

Rapid and High-throughput Evaluation of Diverse Configurations of Engineered Lysins using the VersaTile Technique

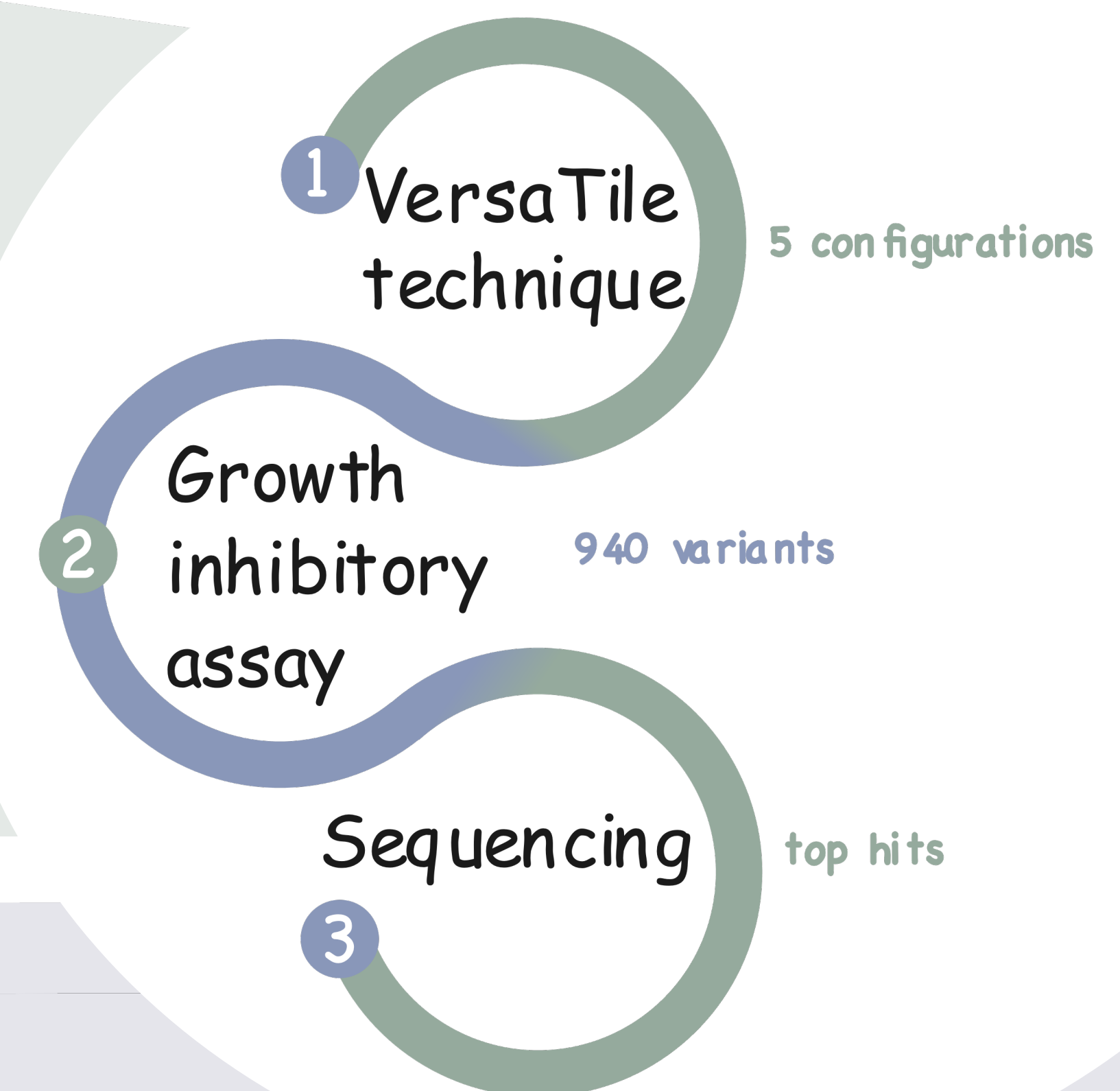
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The **modular composition of lysins** is a hallmark feature enabling

optimization of antibacterial and pharmacological properties by the design and engineering of lysin candidates based on lysin and non-lysin modules. In this regard, the recent introduction of the **VersaTile technique** (Figure 1) allows the rapid construction of large modular lysin libraries based on a premade repository of tiles or building blocks.

In this study, we perform a **high-throughput construction and screening of five combinatorial lysin libraries with different configurations**, targeting *Klebsiella pneumoniae*. An elaborate analysis of the activity distribution of **940** variants and sequencing data of 53 hits inhibiting the growth of *Klebsiella pneumoniae* more than 95% could be associated with specific design rules.



VersaTile Technique: principle

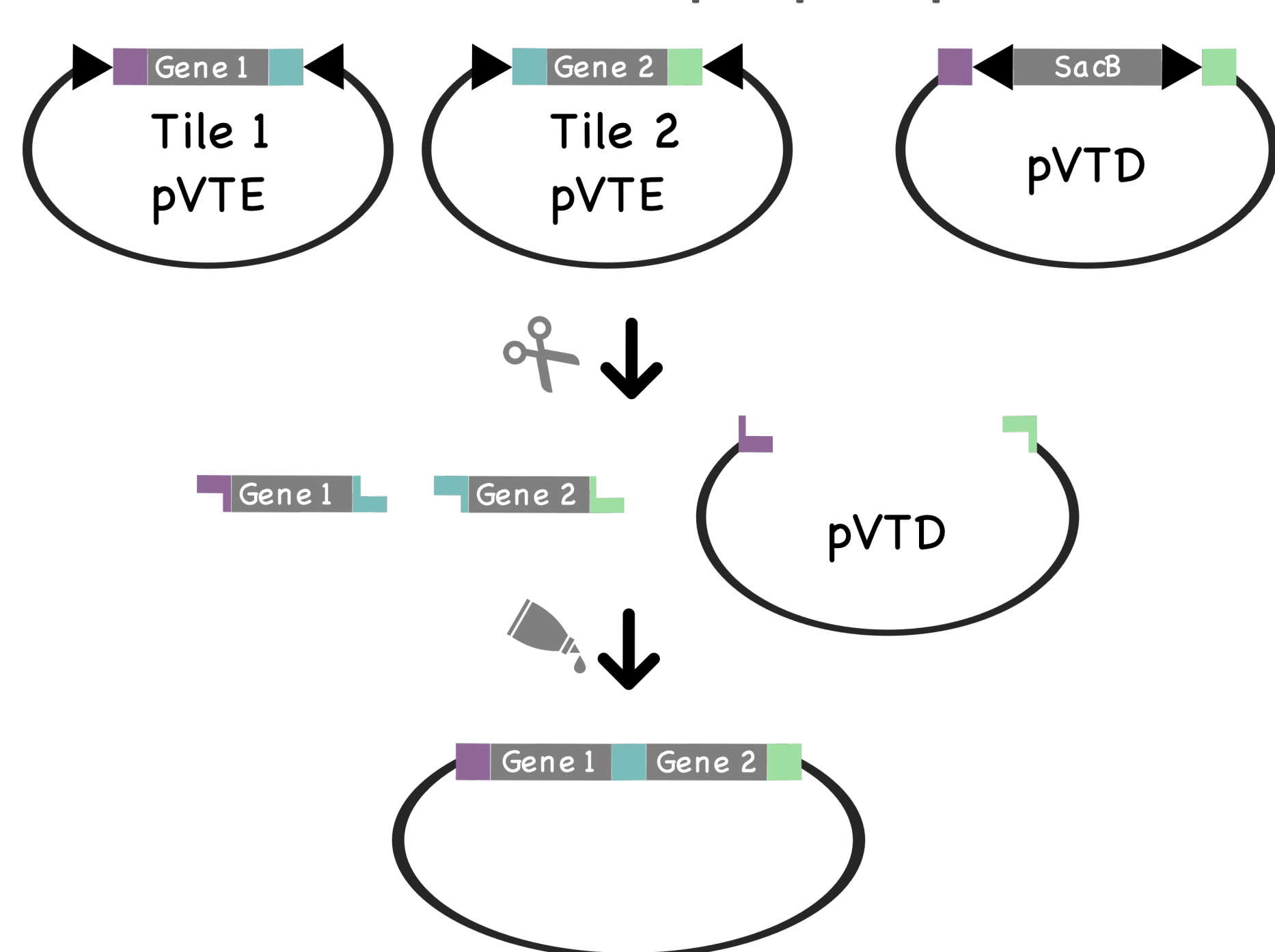


Figure 1. VersaTile technique: simplified representation of 2-way system. pVTE: entry vector (VersaTile cloning); pVTD = destination vector (VersaTile shuffling) (3)

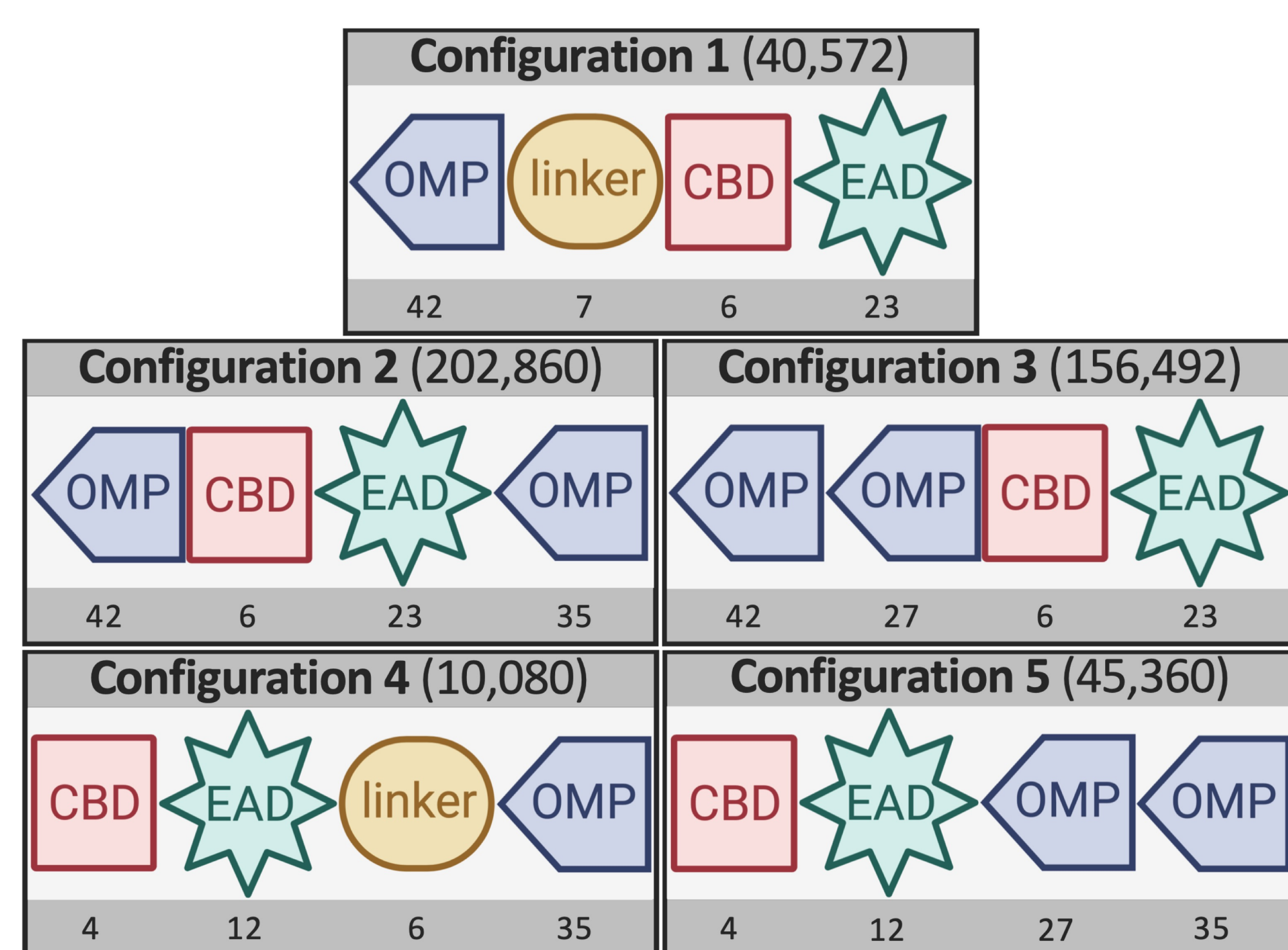


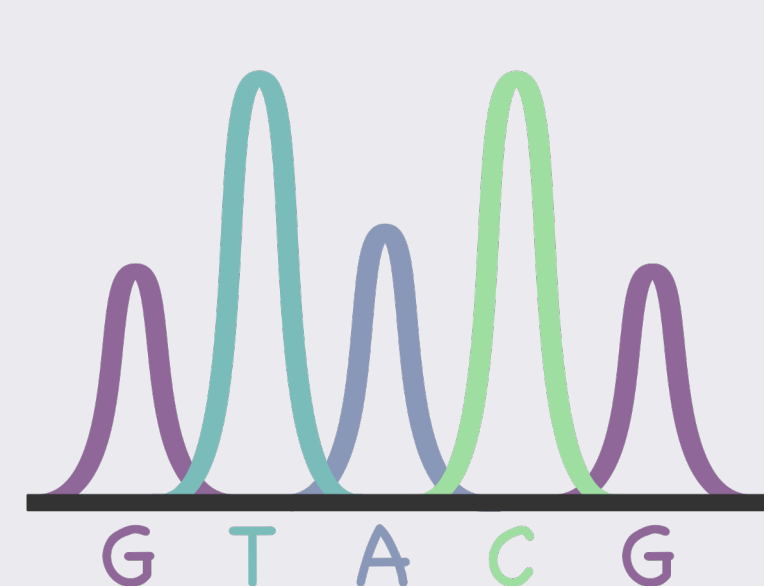
Figure 2. The different configurations tested in this study. Configuration 1: the standard configuration. The total number of available tiles for configurations 1 through 5 are mentioned under each specific position. The resulting possible variants are indicated within the brackets (2).

Analysis of the activity distribution of 940 variants

- **Configuration 1 and 4:** top clusters of highly active variants

Implementation two peptides:

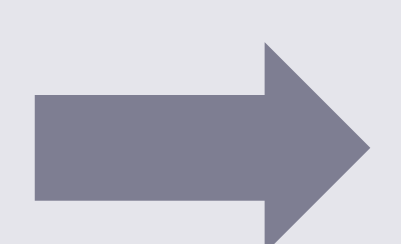
- **Configuration 2 and 5:** bulk of variants with no inhibitory effect & long tail of variants with rising (but limited) activity
- **Configuration 3:** Equal distribution of active variants, maximal GI of $\pm 60\%$
- **EDTA:** Positive impact on GI% (except configuration 2)



Sequencing data of 74 top hits:

- Specific **OMPs** and **EADs** are significantly overrepresented, up to **20-fold**
- CBDs and linkers are equally represented
- No significant overrepresentation of rigid linkers compared to flexible linkers

- Gerstmans et al. (2020) (1): repository of 67 tiles in total for the construction of a library with an **OMP-linker-CBD-EAD** configuration (here configuration 1)
- This study (2): **four additional configurations** by repositioning the same defined modules and/or doubling the OMP module (Figure 2)



As such the **impact of the configuration on the antibacterial activity** can be assessed and specific design rules can be deduced

Method

Experiment

- 188 random variants were randomly selected from each configuration (total: 940)
- Expression & preparation of cleared lysates
- Evaluation for growth inhibitory (GI) activity, both in the absence and the presence of 0.5 mM EDTA
- Conversion values to **GI%**: compare antibacterial activity/protein yield
- Higher GI% = higher GI activity
- Hits = variants inhibiting the bacterial growth for more than 80% (Figure 3: red dashed line)

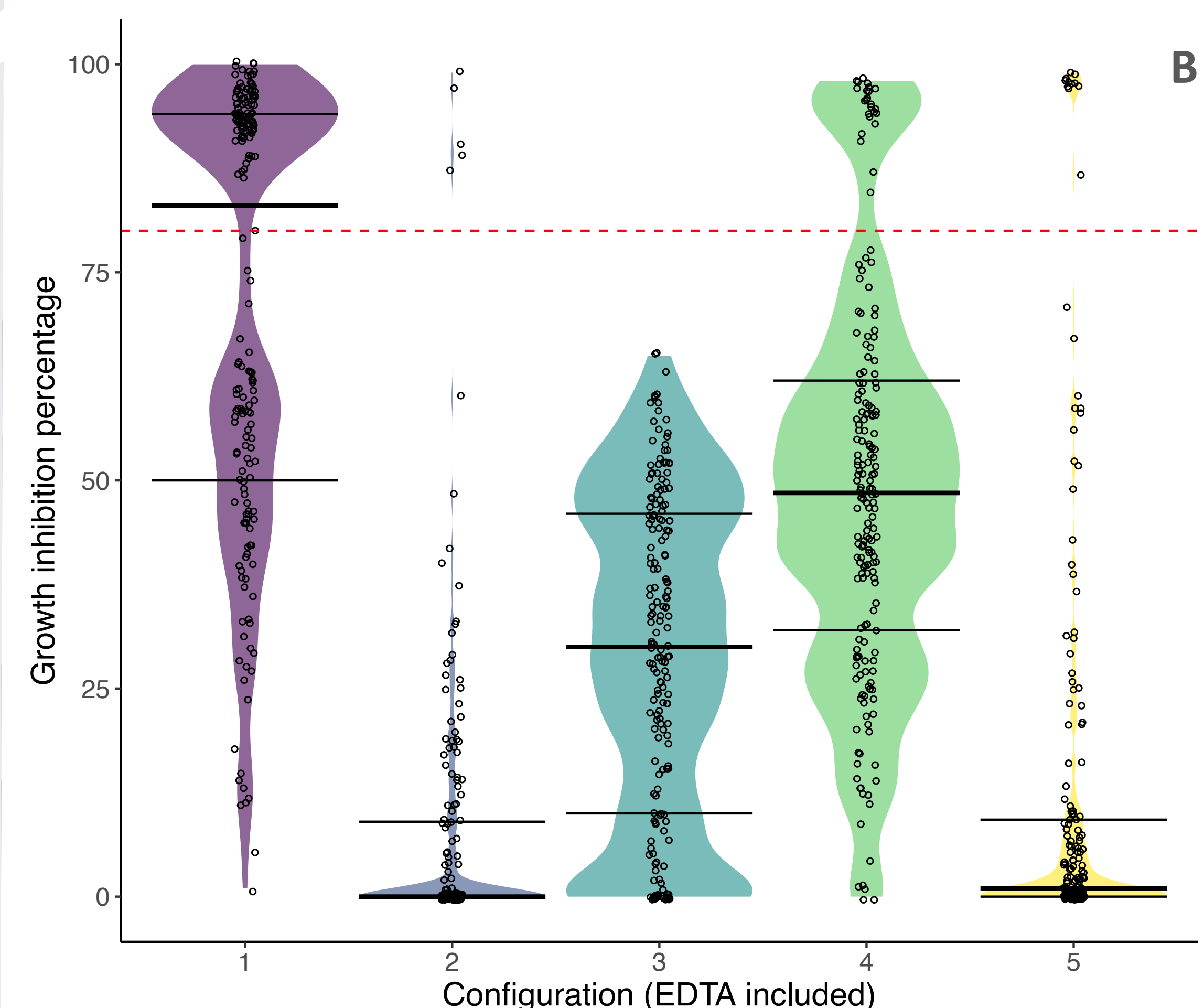
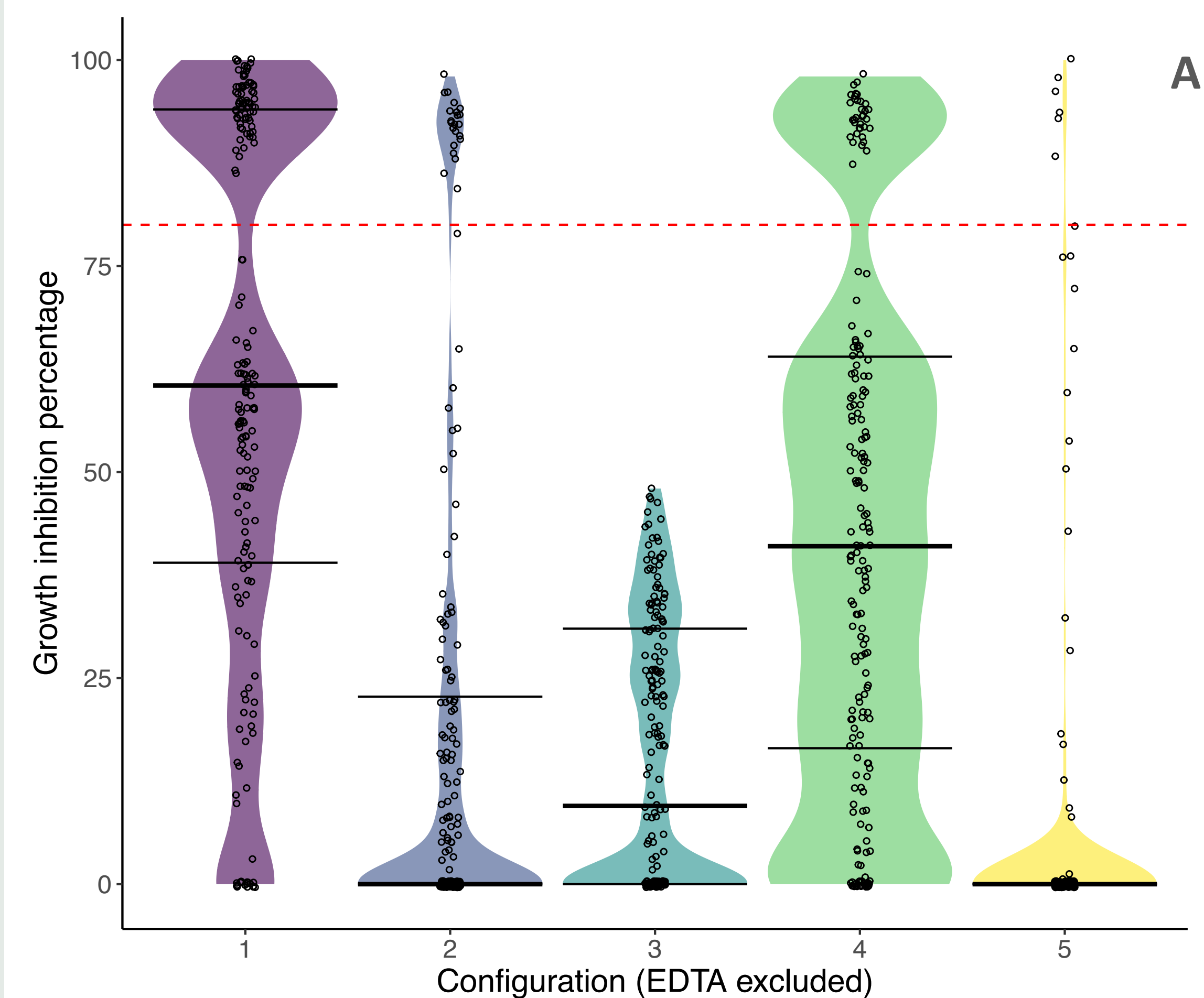


Figure 3. The distribution of the growth inhibitory (GI) activities, expressed as percentages, for 188 variants of each configuration. (A) The replicates tested in the absence of 0.5 mM EDTA. (B) The replicates tested in the presence of 0.5 mM EDTA. Red dashed line: GI threshold set in this study to be considered a hit (80%) (2).

Results

- **Configuration 1 (OMP-linker-CBD-EAD) & inverse (4: CBD-EAD-linker-OMP) most active:**

- OR: Implementing two peptides (2, 3 and 5) strongly reduces the number of active variants
- OR: Introduction of a linker results in higher activity
- Variants are operating by different working mechanisms (EDTA)

Conclusion

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References

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