) can be added. This partitioning scheme separates the DAE DX tile into 4 arm domains, with each domain having 2 arms (*e.g.* the left-most arm domain consists of arms A1 and A5) for a total of eight arms, labeled A1, A2, ..., A8. Furthermore, as can be seen from the figure, each arm consists of segments from two different strands. For instance, the A1 arm consists of segments from the s1 and s2 strands (likewise the A7 arm consists of segments from the s3 and s5 strands). These segments make up the subarms of the structure. The numbering scheme for the subarms is such that if the directionality $(5' \rightarrow 3')$ of the subarm is toward the junction, then it is labeled with ① and if it points away from the junction, then it is labeled with a ②. Now, we are in a position to run SEQMAK.

As of version 0.14, SEQMAK consists of 3 files, `seqmak.py`, `commands.py`, and `functions.py`. Place all three files in the same directory. `seqmak.py` is the main file which is used to run the program. Double-click this file if you want to run it under Windows, or for *nix systems type

```
$ python seqmak.py
```

at the command line prompt (indicated by `$`). The following should appear

```
> Enter a command (type 'help' for help):
```

At any point in the program, the user can type '`help`' (without the apostrophes) and a list of all the possible commands will be shown. Typing `help` gives out the following:

```
Possible commands are:
1. newarms (na)
2. show (s)
3. link (l)
4. crunch (c)
5. strandgen (sg)
6. repeatcheck (rp)
7. dyadcheck (dc)
8. save (sv)
9. load (ld)
10. exit
Use '--help' for more information. e.g. 'na --help'
Enter a command (type 'help' or 'h' for help):
```

With the exception of the `exit` command, there are 9 commands which are used to run the program. Each command may be executed by typing either the full name of the command (*e.g.* 'newarms') or its abbreviated name indicated in parentheses (*e.g.* 'na'). The first step is to create all the arms of the structure. Typing `newarms` asks for the number of arms the user would like:

```
How many arms do you want?
```

From Fig. 3.1(b) we see that a DAE DX tile has 8 arms, so type 8 and press enter.

```
How many arms do you want?
8
What is the length of the subarms of these arms?
```

Next the length (number of bases) of the subarms of each arm needs to be entered. For a DAE DX tile, every arm consists of 2 subarms each of which are 8 bases long, so type 8 and press enter.

```
How many arms do you want?
8
What is the length of the subarms of these arms?
8
Any more arms? (y/n)
```

The next question that comes up is whether the user would like additional arms. The user can type 'y' if they would like additional arms or if the structure requires arms of different lengths, *e.g.* 4 arms consisting of subarms of length 8 and 4 arms consisting of subarms of length 4. Since the length of all the subarms of the DAE DX tile are the same, type 'n'. To check the newly created arms type 'show'.

```
> Enter a command (type 'help' for help):
show

arm1
```

```
xxxxx xxx
xxxxx xxx

arm2
xxxxx xxx
xxxxx xxx

arm3
xxxxx xxx
xxxxx xxx

arm4
xxxxx xxx
xxxxx xxx

arm5
xxxxx xxx
xxxxx xxx

arm6
xxxxx xxx
xxxxx xxx

arm7
xxxxx xxx
xxxxx xxx

arm8
xxxxx xxx
xxxxx xxx
Enter a command (type 'help' for help):
```

The 'show' command lists all the arms along with the sequences of their subarms below each arm. Since the sequences of the subarms have yet to be determined, all the bases are represented by x's, and each subarm sequence is space separated every 5 bases for easy viewing. Next, we must link the arms to form strands. This can be done by making the following table. Not only is each strand a concatenation of subarms, but the subarm indices of each strand alternates between ① and ② (Fig. 3.1(b)). Hence the entries under the $3'$-linked arm lists all the arm numbers of a given strand in the $5'$ to $3'$ direction. For instance, strand s1=A1-①→A2-②→A3①→A4②. The arms in the $5'$ to $3'$ direction (①→②) are A1, A2 and A3, A4 (not A2, A3). Keeping just the arm numbers, we write 1,2 / 3,4 in the intersecting entry of the s1 row and $3'$-linked arm column. Conversely, under $5'$-linked arm, we write down the arm numbers going in the opposite direction ($3'$ to $5'$ or ②→①), which for s1 is A2, A3. In this manner, the information shown in this table allows us to link all the arms to reconstruct the strands. Hence, typing `link` brings up the following prompt

8

strands	$3'$-linked arm	$5'$-linked arm
	① → ②	② → ①
s1	1,2 / 3,4	2,3
s2	5,1	
s3	4,8	
s4	8,7 / 6,5	7,6
s5	2,6 / 7,3	6,7 / 3,2

Table 3.1: Linking table.

```
> Enter the arm and it's 3'-linked arm:
```

under which the user should type all the numbers in the $3'$-linked arm column separated by a comma.

```
> Enter the arm and it's 3'-linked arm:
1,2,3,4,5,1,4,8,8,7,6,5,2,6,7,3
Enter the arm and it's 5'-linked arm:
```

and the same can be analogously done for the $5'$-linked arm.

```
> Enter the arm and it's 3'-linked arm:
1,2,3,4,5,1,4,8,8,7,6,5,2,6,7,3
> Enter the arm and it's 5'-linked arm:
2,3,7,6,6,7,3,2
linker_list [['1', '2', '3', '4'], ['8', '7', '6', '5'],
['2', '6', '7', '3'], ['5', '1'], ['4', '8']]
```

At this point, we can generate the strands with the 'strandgen' command.

```
> Enter a command (type 'help' for help):
strandgen

strand 1 (32 bases)
XXXXX XXXXX XXXXX XXXXX XXXXX XXXXX XX

strand 2 (32 bases)
XXXXX XXXXX XXXXX XXXXX XXXXX XXXXX XX

strand 3 (32 bases)
XXXXX XXXXX XXXXX XXXXX XXXXX XXXXX XX

strand 4 (16 bases)
XXXXX XXXXX XXXXX X
```

```
strand 5 (16 bases)
xxxxx xxxxx xxxxx x

Enter a command (type 'help' for help):
```

5 strands have been produced by concatenating arms according to the directionality given by Tab. 3.1. A couple of caveats are that the numbering of the strands produced by 'strandgen' most likely will not match the numbering of the original strand drawn (*e.g.* strand 1 of the output may actually be strand s3 in Fig. 3.1(b)). This is not a problem since the order of the strands hold no meaning and are relative to each other. The important point is that all the strands are correctly produced. Also, since no specific bases have been determined yet, all the strands consist of x's at this point which will change once the user starts to determine the sequences. Now we can start filling in the bases with the crunch command.

```
> Enter a command (type 'help' for help):
crunch
Please enter the following
arm #, starting base, end base, CRITON size, # of repeats (default: None)
```

The user needs to input 4 digits. The first is the arm number. The second and third are the starting and end base numbers, respectively. The fourth is the CRITON size (which is the desired length of the unit segments that SEQMAK produces). An optional fifth digit is the maximum number of repeats this criton is allowed. If this fifth digit is omitted, then no repeats of this criton are allowed. As an example type 1,1,4,4 and press enter.

```
Please enter the following
arm #, starting base, end base, CRITON size, # of repeats (default: None)
1,1,4,4
crit4
GCAG
(a)ccept or (r)eject or (s)et
```

A random sequence of criton size 4 has been created, namely GATT. We can choose to accept this segment, reject this segment and create a new random segment of criton size 4, or manually set a user determined sequence of bases of criton size 4. Let's reject this choice by typing 'r'.

```
Please enter the following
arm #, starting base, end base, CRITON size, # of repeats (default: None)
1,1,4,4
crit4
GCAG
(a)ccept or (r)eject or (s)et
r
CTGT
(a)ccept or (r)eject or (s)et
```

A new sequence, `ACTT`, has been created. Let's accept this choice by typing 'a'.

```
Please enter the following
arm #, starting base, end base, CRITON size, # of repeats (default: None)
1,1,4,4
crit4
1,1,4,4
crit4
GCAG
(a)ccept or (r)eject or (s)et
r
CTGT
(a)ccept or (r)eject or (s)et
a

strand 1 (32 bases)
CTGTx xxxxx xxxxx xxxxx xxxxx xxxxx xx

strand 2 (32 bases)
xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xx

strand 3 (32 bases)
xxxxx xxxxx xxxxx xxxxx xxxxx xxxxx xx

strand 4 (16 bases)
xxxxx xxxxx xxACA G

strand 5 (16 bases)
xxxxx xxxxx xxxxx x

['CTGT', 'GACA']
```

Not only has the first four bases of A1 has been created (`CTGT`), but its complementary segment (`ACAG`) has also been automatically created and placed in strand 2. These two segments are now stored in memory (`['CTGT', 'GACA']`) and will not be used again as long as repeats of this criton size are not allowed (which is the default). The user can also check which arms have already been determined by typing '`show`' at any time.

```
> Enter a command (type 'help' for help):
show

arm1
CTGTx xxx
GACAx xxx

arm2
xxxxx xxx
```

```
XXXXX XXX

arm3
XXXXX XXX
XXXXX XXX

arm4
XXXXX XXX
XXXXX XXX

arm5
XXXXX XXX
XXXXX XXX

arm6
XXXXX XXX
XXXXX XXX

arm7
XXXXX XXX
XXXXX XXX

arm8
XXXXX XXX
XXXXX XXX
Enter a command (type 'help' for help):
```

By repeating this process over all the arms, we can obtain all the segments to produce strands such as the following:

```
...
> Enter a command (type 'help' for help):
show

arm1
CTGTT GTA
GACAA CAT

arm2
TAAAA AGA
ATTTT TCT

arm3
GCGCT TCG
CGCGA AGC
```

```
arm4
CATTC CTA
GTAAG GAT

arm5
AGGTA TTG
TCCAT AAC

arm6
AGGCG AGC
TCCGC TCG

arm7
CCGGA TCG
GGCCT AGC

arm8
TTACC ACG
AATGG TGC
> Enter a command (type 'help' or 'h' for help):
strandgen

strand 1 (32 bases)
CTGTT GTATC TTTTT AGCGC TTCGT AGGAA TG

strand 2 (32 bases)
TTACC ACGCG ATCCG GAGGC GAGCC AATAC CT

strand 3 (32 bases)
TAAAA AGAGC TCGCC TCCGG ATCGC GAAGC GC

strand 4 (16 bases)
AGGTA TTGTA CAACA G

strand 5 (16 bases)
CATTC CTACG TGGTA A

Enter a command (type 'help' or 'h' for help):
```

With all the strands completed, we can check the strands for repeating segments so as to minimize any crosstalk. This is accomplished with the 'repeatcheck' command. Typing 'repeatcheck', we get

```
> Enter a command (type 'help' or 'h' for help):
repeatcheck
> Enter min. CRITON size, max. CRITON size, min. # of repeats,
max. # of repeats
```

Four input parameters are needed. The first is the minimum criton size. This is the minimum segment length that the user would like to check over all the strands. For example if 'min. CRITON size' = 4, then all segments of length 4 bases or more are checked for repeats over all the strands. The second parameter is the maximum criton size the user would like to check. This sets an upper-bound on the maximum segment length to check. Hence, if 'min. CRITON size' = 4 and 'max. CRITON size' = 6, then criton sizes of 4, 5, and 6 are analyzed over all the strands for repeats. The third and fourth parameters are the minimum and maximum number of repeats the user would like to check. If min. # of repeats = 3 and max. # of repeats = 6, then all segments of criton sizes 4, 5, and 6 which appear between 3 and 6 times throughout the strands are displayed. As an example, type 4,6,3,6

```
> Enter min. CRITON size, max. CRITON size, min. # of repeats,
  max. # of repeats
4,6,3,6
'GCGA' has 3 repeats
strand # => base position
strand3 => 24
strand2 => 8
strand2 => 19


Enter a command (type 'help' or 'h' for help):
```

The results show that of all the segments of criton sizes 4, 5, and 6, only one segment of criton size 4, *i.e.* GCGA, has 3 repeats (since repeats in the range [3, 6] are checked), which are displayed along with the strand number and its starting base position in the strand.

Segments having dyad symmetry (in the same strand) can also be checked for all the strands with the dyadcheck (dc) command.

```
> Enter a command (type 'help' or 'h' for help):
dyadcheck
Enter segment size (in nt's) of dyad symmetry check:
```

By entering the segment size (in nt's), each strand is checked for dyad symmetric repeats of that segment size. If we want to check for dyad symmetric repeat segments of length '5', type '5' and press enter.

```
> Enter a command (type 'help' or 'h' for help):
dyadcheck
Enter segment size (in nt's) of dyad symmetry check:
5


'TTGTA' has 1 dyad symmetric repeats ('TACAA')
strand # => base position
strand4 => 6
9,
```

```
'TGTAC' has 1 dyad symmetric repeats ('GTACA')
strand # => base position
strand4 => 7
8,


'GAGCT' has 1 dyad symmetric repeats ('AGCTC')
strand # => base position
strand3 => 7
8,


'TCCGG' has 1 dyad symmetric repeats ('CCGGA')
strand # => base position
strand3 => 16
17,


'TCGCG' has 1 dyad symmetric repeats ('CGCGA')
strand # => base position
strand3 => 22
23,


'TCCGG' has 1 dyad symmetric repeats ('CCGGA')
strand # => base position
strand2 => 12
13,


'AGCGC' has 1 dyad symmetric repeats ('GCGCT')
strand # => base position
strand1 => 16
17,


Enter a command (type 'help' or 'h' for help):
```

Taking the first output as an example, the segment 'TTGTA' (length 5) starting at the 6th base in strand 4 has 1 dyad symmetric segment, 'TACAA', starting at the 9th base of the same strand. Minimizing dyad symmetric repeats may prove favorable, especially in designing dynamic DNA systems, since they can lead to unwanted secondary structures (*e.g.* hairpins) within the strand.

If the user decides everything is satisfactory, then the strands can be saved to a file by typing 'save'.

```
> Enter a command (type 'help' for help):
save
Type the name of the output file:
```

Choose the name of the output file and press enter.

```
> Enter a command (type 'help' for help):
save
Type the name of the output file:
strands
Save to file (y/n)?
y
Enter a command (type 'help' for help):
```

The sequence map of all the produced strands along with the arm information will be saved to output file. For the case at hand, the output file is `strands`:

```
arm1
CTGTTGTA
GACAACAT

arm2
TAAAAAGA
ATTTTTCT

arm3
GCGCTTCG
CGCGAAGC

arm4
CATTCCTA
GTAAGGAT

arm5
AGGTATTG
TCCATAAC

arm6
AGGCGAGC
TCCGCTCG

arm7
CCGGATCG
GGCCTAGC

arm8
TTACCACG
AATGGTGC

5' -> 3'
strand 1
```

```
CTGTT GTATC TTTTT AGCGC TTCGT AGGAA TG

strand 2
TTACC ACGCG ATCCG GAGGC GAGCC AATAC CT

strand 3
TAAAA AGAGC TCGCC TCCGG ATCGC GAAGC GC

strand 4
AGGTA TTGTA CAACA G

strand 5
CATTC CTACG TGGTA A
```

Now the user may exit the program with the 'exit' command and order the strands!

The commands provided in SEQMAK can also be used on predefined user sequences which can be loaded in with the 'load' command. First, write out the sequences of the strands in a file, one strand per line, *e.g.*,

```
ACGT TGCA TTATA
ATAGTAAAGGA   TTA
  CGCGTGAGTGCAGA
atagtaaaggatta
AGCATGAG agatTTCGGAA
```

The sequences can be written out in either upper or lower case and the whitespaces in-between or at either ends of the sequences don't matter. All that matters is that the user writes one strand per line. Give a name to the file, *e.g.* 'strands.txt', and save it. Run SEQMAK and type 'load' or 'ld'. The output should be the following:

```
> Enter a command (type 'help' or 'h' for help):
ld
Enter the name of the file (e.g. strands.txt) or 'q' to exit command:
```

Type 'strands.txt' to load the saved file and an output of all the predefined strands will appear on the screen (reformatted to uppercase and with whitespaces removed),

```
> Enter the name of the file (e.g., strands.txt) or 'q' to exit command:
strands.txt

strand1: ACGTTGCATTATA

strand2: ATAGTAAAGGATTA

strand3: CGCGTGAGTGCAGA

strand4: ATAGTAAAGGATTA
```

```
strand5: AGCATGAGAGATTTCGGAA

Enter a command (type 'help' or 'h' for help):
```

The sequences of the strands have now been loaded in and commands such as 're-peatcheck' and 'dyadcheck' can be run as described above.

3.2 Multiple Tiles

There are a couple of ways to design structures consisting of multiple tiles, *e.g.* a two-tile DX crystal. One method is to design the tiles individually, save the sequences to a file, and load in the sequences to check for repeat segments. Another method is to first design one tile by numbering the arms from 1 to n and then design the other tile by numbering the arms from $n + 1$ to $2n$ (without exiting the program). Since the sequences of the first tile are retained in the memory of the program, checking for repeat segments will scan the sequences of both tiles. Below is an illustration of the second method.
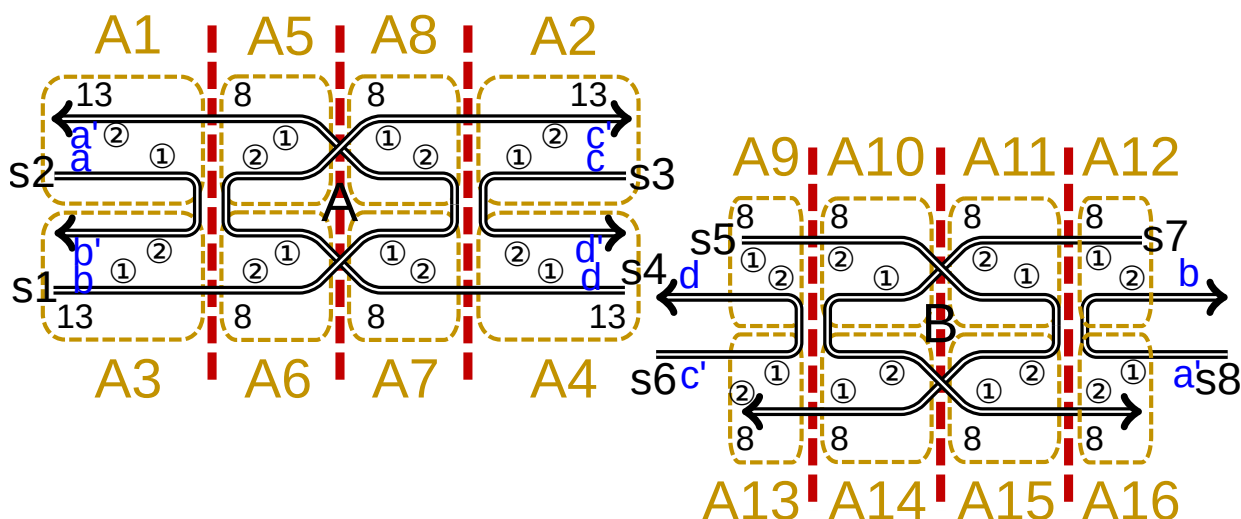

Figure 3.2: An AB-type (DAE-O) DX tile system. Each tile is divided into arms and further subdivided into subarms. The A-tile has 4 arms which include the sticky-ends (A1, A2, A3, and A4) making the lengths of these arms 13 bases. The numbers inside each dashed rectangle denote the length of the arms (in bases). Since all the sticky-ends are produced when creating the A-tile, only the body of the B-tile (excluding the sticky-ends) needs to be designed, which makes the length of all the arms of the B-tile 8 bases in length. The strands are labeled as s1 $\sim$ s8 and the sticky ends are labeled as lowercase letters (a $\sim$ d) with primes indicating their complements (a' $\sim$ d').

Fig. 3.2 shows a DX crystal scheme comprising two tiles. Since we have more than one tile, complementary sticky-ends of the tiles must be taken into account. This can

be done by including the sticky-ends as part of the strands when creating the first tile. This leaves 4 extra sticky-ends which can be cut out and manually concatenated to the appropriate positions of second DX tile.

To implement this, we first create the A tile following the steps of the DAE protocol. Create 4 arms of length 13 and then 4 arms of length 8. Next connect the arms according to the linking information given in Tab. 3.2. Make sure the lengths of the created arms match those of the designed structures. In our example, since 4 arms of length 13 were created first, arms $1 \sim 4$ should be designated to be length 13, as shown in Fig. 3.2. By

strands	3′-linked arm ① → ②	5′-linked arm ② → ①
s1	3,6 / 7,8 / 5,1	6,7 / 8,5
s2	1,3	
s3	2,4	
s4	4,7 / 6,5 / 8,2	7,6 / 5,8

Table 3.2: Linking table for the A-type DX tile.

providing the linking table information into the program and following the steps given above, the resulting strands should produce the A-type DX tile.

To create the second DX tile, create new arms (without exiting the program), but this time numbering the arms from 9 to 16 (arms $1 \sim 8$ were already used for the A tile). Also, since the sticky-ends were already created when producing the A tile, only the body of the B tile needs to be designed, therefore 8 arms with a length of 8 bases need to be generated. The strands can be produced with the linking information from Tab. 3.3. Once

strands	3′-linked arm ① → ②	5′-linked arm ② → ①
s5	9,10 / 11,15 / 14,13	10,11 / 15,14
s6	13,9	
s7	12,11 / 10,14 / 15,16	11,10 / 14,15
s8	16,12	

Table 3.3: Linking table for the B-type DX tile.

all the sequences for the B tile have been produced, the information for both the A and B tiles are now in the memory of the program. Checking for any repeating segments will scan through both the A and B tiles. Finally, the sticky-ends corresponding to a′, b, c′, and d should be cut out from the A tile and added to the appropriate strands of the B tile.

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