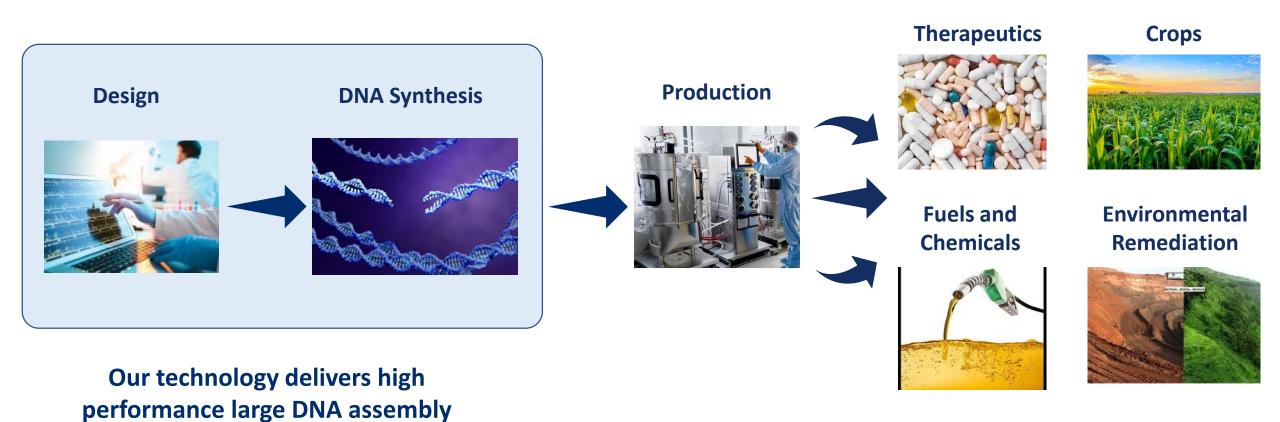
Our assembly technology



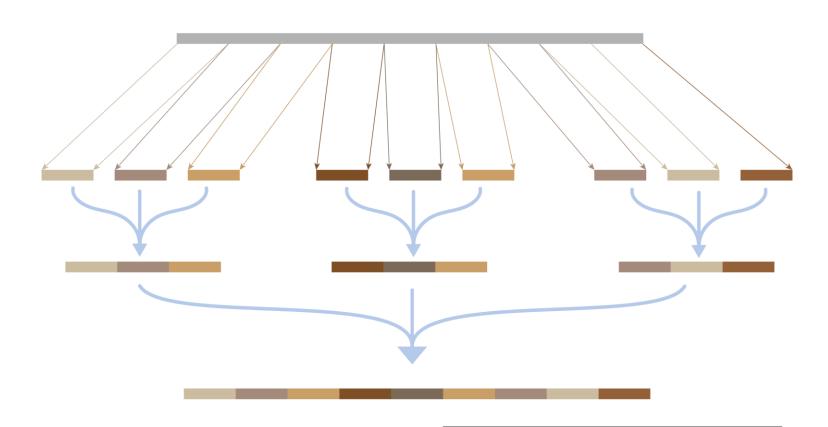
Hierarchical one-pot DNA assembly of any sequence

Sequence to be assembled

Short fragments synthesised

First round of assembly

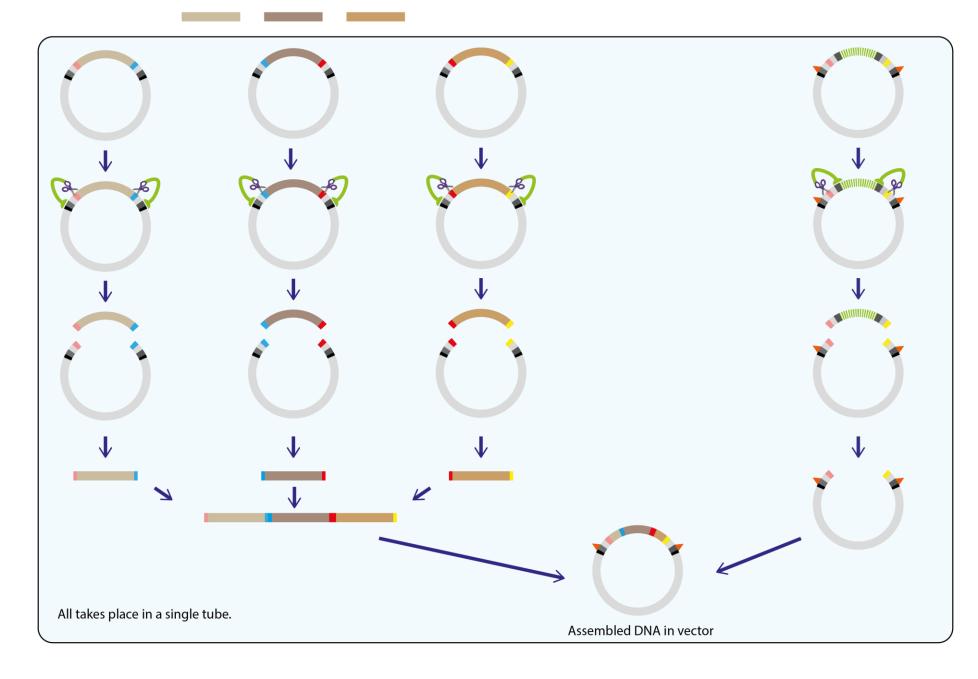
Second round of assembly



- can do multiple rounds
- assembly is a one pot reaction

DNA fragments to assemble

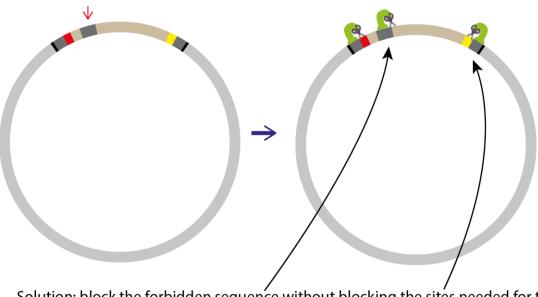
1. Flexible assembly with only one type **IIS restriction** enzyme: Design of vectors with two outer and two inner type **IIS sites. The** outer are methylated and the inner cut during the assembly



2. Assembly of fragments containing type **IIS internal sites** for the enzyme used during the assembly: Use of CRISPR/dCas9 to protect from methylation the flanking sites required for the assembly and methylases to methylate the internal sites

Problem: a forbidden sequence (ψ) causes cutting up of the DNA to be assembled

All sites can be blocked, but then no fragment can be cut out





Solution: block the forbidden sequence without blocking the sites needed for the assembly process

