**Supplementary information S3 (box) | Methods for constructing DNA encoding TALE repeat arrays**

*Standard cloning-based methods*

We[1](#_ENREF_1) and another group[2](#_ENREF_2) have described methods that utilize standard cloning methods to construct TALE repeat arrays (**Supplementary information S4, Aa-Ae**). These platforms rely on an archive of plasmids encoding single or multiple TALE repeat domains and the joining of coding sequences for these repeats together using traditional restriction digestion and ligation reactions that are performed in a parallel hierarchical fashion. The primary advantage of these approaches is that they utilize methods already practiced by most laboratories. The assembly process typically takes one or more weeks to perform but it is difficult to use these methods to make large numbers (e.g.—hundreds or thousands) of TALENs.

*“Golden Gate” cloning methods*

More than half a dozen groups have described TALE repeat array assembly platforms based on the “Golden Gate” multi-fragment ligation strategy.[3](#_ENREF_3), [4](#_ENREF_4) With these various platforms, a researcher can simultaneously ligate anywhere from 3 to 10 TALE repeat-encoding DNA fragments in a particular linear order into a plasmid vector.[5-10](#_ENREF_5) As shown in **Supplementary information S4, Ba-Bd)**, the Golden Gate strategy relies on the presence of orthogonal 5’ overhangs (generated by digestion with Type IIS restriction enzymes) on each end of each fragment to be ligated. The use of these different 5’ overhangs dictates ligation of the various fragments into a vector in a specified order. Because there appears to be an upper limit of 10 on the number of fragments that can be efficiently ligated together in a single reaction using this approach, Golden Gate strategies typically require the construction of intermediate plasmids encoding subsets of repeat domains that ultimately become part of the final desired array (**Supplementary information S4, Bc**). Golden Gate methods allow the construction of plasmids in two weeks or less but, as with standard cloning-based methods described above, it would be challenging to make large numbers of TALENs using these methods.

*Solid-phase assembly methods*

We recently described Fast Ligation-based Automatable Solid-phase High-throughput (**FLASH**) assembly, an automated, high-throughput method for assembling DNA encoding TALE repeat arrays on a solid-phase support[11](#_ENREF_11) (**Supplementary information S4, Ca-Ce**). This type of assembly approach is well suited for high-throughput use because it avoids the need for construction and passaging of intermediate constructs. With FLASH, we perform iterative ligation of DNA fragments encoding one or more repeat domains to construct a final DNA encoding the full-length TALE repeat array, which can be released from the solid support and then cloned directly into an expression vector of interest. The method relies on an archive of plasmids encoding one, two, three or four TALE repeat domains (available by request from our lab). FLASH can be practiced manually with a multi-channel pipet to construct dozens of DNA fragments encoding TALE repeat arrays in a single day. We have also established an automated FLASH platform that enables us to make as many as 96 DNA fragments encoding different TALE repeat arrays in one day.[11](#_ENREF_11) Using FLASH, our lab constructed 96 TALEN plasmids for EGFP disruption and 192 TALEN plasmids for disruption of endogenous human genes involved in cancer or epigenetics. To date, we have constructed over 1600 TALEN plasmids using the FLASH method (unpublished data).

Two other more recently published studies have also described methods for constructing DNA encoding multiple TALE repeats on a solid-phase support. The Iterative Capped Assembly (**ICA**) method uses DNA fragments encoding single TALE repeats and incorporates a “capping” step that blocks extension of incompletely ligated DNA fragments.[12](#_ENREF_12) ICA has been used manually with a multi-channel pipet to make 20 plasmids encoding TALENs. Another group has also described a solid-phase iterative ligation strategy similar to FLASH that uses pre-assembled DNA fragments encoding four TALE repeats.[13](#_ENREF_13) This latter strategy has been used to construct four plasmids encoding TALENs and two plasmids encoding TALE-based transcriptional activators.

*Commercial DNA synthesis*

Plasmid DNA encoding full-length TALE repeat arrays can also be obtained through commercial DNA synthesis companies[14](#_ENREF_14). Synthesis may be complicated by the highly repetitive nature of the TALE repeat array coding sequence and therefore may be more expensive than other DNA constructs of comparable size.

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