

ANALYTICAL SEMINAR



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“Construction of complex and diverse DNA sequences using DNA three-way junctions”

DNA assembly is an essential tool in synthetic biology, but constructing long, repetitive, or sequence-complex DNA remains difficult because most conventional methods rely on sequence-dependent overlaps between fragments. In this study, the authors developed Sidewinder, a DNA assembly strategy that uses programmable DNA three-way junctions to guide the joining of multiple DNA fragments while separating the assembly instructions from the final DNA sequence. This design enables highly specific and scalable assembly with reduced dependence on the sequence composition of the target DNA. The method was demonstrated through the construction of complex and repetitive DNA sequences, assembly of up to 40 fragments, and generation of a large combinatorial DNA library containing hundreds of thousands of possible variants. Long-read sequencing was used to quantitatively evaluate assembly fidelity and revealed a very low frequency of incorrect junction formation. Together, these results show that DNA three-way junctions provide a robust and flexible platform for assembling complex DNA molecules and generating diverse sequence libraries, offering a useful approach for applications in synthetic biology, DNA engineering, and high-throughput biological screening.