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An Efficient, Low-cost and Scalable Method for Constructing a Thousand of Synthetic Communities (SynComs)

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Abstract

Recent advances in the study of complex microbial communities have highlighted the necessity of large-scale development and application of synthetic communities (SynComs) to investigate microbiome functionality and ecology within various fields such as agriculture, medicine, and environmental sciences. Despite the exponential increase in potential combinations within SynComs correlating with the number of involved strains (totaling 2^N combinations for N strains), research has often focused on a limited subset of possible combinations due to these constraints. This limitation could potentially impact the conclusions drawn about mechanisms of emergent functionsy within microbial communities. In order to exhaustively construct SynComs from multiple species, this paper integrates the permutation and combination method with commonly used laboratory materials such as microtiter plates and pipettes, and develops a program to perform tasks like unique microbial community id assignment and data collection form generation. This simplifies the process of manually constructing over 1000 different SynComs in the laboratory. Compared to conventional methods, this method is more efficient, cost-effective, and scalable, and it avoids the sampling process confusion that can occur with conventional methods. Herein, we provide detailed experimental procedures for constructing 16-2048 different SynComs using 4-11 species as examples. Moreover, the program 'syncons' is provided as an R package that can be operated locally or accessed via cloud service, offering functionalities such as procedure overview, SynComs id assignment, and data collection form generation. Given the significant potential of SynComs, this method can serve as a fundamental experimental operation in research topics such as microbial co-cultivation and multispecies biofilms, offering broad application value.

Keywords: SynComs, emergent properties, Co-culture experiments, Multispecies biofilms, Microbial community construction

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Background

Advancements in the investigation of complex microbial community structures and functions have rendered the development and utilization of synthetic communities (SynComs) [1], which are essentially employed for exploring microbiome functionality and microbial ecology across diverse sectors, including agriculture [2], medicine [3], ecology [4], environmental science [5], and energy [6]. Future applications in designing SynComs are anticipated to grow, potentially involving combinations of dozens or hundreds of strains[7]. Theoretically, possible combinations within SynComs rise exponentially as a function of the number of strains used, for example, a system with 10 strains yields 1,024 possible different combinations is sum. This combinatorial explosion has historically confined research to only a fraction of potential combinations, and this may potentially limit understanding of the emergence and dynamics of microbial communities. To meet specific research needs, it is sometimes necessary to exhaustively construct all possible combinations of synthetic microbial communities.

Currently, the construction of many different SynComs is primarily accomplished using methods such as manual assembly, automated devices, and microdroplet technologies. Among these, automated equipment, such as the apricot DC1 pipetting workstation, has achieved a degree of high repeatability and precision, but at the expense of some flexibility and at a cost not all laboratories can afford. Furthermore, while microdroplet technology has shown immense potential in high-throughput screening of SynComs, its application is currently limited by numerous technical challenges[8]. Following traditional construction paradigms, manual assembly is undoubtedly the least efficient and most error-prone approach. However, this method, through judicious planning of the addition sequence, can significantly enhance the efficiency of manually constructing SynComs, while substantially reducing the error rate. This provides researchers in related fields with a convenient and practical experimental strategy.

Principles and Methods

SynComs are artificially constructed simple microbial consortia, typically containing between 3 to 7 species. To exhaustively construct all possible species combinations of n species, it is necessary to consider scenarios including zero species, one species, two species, multiple species, and up to n species. Taking n=10 as an example, the total number of species combinations N can be calculated using the following formula (Equation 1):

$$N = C_{10}^{0} + C_{10}^{1} + C_{10}^{2} + \cdots + C_{10}^{9} + C_{10}^{10} = \sum_{k=0}^{n} \binom{10}{k} \quad \text{(Equation 1)}$$

As shown in Figure 1, within this exhaustive construction scheme, the number of combinations without any species (blank control) is 1, single-species combinations are 10, two-species combinations are 45, three-species combinations are 120, and so on, totaling 1,024 combinations. Although blank control and single-species combinations may not be considered microbial communities in the traditional sense, they are essential controls for research and thus are important members of this SynCom system.

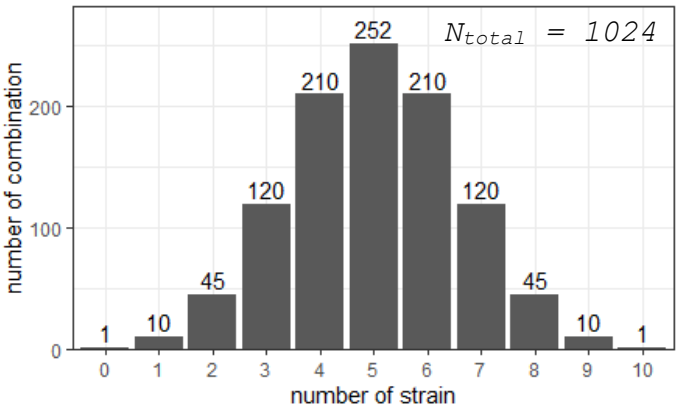

Figure 1. The number of SynCom combinations with different numbers of species when exhaustively constructing SynComs with 10 different strains.

More generally, if the total number of possible SynCom combinations constructed from n strains is denoted as N, then according to the binomial theorem, the value of N is equal to 2^n (Equation 2). Therefore, the number of combinations of SynComs increases exponentially with the number of strains. Given this, while it may be relatively easy to manually construct dozens of different SynComs in a short time, the task becomes prone to errors without proper design when dealing with hundreds of different combinations as the number of species increases.

$$N = \sum_{k=0}^{n} \binom{n}{k} = 2^n \quad \text{(Equation 2)}$$

From a combinatorial mathematics perspective, 2^n represents the total number of ways to select subsets from n elements. Each subset corresponds to a 0–1 vector, where 0 indicates the absence of an element and 1 indicates its presence. Thus, when these vectors are sorted, they can be orderly arranged on a $2^{(n-1)} \times 2^{(n-1)}$ layout on a two-dimensional plane, providing important guidance for planning the order of additions during the construction of SynComs. Since there are no $2^{(n-1)} \times 2^{(n-1)}$ microtiter plates in laboratories, and common microtiter plates are available in formats like 24-well, 96-well, and 384-well, experimental operations are orderly carried out on the available space of 24-well, 96-well, and 384-well plates by rows and columns. Each SynCom is given a unique id based on its position on the plate. Ultimately, this method can construct over a thousand different combinations of SynComs efficiently. When constructing SynComs on the latter two types of microtiter plates, using multi-channel pipettors can significantly improve work efficiency. To clearly identify the composition of the synthetic microbial communities in each well after addition, we also developed an R package ‘syncons’. This package can be run locally or accessed through cloud services, and in addition to providing SynComs ids, it also offers functions such as sampling process inquiry and data collection form generation.
Overall, this method aims to improve the efficiency of manually constructing SynComs using common laboratory materials and equipment, with careful planning of the addition order, while also reducing errors and avoiding cross-contamination, providing researchers in related fields with a convenient and practical experimental strategy.

Materials and reagents

1. 24-well plate (Corning, catalog number: 3524)
2. 96-well plate (Corning, catalog number: 3599)
3. 384-well plate (Axygen®, catalog number: P-384-240SQ-C-S)
4. Pipette tip (Biosharp, catalog number: BS-200-T)
5. Reagent Reservoir (Biosharp, catalog number: BS-PS50-RR)

6. Culture media (TSB media, LB media, etc.) (BD, catalog number: GD-211825)
7. Strains
   Different strains are distinguished by their IDs, herein represented as Strain S1 through S11, totaling eleven distinct bacterial strains.

Equipment

1. Shaking incubator (Huamei Biotech, catalog number: THZ-701B)
2. Single-channel pipette (Thermo Scientific™, catalog number: 4651140N)
3. 8-channel pipette (Thermo Scientific™, catalog number: 4661010N)
4. 16-channel pipette (EVOLVE, catalog number: 3044)
5. Laminar Flow Cabinet (AIRTECH, catalog number: ES-A01-VS-1300L-U)

Procedures

(I) Dispense the prepared bacterial suspensions into the reagent reservoirs;
(II) Follow the illustrated steps to add strains using the corresponding pipettes. An 8-channel pipette can be utilized for the 96-well plate, and a 16-channel pipette is suitable for the 384-well plate. All additions should be conducted within a Laminar Flow Cabinet.

Method M1: Construction of SynComs with 4 strains (totaling 16 combinations)
The procedure is as follows (Figure 2):

1. In a 24-well plate, add samples to wells in columns 1–4 of rows 1–4, totaling 16 wells.
2. Add the S1 strain to columns 1–2.
3. Add the S2 strain to columns 1 and 3.
4. Add the S3 strain to the first 4 columns in rows 1–2;
5. Add the S4 strain to the first 4 columns in rows 1 and 3.
6. Table 1 shows the strain combinations of SynComs in all the wells. The resulting SynComs consisted of one negative control (D4 wells), four monocultures (B4, C4, D2, and D3 wells), six 2-strain SynComs (A4, B2, B3, C2, C3, and D1 wells), four 3-strain SynComs (A2, A3, B1, and C1 wells), and one 4-strain SynComs (A1 wells), for a total of 16 SynComs.

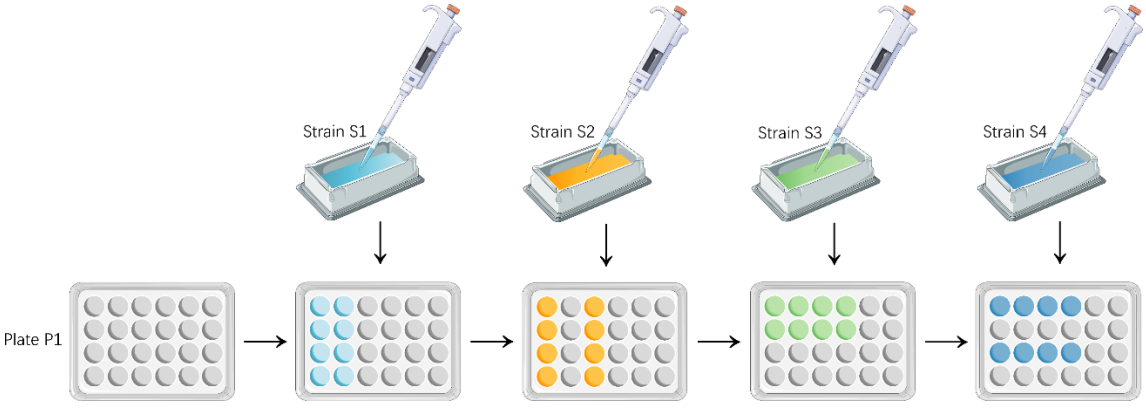

Figure 2. Operational procedure for constructing SynComs with 4 strains using a 24-well plate. The combination IDs and compositions of these SynComs are detailed in Supplementary Excel Sheet 1.

Table 1. Construction of 16 SynComs with 4 strains.

rows/columns	1	2	3	4
A	S1/S2/S3/S4	S1/S3/S4	S2/S3/S4	S3/S4
B	S1/S2/S3	S1/S3	S2/S3	S3
C	S1/S2/S4	S1/S4	S2/S4	S4
D	S1/S2	S1	S2	Control

Method M2: Construction of SynComs with 5 strains (totaling 32 combinations)

The procedure is as follows (Figure 3):

1. The first 16 SynComs were adopted from the result of method M1, and the used 24-well plate was labeled as P1.
2. Adding strain S5 to the first 4 columns of another 24-well plate P2, repeat method M1, which yields the remaining 16 SynComs.
3. In total, 32 SynComs are obtained with 5 strains (S1-S5); the combination IDs and compositions of them are detailed in Supplementary Excel Sheet 2.

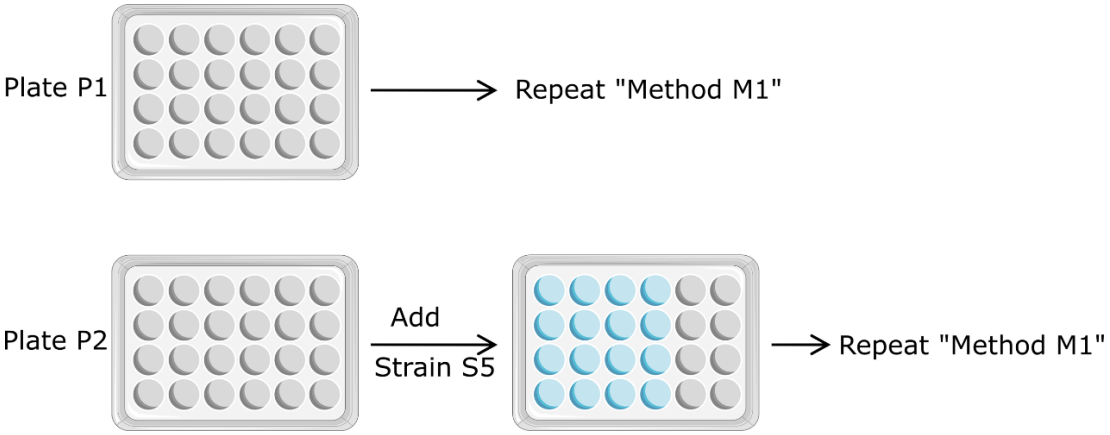

Figure 3. Operational procedure for constructing SynComs with 5 strains using two 24-well plates. Please refer to Figure 1 for “Method M1”.

Method M3: Construction of SynComs with 6 strains (totaling 64 combinations)

The procedure is as follows (Figure 4):

1. In a 96-well plate, add strains to wells in columns 1–8 of rows 1–8, totaling 64 wells.
2. Add strain S1 to columns 1–4.
3. Add strain S2 to columns 1–2 and 5–6.
4. Add strain S3 to columns 1, 3, 5, 7.
5. Add strain S4 to the first 4 columns in rows 1–4;
6. Add strain S5 to the first 4 columns in rows 1–2 and 5–6.
7. Add strain S6 to the first 4 columns in rows 1, 3, 5, 7.
8. A total of 64 SynComs were obtained. The distribution of them in 96-well plate is given in Table 2, and the combination IDs and compositions of them are detailed in Supplementary Excel Sheet 3.

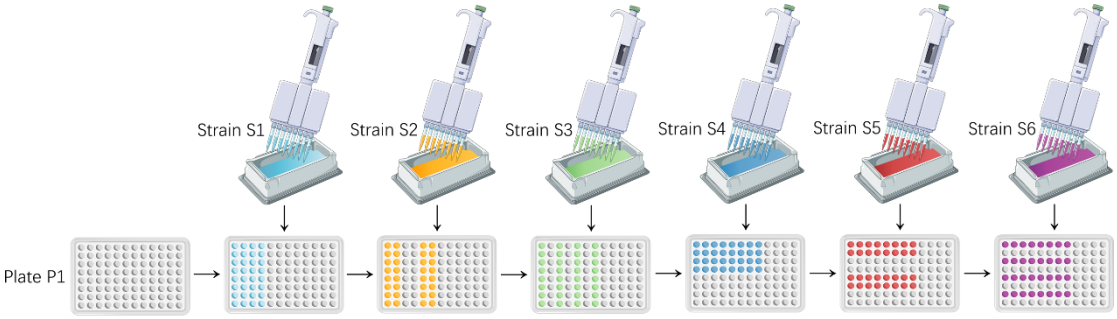

Figure 4. Operational procedure for constructing SynComs with 6 strains using a 96-well plate and 8-channel pipette.

Table 2. The distribution of 64 SynComs constructed with 6 strains in a 96-well plate. The data collection spreadsheet can be found in the supplementary Sheet 3.

	1	2	3	4	5	6	7	8
A	S1/S2/S3/S4/S5/S6	S1/S2/S4/S5/S6	S1/S3/S4/S5/S6	S1/S4/S5/S6	S2/S3/S4/S5/S6	S2/S4/S5/S6	S3/S4/S5/S6	S4/S5/S6
B	S1/S2/S3/S4/S5	S1/S2/S4/S5	S1/S3/S4/S5	S1/S4/S5	S2/S3/S4/S5	S2/S4/S5	S3/S4/S5	S4/S5
C	S1/S2/S3/S4/S6	S1/S2/S4/S6	S1/S3/S4/S6	S1/S4/S6	S2/S3/S4/S6	S2/S4/S6	S3/S4/S6	S4/S6
D	S1/S2/S3/S4	S1/S2/S4	S1/S3/S4	S1/S4	S2/S3/S4	S2/S4	S3/S4	S4
E	S1/S2/S3/S5/S6	S1/S2/S5/S6	S1/S3/S5/S6	S1/S5/S6	S2/S3/S5/S6	S2/S5/S6	S3/S5/S6	S5/S6
F	S1/S2/S3/S5	S1/S2/S5	S1/S3/S5	S1/S5	S2/S3/S5	S2/S5	S3/S5	S5
G	S1/S2/S3/S6	S1/S2/S6	S1/S3/S6	S1/S6	S2/S3/S6	S2/S6	S3/S6	S6
H	S1/S2/S3	S1/S2	S1/S3	S1	S2/S3	S2	S3	空白

Method M4: Construction of SynComs with 7 strains (totaling 128 combinations)

The procedure is as follows (Figure 5):

1. 64 SynComs were adopted from method M3 on a 96-well plate labeled P1.
2. After adding strain S7 to the first 8 columns of another 96-well plate labeled P2, repeat M3, resulting in the remaining 64 SynComs.
3. A total of 128 SynComs were obtained. The combination IDs and compositions of them are detailed in Supplementary Excel 4.

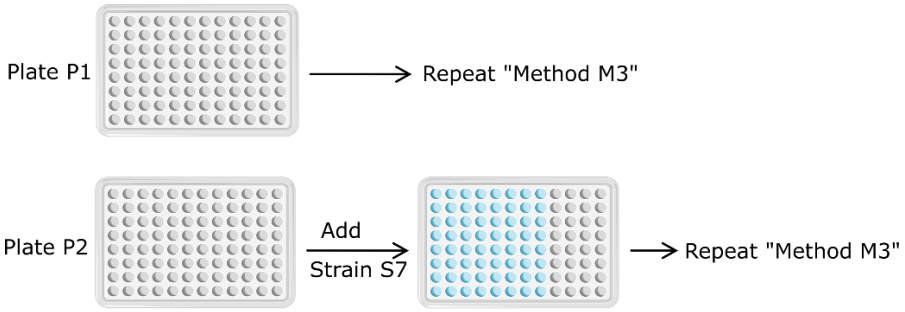

Figure 5. Operational procedure for constructing SynComs with 7 strains using two 96-well plates and 8-channel pipette. Please referred to Fig. 4 for “Method M3”.

Method M5: Construction of SynComs with 8 strains (totaling 256 combinations)

The experiment can be performed using either four 96-well plates (Plan A) or one 384-well plate (Plan B).

Method M5, Plan A

The procedure is as follows (Figure 6):

1. 64 SynComs were constructed using method M3 on a 96-well plate labeled P1.

2. After adding strain S7 to the first 8 columns of the second 96-well plate labeled P2, repeat method M3, resulting in the second portion of 64 SynComs.
3. After adding strain S8 to the first 8 columns of the third 96-well plate labeled P3, repeat method M3, resulting in the third portion of 64 SynComs.
4. After adding strain S7 and S8 to the first 8 columns of the fourth 96-well plate labeled P4, repeat method M3, resulting in the fourth portion of 64 SynComs.
5. A total of 256 SynComs were obtained. The combination IDs and compositions of them are detailed in Supplementary Excel Sheet 5–1.

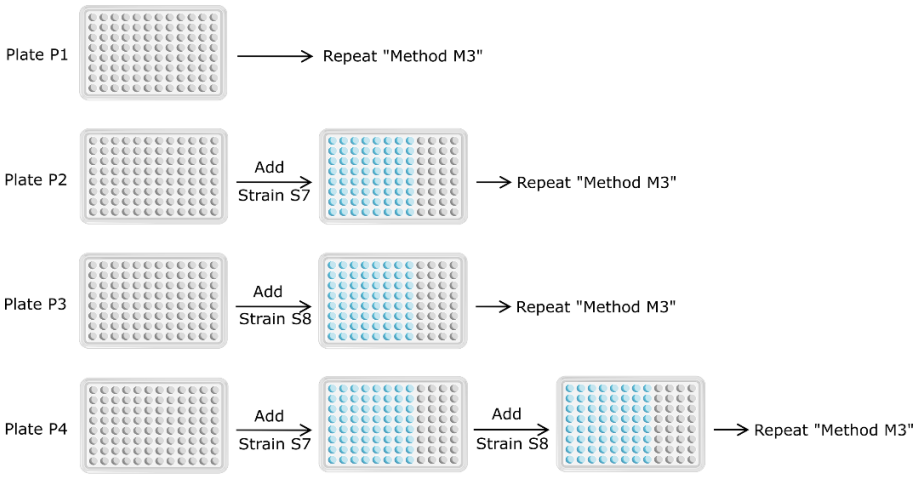

Figure 6. Operational procedure for constructing SynComs with 8 strains using four 96-well plates and 8-channel pipette. Please referred to Figure 4 for "Method M3".

Method M5, Plan B

The procedure is as follows (Figure 7):

1. In a 384-well plate, add samples to wells in columns 1–16 of rows 1–16, totaling 256 wells.
2. Add strain S1 to columns 1–8.
3. Add strain S2 to columns 1–4 and 9–12.
4. Add strain S3 to columns 1–2, 5–6, 9–10, and 13–14.
5. Add strain S4 to columns 1, 3, 5, 7, 9, 11, 13, and 15.
6. Add strain S5 to the first 8 columns in rows 1–8;
7. Add strain S6 to the first 8 columns in rows 1–4 and 9–12.
8. Add strain S7 to the first 8 columns in rows 1–2, 5–6, 9–10, and 13–14.
9. Add strain S8 to the first 8 columns in rows 1, 3, 5, 7, 9, 11, 13, and 15.
10. A total of 256 SynComs were obtained. The combination IDs and compositions of them are detailed in Supplementary Excel Sheet 5–2.

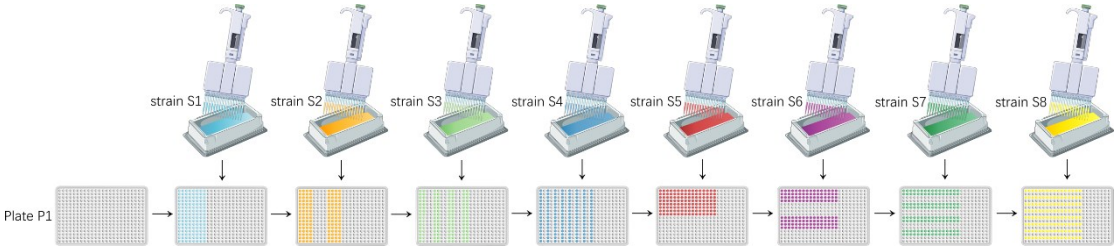

Figure 7. Operational procedures for constructing SynComs containing 8 strains using a 384-well plate and 16-channel pipette.

Method M6: Construction of SynComs with 9 strains (totaling 512 combinations)
The experiment can be performed using either eight 96-well plates (Plan A) or two 384-well plates (Plan B).

Method M6, Plan A
The procedure is as follows (Figure 8):

1. 64 SynComs were created using method M3 on a 96-well plate labeled P1.
2. After adding strain S7 to the first 8 columns of the second 96-well plate labeled P2, repeat method M3, resulting in the second portion of 64 SynComs.
3. After adding strain S8 to the first 8 columns of the third 96-well plate labeled P3, repeat method M3, resulting in the third portion of 64 SynComs.
4. After adding strain S9 to the first 8 columns of the fourth 96-well plate labeled P4, repeat method M3, resulting in the fourth portion of 64 SynComs.
5. After adding strain S7 and S8 to the first 8 columns of the fifth 96-well plate labeled P5, repeat method M3, resulting in the fifth portion of 64 SynComs.
6. After adding strain S7 and S9 to the first 8 columns of the sixth 96-well plate labeled P6, repeat method M3, resulting in the sixth portion of 64 SynComs.
7. After adding strain S8 and S9 to the first 8 columns of the seventh 96-well plate labeled P7, repeat method M3, resulting in the seventh portion of 64 SynComs.
8. After adding strain S7, S8 and S9 to the first 8 columns of the eighth 96-well plate labeled P8, repeat method M3, resulting in the final 64 SynComs.
9. A total of 512 SynComs were obtained. The combination IDs and compositions of them are detailed in Supplementary Excel Sheet 6–1.

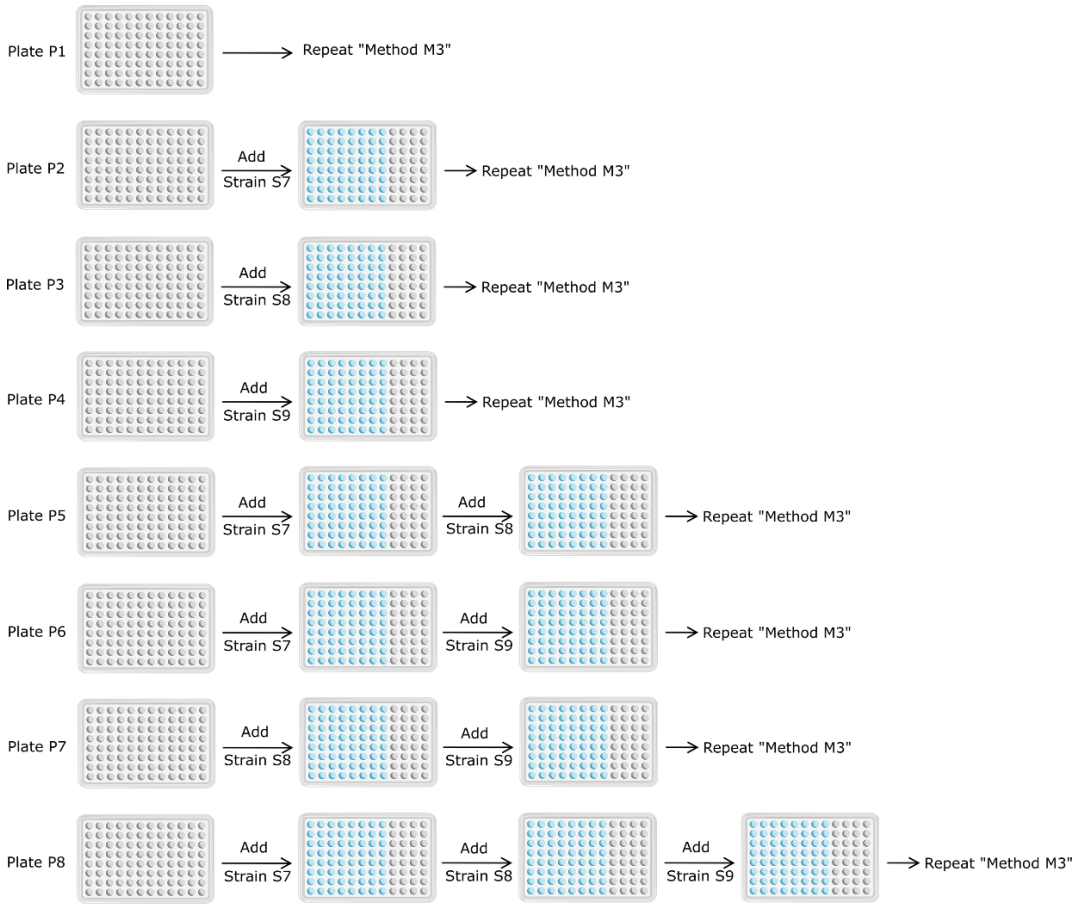

Figure 8. Operational procedures for constructing SynComs containing 9 strains using eight 96-well plates and 8-channel pipette. Please refer to Figure 4 for Method M3.

Method M6, Plan B
The method has been described in Method M4, involving the use of a 16-channel pipette on two 384-well plates. Details will not be repeated here. The combination IDs and compositions of them are detailed in Supplementary Excel Sheet 6–2.

Method M7: Construction of SynComs systems with 10 strains (totaling 1,024 combinations)
The method has been described in Method M5, Plan B, involving the use of a 16-channel pipette for construction on four 384-well plates, details of which will not be repeated here. The combination IDs and compositions of them are detailed in Supplementary Excel Sheet 7.

Method M8: Construction of SynComs with 11 strains (totaling 2048 combinations)
The method has been described in Method M6, Plan A, involving the use of a 16-channel pipette for strain additions on eight 384-well plates, details of which will not be repeated here. The combination IDs and compositions of them are detailed in Supplementary Excel Sheet 8.

SynComs IDs and strain compositions
The Supplementary Excel file provided with this protocol lists the combination IDs and bacterial compositions of all SynComs. To generate the Excel file, we developed a R package namely “syncons” (https://github.com/gaospecial/syncons), which can generate the IDs and combination of SynComs with given plate type and strain names in batch, thereby facilitating the collection of experimental data in subsequent experiments. The package includes a Shiny App (Figure 8), which can directly be accessed through a public cloud service at Shinyapps.io (https://bio-spring.shinyapps.io/SynComsConstructor/)。

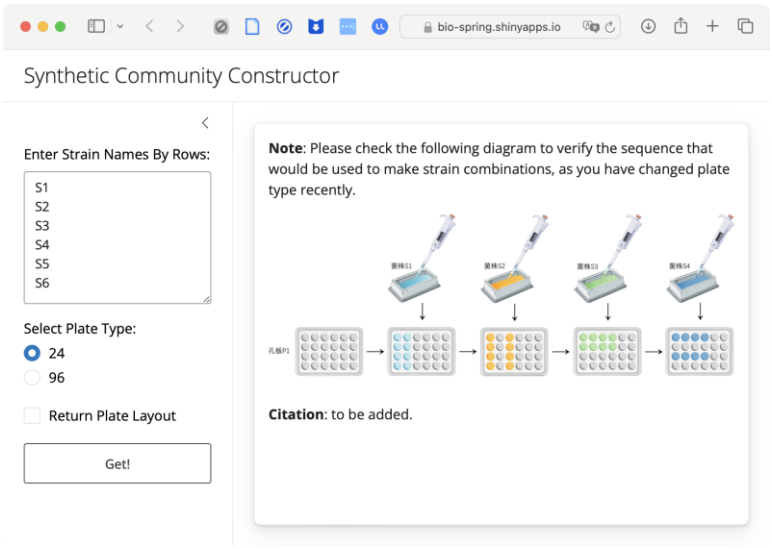

Figure 8. A Shiny application for generating data collection tables of SynComs constructions. Users can input their own strain names and specify the type of plate to be used during the construction process (e.g., 24-well plate, 96-well plate, or 384-well plate). Following the addition sequence specified by the above-mentioned methods, all SynComs IDs and compositions can be obtained via the Get! button.

Notes

To prevent contamination, improve efficiency, and ensure accuracy during strain addition, these tips can be followed in experiments:

1. To avoid overfilling of plate wells, one should use a suitable volume of each strain based on the number of strains used. Typically, the maximum applicable volume of a well in a 96-well plate is around 300 μL, under which circumstances, setting the addition volume to each strain at 20–30 μL should sufficient; for deep 384-well plates (e.g., Greiner 781270, 781271), with a well capacity of approximately 220 μL, setting the addition volume for each strain at 20 μL is also adequate for constructing 2048 SynComs from 11 strains.
2. During additions, proceed to add a second strain into the reservoir only after you have completed the previous strain and cleaned the workspace. Taking Strain S1 as an example, after adding S1 to the reservoir, one should add it to all wells that require S1 before proceeding with the addition of Strain S2. When adding Strains S1 or S2, reusing the pipette tips can speed up the process. However, it is crucial to stabilize the pipettor and avoid touching the inner walls of the wells, and ensure a tight connection between the pipettor and tips to prevent cross-contamination and inaccurate volume transfers.
3. In this method, the initial ratio of all strains included in SynComs is 1:1 by volume. If a 1:1 initial ratio of strain population is required, and there are differences in cell concentrations across different strain cultures, adjustments can be made by adding fresh medium before additions. However, based on our previous studies, due to the convergent succession during community assembly, minor differences in initial ratios typically do not significantly affect the functionality of SynComs [9]. By contrast, significant functional differences usually only result from the situations when initial ratios differ by orders of magnitude[10]. Therefore, a volume ratio of 1:1 should meet the needs of most studies.
4. When a construction method involves multiple strains and plates, ensure to label each strain and plate accordingly to avoid confusion or omission during additions. The Supplementary Excel document lists all the combinations IDs and compositions derived from the above-mentioned procedures.
5. After additions, the plates need to be shaken to mix thoroughly before proceeding to subsequent experiments. If not used immediately, they can be sealed with a lid and stored at 4 °C for 1–3 days. Theoretically, they can also be preserved at -20 °C or -80 °C after adding glycerol for future use. However, for consistency, we recommend preparing and using them as needed.
6. Only part of the plate area is used in this protocol. The remaining wells can be used for setting up controls. Recommended controls typically include a medium control (to ensure the medium is uncontaminated), a sampling control (to ensure the experimental materials used during sampling are uncontaminated), and more single-strain controls (to allow statistical comparison between single strain and multispecies SynComs).

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Supplementary information

The following supporting information can be downloaded here

1. Supplementary Excel Sheet 1
2. Supplementary Excel Sheet 2
3. Supplementary Excel Sheet 3
4. Supplementary Excel Sheet 4
5. Supplementary Excel Sheet 5
6. Supplementary Excel Sheet 6
7. Supplementary Excel Sheet 7
8. Supplementary Excel Sheet 8