**Serine Integrases: Advancing Synthetic Biology**

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* Can have site specific recombination
  + This switches orientation of sequences, doesn’t make it bigger
* Dna assembly by site specific recombination
  + Construction of genome of first synthetic organism, metabolic engineering of microbes for production of high-value compounds, establishment of DNA registries for the distribution of reusable DNA parts,and cloning of repetitive DNA sequences
  + DNA assembly methods
    - **endonuclease-mediated assembly** methods including BioBrickbased methods,70,75,76 and Golden Gate-based Assembly;77−79 **homology-based methods** including sequence and ligation independent cloning (SLIC),80 Gibson Assembly,81 DNA Assembler in yeast,82 and **PCR-based methods** like circular polymerase extension cloning (CPEC)83 and PaperClip;84 and site-specific recombination-based methods including Gateway,85−87 site-specific recombination-based tandem assembly (SSTRA)88 and serine integrase recombinational assembly (SIRA).20
  + LIMITATIONS:
    - Endonuclease based methods – must remove incompatible restriction sites from DNA parts prior to assembly – DNA parts have to be synthesized, precloned and mutated
    - *in vitro* homology-based methods are associated with size of DNA parts
      * SLIC and Gibson use exonuclease activity to generate ss complementary overhangs on DNA. – can degrade small DNA parts
    - PCR based methods – limited to DNA parts short enough to be amplified in vitro with DNA polymerase, mutations can occur. Once assembled, not easy to exchange dna sections
* Can have gateway assembly up to 5 DNA parts in single reaction
  + Precloning into donor and entry vectors is time-consuming, *att* sites are large – 200 pb(attr)
* Serine integrases – *att* sites are small (<50 bp) – require no accessory proteins to bind or bend them for recombination
* Can recombine serine integrase *att*  sites on linear pieces of DNA, eliminating need to preclone DNA parts into entry vectors
* **Logic and Memory**
* Drawback: in order to main a ss, systems rely on levels of tx that could impose considerable resource burden on host cell by redirecting nucleic acids, amino acids, and other metabolites away from native cellular processes can be solved by using syn cells (minimal resource burden)
* Intrinsic issue of logic and memory devics that rely on switching of states is noise generated by heterogeneity in biological populations a lot less noise in syn cells
* Maybe can you have a syn cell within a population of bio cells recording environmental things around it, bc more clear data collection, less noise etc
* Accumulating mutations form record of analog data, outputted as some reporter signal - Limitations of these approaches are associated with in situ sequencing for analysis of a mixed population of cells and the restricted diversity that can be generated in the guide RNAs. Some mutations in these systems occur with a higher frequency than others,108 and NHEJ can repair DNA cleavage without introducing mutations at all. It is also not known if Cas9 would have any off-target activity, or if integrated arrays of nuclease targets would affect genome stability.
* s. Data stored in DNA can be reliably retained throughout cell divisions, transferred across species, and easily detected after cell death. Importantly, these devices have the potential to detect transient signals and to record data in otherwise inaccessible environments.

**DNA assembly for synthetic biology: from parts to pathways and beyondw**

<https://pubs-rsc-org.clsproxy.library.caltech.edu/en/content/articlepdf/2011/ib/c0ib00070a>

* small fragments into large constructs
* standardized restriction enzyme assembly protolcl – bioBricks, bglBricks, golden gate
* sequence independent overlap techniques – in-fusion, SLIC, Gibson are popular for larger assemblies
* *in vivo* DNA assembly in yeast and bacillus for chromosome fabrication
* each ORF is considered in isolation
* want to combine parts to produce genes, linking genes to make pathways and devices, finally arranging these together to create synthetic chromosomes and genomes
* need an ability to reliably assemble and test DNA components in a high throughput manner
* BioBrick – dna unit with standardized flanking sequences that enabled assembly to be achieved by a cheap, simple, and standardized restriction/ligation method
  + Becomes bad when trying to do intermediate assembly scale involving several genes and regulatory elements
* Parallel assembly – user given self-assembly of many partsin single reaction
* Ordered assembly – construcst with pre-defined physical arrangemet
* Combinatorial assembly – multiple versions of parts to be used simultaneously
* Assembly needs to work at all levels of abstraction (genes, pathways, and genomes)and to clearly understand context dependencies when parts are physically placed next to other parts
* Functional gene – promoter, translation start site (RBS for prok), protein coding ORF, and terminator
* Need ordered assembly
* Need it to be perfect- ‘scar’ sequences (bases left behind) are undesirable
* Close proximity of parts beings up issue of context dependency
* RBS has to be exactly before ORF
* Biobrick downside – 8 bp scare sequence found at every junction, can be acceptable
* BglBrick has 6 bp scare, advantage: common restriction enzymes whose recognition sequences are not blocked by the most common DNA methylases, Dam and Dcm
* Neither can assemble scarless gene from parts and crucially cannto aseembly every sequence of DNA as the use of restriction enzymes means sequences they use sa recognition sites are forbidden within a part.
* However, scarless assembly without any ‘forbidden sites’ is possible using other methods – notably overlap extension polymerase chain reaction (OE-PCR).
  + Create homologous ends
* For parts to genes, OE-PCR is always good compared to restriction-ligation assembly.
  + Dependent on ordering custom oligonucleotide primers for each concatenation, decreasing in cost but are stille xpensive when scaled to library size batches
* OE PCR USED TO assemble 0.5 to 5 kb genes fragment *in vitro* – ligated to plasmids using restriction methods
* OE PCR can assemble a whole plasmid (CPEC)
* The huge disadvantage this has for synthetic biology is that individual parts, like promoters and ORFs, cannot be combinatorially swapped within constructs in order to create gene libraries without a large increase in synthesis costs. At this point the cost-balance switches and it is more common to have a codon-optimised ORF synthesised, which can then be assembled into a device using combinatorial techniques.
* Most operate at a higher scale of linking genes to construct pathways and devices
* Importance is to be able to assemble genes combinatorially so that libraries of genes can. Yield massive diversity in possible pathways and devices
* Nature of scar site means that individual parts cannot be replaced once assembled. OE PCR Methods can be used but limited in their ability to scale up, plasmids become less efficient atlarger sizes, error rate oF PCR, erroneous for reliable perfect amplification of 10+ kb constructs
* Repeating the pairwise selection cycle with 1 to 2 kb starting fragments can build a 91 kb assembly in only 6 rounds of transition between cassette vectors.
* The use of tags containing Type IIs sites that double as promoters for stringent antibiotic resistance markers allows this method to be done entirely in liquid culture, facilitating faster throughput using automation with a liquid handling robo
* , Gibson et al. successfully assembled a complete synthetic 583 kb M

r combinatorial construction from standarised part libraries, have no forbidden site requirements, and allow for pre-determined order in the final product. Yet it would also need to be assembled rapidly in a parallel reaction, applicable for work at any scale and only leave scars between parts that can tolerate them.

**A single-input binary counting module based on serine integrase site-specific recombination**

<https://academic.oup.com/nar/article/47/9/4896/5430839>

* Dna based ‘latch’ that switches between two states upon each exposure to a repeated stimulus
  + By integrase and rdf
* N latches could form binary ripple counter that can count to 2n-1
* Read before, check senior thesis ideas doc

**Logic Synthesis of Recombinase-Based Genetic Circuits**