TruPool®
Sequence Template Guidelines

LinkZill

Template File Versions

Universal template	Fills up to 65,536 sequences	Download: TruPool_Universal.xlsx

File Format

The file is an Excel file with a single sheet. You can rename the file, but please do not modify the sheet names.

Oligo_ID_and_Seq The sheet ontains four columns of information:

Oligo_No	Oligo_ID	Oligo_Seq	Redundancy
The index number for each oligonucleotide. 0 represents a demo oligo; 1 to 4,096/65,536 represents the sequences to be synthesized. Do not modify, add, or delete anything in this column.	The name of each oligonucleotide, equivalent to the content after ">" in FASTA format.	The sequence of each oligonucleotide, provided in the 5' -> 3' direction.	The redundancy/copy number of each oligonucleotide. The default is 1.

Filling and Validation Rules

Oligo_No	Must be continuous, non-repeating positive integers.
	Should not be modified.
Oligo_ID	If you do not wish to set names, you may leave the entire column blank (IDs will be assigned automatically); however, you cannot fill some rows and leave others blank.
	Duplicate Oligo_IDs are not allowed. If a sequence needs to be synthesized multiple times, please set the Redundancy instead of duplicating the row.
	Allowed characters: Uppercase and lowercase letters (A-Z, a-z), Arabic numerals (0-9), and certain symbols (+-=_.:). Spaces, full-width characters (including Chinese), line breaks, tabs, and other special characters are prohibited.
	Due to limitations in most bioinformatics software, the first character of the Oligo_ID must be a letter (A-Z, a-z) and cannot be a number or special character.
Oligo_Seq	Allowed characters: Letters (A-Z, a-z) and Arabic numerals (0-9).
	Unless there are special requirements, use uppercase A, C, G, T for natural bases. By default, lowercase letters will be converted to uppercase.
	Uppercase R, Y, S, W, K, M, B, D, H, V, N represent condensed bases. By default, lowercase letters will be converted to uppercase.
	You may use N for random condensed bases. However, if you need to synthesize non-N condensed bases (e.g., R, Y, S, W, K, M, B, D, H, V), please contact our customer manager for clarification and confirmation.
	Characters other than A, C, G, T, R, Y, S, W, K, M, B, D, H, V, N (including numbers) may represent special monomers, specific synthesis recipe, or modification groups. If required, please contact our customer manager.
	If your lower- and uppercase letters represent different monomers or recipes, please contact our customer manager.
Redundancy	Must be a positive integer.
	If left blank, the default value is set to 1.
	The sum of the Redundancy column should not exceed the throughput limit (e.g., 4,096 or 65,536).

Additional Suggestions and Rules

[1] In the **Oligo_ID_and_Seq** sheet, **each row represents one unique oligonucleotide sequence**.
For example, if a user needs to synthesize 1,800 different sequences using a 4K chip with 2 copies of each, they should fill rows with **Oligo_No** 1 to 1,800 and set **Redundancy** to 2, rather than filling rows with **Oligo_No** 1 to 3,600.

[2] The row where **Oligo_No** is "0" and **Oligo_ID** is "Demo_Oligo" is an **example row**. Please do not modify it.
This row is not synthesized and will not appear in the final product.

[3] In principle, **Oligo_Seq** should correspond one-to-one with **Oligo_ID**.
A single **Oligo_ID** can only correspond to one **Oligo_Seq**.
While different **Oligo_IDs** corresponding to the same **Oligo_Seq** are permitted, we recommend verifying your design.

[4] Avoid cases where **Oligo_ID** is empty while **Oligo_Seq** contains data.

Product Quality Related Tips

[1] If you have certain requirements for product yield or if downstream experiments involve amplification, we strongly recommend including **universal primer sequences**.

[2] Within an oligo pool or subpool amplified with the same primer pair, significant **differences in sequence length** may cause variations in synthesis yield and significant **amplification bias**, affecting the **uniformity** of the product.

[3] If you need to use **polyX** sequences to normalize sequence length, **dT** is the recommended base type.

[4] Excessive **dA** or **dG** content may negatively impact synthesis quality.

[5] **GC content** that is too high, too low, or varies significantly among sequences may severely affect the **uniformity** of amplification products.