Semi-automated Tip Snip cloning of restriction fragments into and out of plasmid polylinkers

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Synthetic biologists rely on semi-synthetic recombinant plasmids, but DNA synthesis is constrained by practical limits on length, accuracy, and sequence composition. Cloned DNA parts can be assembled into longer constructs via subcloning, but conventional methods are labor-intensive. One-pot recombination reactions are more convenient but harder to troubleshoot, and those that depend on PCR to create fragments with compatible ends necessitate re-sequencing. The Tip Snip protocol described here enables the subcloning of an insert from one plasmid polylinker into another without PCR or gel purification steps. Cohesive ends of unwanted restriction fragments are snipped off by additional restriction endonucleases. The resulting short fragments (snippets) are eliminated by hybridization to complementary oligonucleotides (anti-snippets) and subsequent size-selection spin-column chromatography. Unwanted linear donor vectors are ligated to double-stranded oligonucleotides (unlinkers) so that only the desired insert and recipient plasmid form circular DNA capable of transforming bacteria. This new method is compatible with high-throughput processing and automated liquid handling, and because no specialized vectors, reagents, selection schemes, or analytical techniques are required, the barriers to adoption are low.

DNA synthesis costs are decreasing (1), but the assembly and cloning of synthetic DNA remains relatively labor-intensive and expensive. Nucleoside phosphoramidites are chemically synthesized on large scales. Single-stranded oligonucleotides and double-stranded synthetic genes are custom manufactured by machines, so turnover is rapid, throughput is high, and production costs are relatively low. Automation and miniaturization have decreased the per-unit cost of synthesizing gene-length (≤ 2 kb) DNAs, but further innovations are required to overcome practical limitations in length, nucleotide composition, accuracy, and yield (1). It is not yet feasible to have every new construct synthesized with its vector *de novo*, so PCR products and synthetic genes are most often cloned into plasmids and then sequenced.

Cloned parts are often assembled into larger constructs by subcloning, but this classical approach is recalcitrant to automation for three reasons. First, robots that can load agarose gels and purify particular restriction fragments have not yet been invented. Furthermore, DNA purification, restriction digests, and ligation reactions aren't reliably efficient, making monitoring and troubleshooting necessary. Finally, the design of cloning experiments is idiosyncratic, so the development of software algorithms that emulate decision making by experienced molecular biologists is non-trivial. The per-unit labor cost of subcloning (~10 h of labor

per attempt, not including incubation times) far exceeds those of reagents (e.g., enzymes and purification kits).

The high cost of molecular cloning has motivated the invention of new methods (2–5). In general, one-pot sequence-specific recombination reactions, such as those catalyzed by recombinases (e.g., Gateway cloning) (6, 7), thermostable polymerases (overlap extension PCR) (8), thermostable ligases (ligase chain reaction) (9), or combinations of exonuclease, polymerase, and ligase (Gibson assembly or ligase-independent cloning (10,11) are the most amenable to high-throughput and automated techniques (12). These protocols are less labor-intensive than traditional cloning workflows with discrete steps but are more difficult to troubleshoot. Another drawback of many seamless assembly techniques is their reliance on PCR or gene synthesis to create fragments with compatible ends. Every part must be re-sequenced each time it is seamlessly combined with another element and re-cloned (Special News Report. Weaver, J. 2015. *BioTechniques*. 59:II-III.), because the DNA polymerase I homologs used in PCR are three to five orders of magnitude less accurate than those responsible for *in vivo* plasmid replication and repair. Next-generation sequencing techniques lower per-unit cost, but they cannot be applied to individual plasmids. Thus, many synthetic biologists continue to assemble parts by manual subcloning.

METHOD SUMMARY

Tip Snip cloning uses restriction enzymes to shorten unwanted DNA fragments; the unwanted sticky ends are then neutralized by synthetic oligonucleotides. By eliminating the need to gel purify the desired restriction fragments, Tip Snip enables automation of the entire subcloning workflow.

I therefore sought a way to automate the conventional restriction endonuclease/T4 DNA ligase-dependent subcloning workflow. Golden Gate assembly, which utilizes type IIS restriction endonucleases (13), and 2ab assembly (14), which utilizes plasmids with two selectable markers separated by a unique restriction site, obviate gel purification but necessitate the employment of specialized vectors incompatible with those of other cloning standards. The three antibiotic assembly (3A) protocol (15) was specifically designed to assemble parts compatible with the seminal BioBrick Assembly Standard (RFC10) used by many synthetic biologists (16). Two donor plasmids carrying parts and a recipient plasmid encoding a counter-selection marker along with a selectable marker different than those of the donors are digested with different pairs of restriction enzymes. All six of the resulting restriction fragments are ligated together and used to transform *Escherichia coli*. The desired recombinant construct is distinguished from the parental plasmids using an antibiotic and the counter-selection scheme. This technique circumvents gel purification, but sacrifices efficiency for convenience. Three-fragment ligations don't occur as frequently as two-fragment reactions, particularly when three other unwanted fragments with compatible cohesive ends are present. The extraneous DNA also inhibits heat shock transformation of chemically competent *E. coli* (17), and electroporation is sensitive to salts in ligation reactions so it is less amenable to high-throughput experiments. Here, I describe a set of expedients that in combination facilitate efficient and reliable cloning of DNA into or out of almost any existing plasmid polylinker (multiple cloning site) without the need for PCR amplification or gel purification.

Materials and methods

The approach described here builds upon the following classical cloning techniques (18), except as noted. A more detailed step-by-step protocol is included in the Supplementary Material. Plasmids were purified from transformed *E. coli* using silica spin columns (QIAGEN, Valencia, CA) and hydrated Sephadex G-50 (GE Healthcare Life Sciences, Pittsburgh, PA) in empty spin columns (Epoch Life Science, Missouri City, TX) as directed by their manufacturers. Restriction digests were set up as recommended by the supplier [New England BioLabs (NEB), Ipswich, MA]. Whenever possible 2 µg of DNA (6 nM for a 5 kb plasmid) were digested to completion (or nearly so) at 37°C overnight with 20–40 U of each restriction enzyme (1–2 nM) in 100 µL total reaction volume. Approximately 20 fmol of digested, purified recipient plasmid and 20 fmol of digested insert (and donor plasmid) were reacted with 0.3–1 Weiss units of T4 DNA ligase in NEB T4 DNA ligase buffer containing 5% polyethylene glycol (molecular weight: 8000) (19). The reactions were temperature cycled in a Bio-Rad (Hercules, CA) MJ mini thermocycler between 10°C for 30 s and 30°C for 30 s for a total of 4–18 h (20).

Table 1. Anti-snippet oligonucleotide sequences.

Name	Snippet fragment	Anti-snippet (5' to 3')
BioBrick prefix <i>EcoRI-XbaI</i>	5'-AATTGCGGCCGCTT gcgcgcgcgaagatc-5'	aagcgcgcgcgaattG
BioBrick suffix <i>SpeI-PstI</i>	5'-CTAGTAGCGGCCGCTGCA atcgccggcg-5'	ctgcagcggccgctac- tagt
External* <i>KpnI-EcoRI</i>	5'-CTTAATTAAGACGTCGG catggaattaattctgcagccttaa-5'	attccgacgtcttaaat- taaggtta
External* <i>PstI-SacI</i>	5'-GCATCGATGTTTAAACGAGCT acgtcgtagctacaaatttgc-5'	TGCAGCATCGATGTT- TAAACGAG

*External anti-snippets that bind short restriction fragments (snippets) released by *KpnI/EcoRI* or *PstI/SacI* are specific for IMB2.4 and were not used in this study.

Figure 1. Conventional (middle to top) and Tip Snip (middle to bottom) subcloning workflows. Conventional digestion of donor and recipient plasmids with two restriction endonucleases each (A and B) creates four fragments: insert, unwanted donor plasmid, recipient plasmid and unwanted stuffer fragment. The unwanted fragments are traditionally eliminated manually via agarose gel electrophoresis and subsequent gel extraction of the desired bands. The Tip Snip strategy enables the automated subcloning of an insert from one polylinker (multiple cloning site) to another. Unwanted fragments are shortened slightly by additional restriction endonucleases (C and D or E and F). The unwanted short fragments (snippets) are heat denatured, hybridized to complementary oligonucleotides (anti-snippets), and eliminated via automated size-selection chromatography. The cohesive ends of the unwanted donor plasmid are made blunt by ligating them to oligonucleotide pairs that form cohesive ends (unlinkers). Only the recipient vector and insert remain intact to form circular plasmids capable of transforming *Escherichia coli*.

Chemically competent *E. coli* OmniMax2 cells (Thermo Fisher Scientific, Waltham, MA) were prepared according to Inoue et al. (17). For each transformation, up to 1.25 ng total DNA in ligation reactions were used to transform 25 µL of competent cells in the thermocycler. The transformants were spread on lysogeny broth medium (LB) agar plates containing 100 µg/mL ampicillin. Some agar plates also contained inducer and a histochemical substrate as described below. Some colonies were adsorbed to a nitrocellulose filter and transferred colony-side up to fresh LB-ampicillin plates supplemented with inducer (1 mM IPTG, 10 µg/mL tetracycline, 0.4% L-arabinose or 0.4% rhamnose) and 2 mg/25 mL plate X-gal (for colonies carrying *lacZ* expression vectors). Additional information the reagents and materials used to culture the bacteria can be found in the Supplementary Material.

Results and discussion

The Tip Snip cloning workflow begins with plasmids prepared via alkaline lysis and silica spin-column chromatography. A QIAcube robotic workstation (QIAGEN) can be used to automate this and other purification protocols. To eliminate small molecules that might inhibit restriction endonucleases or broaden their sequence specificity (21), plasmids are further purified via manual gel-filtration spin-column chromatography. Recipient and donor plasmids are digested as usual

Figure 2. Tip Snip cloning techniques demonstrated via ligation and bacterial transformation. (A) Cloning plasmid IMBB2.4-pUC57-mini (Supplementary Material) was digested with four different pairs of restriction endonucleases, thereby releasing small restriction fragments (Column 1 from the top). The digestion products were reacted with T4 DNA ligase in the presence of buffer (Column 2), and unlinker (Column 3), or unlinker with overhangs that did not match those of the restriction fragments (Column 4). Alternatively, the digestion products were heat-denatured and cooled in the presence of buffer (Column 5), anti-snippets (Column 6), or anti-snippets that failed to complement either strand of the small restriction fragment (Column 7) and then reacted with T4 DNA ligase. Finally, the digestion products were purified via the QIAGEN GeneRead Size Selection protocol. The purified fragments were reacted with either buffer (Column 8) or T4 DNA ligase (Column 9). All samples were used to transform *Escherichia coli* cells, which were spread on lysogeny broth (LB) agar plates supplemented with ampicillin. The numbers represent average colony counts from four experiments, each of which employed a different pair of restriction endonucleases. (B) Four recipient plasmids, each encoding a different promoter, were digested with *SpeI* and *PstI*, thereby releasing short restriction fragments. Four donor plasmids, each encoding a different reporter enzyme, were digested with *XbaI*, *PstI*, and *EcoRI*, thereby releasing insert and a short restriction fragment. The digestion products were heat denatured and cooled in the presence of a complementary oligonucleotide (anti-snippet), then purified via the QIAGEN GeneRead Size Selection protocol. The recipient plasmid (Column 2), the insert (and donor plasmid, Column 3) or combinations of recipient and insert (Columns 4–7) were reacted with T4 DNA ligase. Each reaction, as well as the unligated recipient plasmid (Column 1), were separately used to transform *E. coli*. The numbers represent average colony counts from four experiments, each employing a different recipient plasmid.

Table 2. Unlinker oligonucleotide pair sequences.

Restriction site	Overhangs	Unlinker pair
<i>EcoRI</i>	5'-G AATTC-3' 3'-CTTAA G-5'	5'-AATTaacgcctgaaggcagatt-3' ttgcgggacttcgcgctaa-5'
<i>PstI</i>	5'-CTGCA G-3' 3'-G ACGTC-5'	5'-gcagcaggtggacgtGTCA-3' 3'-cgtcgtccacctgcac
<i>KpnI</i> *	5'-GGTAC C-3' 3'-C CATGG-5'	5'-cgagatgcccacgacGTAC-3' 3'-gctctacgggttcgtcg
<i>SacI</i> *	5'-GAGCT C-3' 3'-C TCGAG-5'	5'-agctcacgcgggtgctAGCT-3' 3'-tcgagtggcgccacga

*External unliners that ligate to donor plasmids digested with *KpnI* or *PstI* are specific for IMBB2.4 and were not used in this study.

with restriction enzymes that produce fragments with compatible cohesive ends. Additional restriction enzymes recognizing sites in the polylinker are used to shorten undesired restriction fragments (the stuffer fragment of the recipient plasmid or the donor plasmid of a subcloning experiment) (Figure 1).

The digests are heated to denature the short restriction fragments (called snippets) and then cooled to anneal one strand to a molar excess of a synthetic oligonucleotide (anti-snippet), thereby displacing one strand of the original fragment and forming short double-stranded hybrid DNAs with ends that cannot anneal to those of the desired vector or insert fragments (Table 1). All short single- and double-stranded DNAs are then eliminated using the QIAGEN GeneRead Size Selection Kit; a custom protocol for the QIAcube automates the addition of anti-snippets, denaturation, annealing, and silica spin-column chromatography (Supplementary Material). Purified mixtures of restriction fragments are combined and reacted with T4 DNA ligase in a thermocycler (20). Inclusion of oligonucleotide pairs that hybridize to form cohesive ends compatible with those of unwanted donor plasmids (unliners) (Table 2) prevent the re-circularization of those fragments (Figure 1). Ligation reactions are used to transform chemically competent *E. coli*, and recombinant plasmids are confirmed via plasmid mini-preps and restriction mapping.

The Tip Snip strategy is intentionally redundant in several ways. Purification of plasmids via silica and size-exclusion chromatography diminishes the probability that subsequent restriction digests will be incomplete. Snippets are neutralized with anti-snippets and eliminated via size-selection spin-column chromatography. Unliners are used to prevent multiply digested donor plasmid fragments from ligating to other copies of themselves; such ligations produce long inverted repeats, which are not usually viable in vivo unless some further rearrangement occurs (22). These procedural redundancies could be eliminated at the risk of increasing costs associated with screening clones, troubleshooting, and repeating experiments.

The utility of size-exclusion chromatography for the elimination of restriction endonuclease inhibitors, size-selection chromatography for the elimination of small DNA fragments, and unliners for the inhibition of ligation reactions was initially demonstrated in agarose gels (Supplementary Figure S1). Unliners, size selection, and anti-snippets were also characterized by digesting plasmids, re-ligating them under various conditions, and transforming *E. coli* as follows: Cloning plasmid IMBB2.4-pUC57-mini was digested with pairs of restriction endonucleases (*EcoRI/XbaI*, *SpeI/PstI*, *KpnI/EcoRI* or *PstI/SacI*) so that snippets were released. Aliquots of each digest were mixed with a molar excess of compatible unlinker (or water or mismatched unlinker). Other aliquots were combined with the corresponding anti-snippets (or water or mismatched anti-snippets), incubated for 30 s at 70°C, and cooled slowly to room temperature. One additional aliquot of each digest was purified using the QIAGEN GeneRead Size Selection protocol. All aliquots were reacted with T4 DNA ligase, diluted, heat killed, and used to transform *E. coli* OmniMax2 cells, which were spread on LB plates supplemented with ampicillin. The sequence-matched unlinker, anti-snippets, and size selection reduced the re-ligation frequency of the plasmid to its snippet insert by 92%, 99%, and 99%, respectively, compared with the corresponding untreated control (Figure 2A). Mismatched unlinker and anti-snippets exhibited no inhibitory effects, demonstrating that their activities are sequence-dependent as expected.

A series of reporter gene expression vectors were constructed using only the four BioBrick restriction endonucleases, namely *EcoRI*,

XbaI, *SpeI*, and *PstI*, to demonstrate compatibility with this widely used cloning standard (Supplementary Figure S1). Four BioBrick-compatible variants of promoter regions, *tacl* (23), *tac-tetO* (identical to *tacl* but with a *tet* instead of *lac* operator), *araBAD* (24), and *rhaBAD* (25) were synthesized (see Supplementary Material for sequences) and digested overnight with restriction endonucleases *EcoRI* and *PstI*. The QIAquick PCR purification protocol was used to purify the large restriction fragments. Cloning vector IMBB2.4-pUC57-mini was digested with *EcoRI*, *PstI*, *XbaI* and *SpeI*. A custom QIAcube protocol (Supplementary Material) then added anti-snippets that partially complement the BioBrick prefix and suffix. The reaction was heated to 70°C to denature the snippets and then cooled slowly to room temperature.

The large fragments were purified with the GeneRead Size Selection protocol as directed by the manufacturer (QIAGEN), albeit with the custom QIAcube protocol. The concentrations of purified plasmid and synthetic DNA fragments were measured with a BioTek Take3 plate in a Synergy 2 microtiter plate spectrophotometer. The fragments were then ligated together in reactions with T4 DNA ligase. The ligation reactions were used to transform *E. coli* strain OmniMax2 and the cells were spread on LB agar plates supplemented with ampicillin. The number of colonies on plates corresponding to vector + insert + ligase reactions (1450 ± 159 cfu/ng) exceeded those on the vector + ligase control plates (156 cfu/ng) by nearly an order of magnitude. The number of background colonies would have been lower if the ligation products had been digested with a restriction endonuclease that recognized an internal site within the polylinker (namely *SphI*, *Apal*, *NdeI*, *BglII*, *NcoI*, *SfiI*, *EcoRV*, *HindIII*, *BamHI* or *XhoI*) (26,27); post-ligation digestion was eschewed to show that the approach works with any BioBrick accepting vector.

Tip Snip subcloning is particularly useful for the assembly of DNA constructs because fragments don't need to be re-amplified or re-sequenced after they are recombined with others. Four BioBrick-compatible ribosome binding sites and reporter genes, those encoding tagRFP, GFP, rpmH-sfBFP, and β -galactosidase, were previously synthesized or PCR amplified (*lacZ*) and cloned via classical techniques into pUC-based plasmids that confer upon host cells resistance to ampicillin (Supplementary Material). The reporter genes were excised from donor plasmids by overnight digestion with *XbaI*, *PstI* and *EcoRI* (Supplementary Figure S2). The recipient plasmids, which encode promoters (see above), were digested overnight with *SpeI* and *PstI*.

The custom QIAcube protocol was used to add anti-snippets complementary to the small restriction fragments (BioBrick prefix and suffix), incubate the mixture at 70°C, cool it slowly to room temperature, and then purify the larger fragments with the GeneRead Size Selection protocol. The purified restriction fragments were analyzed by agarose gel electrophoresis. Each of the four cloned promoters was ligated to each of the four reporter genes. These ligation reactions, including controls (vector only, vector plus ligase, insert plus ligase), were incubated at 65°C for 10 minutes to inactivate the T4 DNA ligase and used to transform *E. coli* OmniMax2 cells. The transformed bacteria were spread on LB agar plates supplemented with ampicillin and incubated at 37°C overnight. The number of colonies on the plates corresponding to the complete ligation reactions (vector plus insert plus ligase) exceeded those on control plates (vector plus ligase or insert plus ligase) by nearly an order of magnitude (Figure 2B).

The transformed colonies were filter-lifted onto LB agar plates supplemented with inducer (IPTG, tetracycline, L-arabinose, or

Figure 3. Filter-lifts of transformed colonies illustrate the high frequency of desired recombinant constructs. Colonies putatively transformed with recombinant plasmids—(A) *araBAD*-tagRFP, (B) *Ptac-tetO*-gfp, (C) *rhaS*-*rhaB*-rpmH-sfBFP, (D) *Ptac-lacO*-12his-*lacZ*—were adsorbed to nitrocellulose filters and transferred colony-side up to fresh lysogeny broth (LB) agar plates containing ampicillin, an appropriate inducer—(A) L-arabinose, (B) tetracycline, (C) L-rhamnose, (D) IPTG—and X-gal (for clones expected to express *lacZ*). The transferred colonies were induced for 16 h and photographed under white (tagRFP, *lacZ*/X-gal) or 360 nm light (gfp, sfBFP). Uncolored colonies are located with squares. Colony counts from all filters are listed in Supplementary Table S1.

L-rhamnose) and the histochemical marker X-gal for cells harboring plasmid-borne *lacZ* genes. Colonies were incubated overnight at 37°C then photographed under white (tagRFP, *lacZ*) or 360 nm (gfp, sfBFP) light (Figure 3). The majority of filter-bound colonies that putatively carried *tac*-tagRFP, *tac-tetO*-tagRFP, *araBAD*-tagRFP, or *rhaBAD*-tagRFP turned red, while those containing *tac*-rpmH-sfBFP, *tac-tetO*-rpmH-sfBFP, *araBAD*-rpmH-sfBFP, or *rhaBAD*-rpmH-sfBFP turned blue. These results confirm that all four promoters can drive downstream open reading frames and that the tagRFP and rpmH-sfBFP reporters can produce detectable signals in *E. coli* colonies. Most colonies that putatively carried *tac-lacZ* or *araBAD-lacZ* cassettes turned blue on plates containing inducer and X-gal, but those that carried *tac-tetO-lacZ* or *rhaBAD-lacZ* did not. Similarly, most colonies that putatively carried *tac-tetO-gfp* turned green, but those that carried *tac-gfp*, *araBAD-gfp*, and *rhaBAD-gfp* did not.

Overall, 11 of the 16 promoter-reporter gene combinations that were assembled produced signals that could be visualized within individual colonies (Supplementary Table S1). The vast majority of colonies on those 11 filters ($94\% \pm 1\%$) were colored, representing desired promoter-reporter gene assemblies. No color was observed in control colonies that carried the parental plasmids (promoter only or reporter gene only). Furthermore, 5 red and 12 white colonies were picked from the *araBAD*-tagRFP filter (and a replicate) and propagated in liquid LB-ampicillin. Plasmids were prepared from each culture and restriction mapped. All five plasmids derived from the red colonies showed restriction fragments corresponding to *araBAD*-tagRFP as expected, suggesting that all of the colored colonies were recombinant. Among the 12 plasmids from the white colonies, 6 contained fragments corresponding to tagRFP only, 4 encoded *araBAD* only, and 2 did not produce restriction fragments consistent with either parent plasmid. These results suggest that the restriction digests were incomplete and that optimization of these reactions would have decreased background. The inclusion of *SacI* (or plasmid-cleaving Cas9/single-guide RNA complex) in the initial digest of the donor plasmid (Figure 1 and Supplementary Figure S2) and the subsequent addition of the appropriate anti-snippet would also have eliminated the six tagRFP-only plasmids.

Figure 4. Induction of clones expressing tagRFP. Four individual colonies transformed with plasmids encoding (A) tac-lacO-tagRFP, (B) tac-tetO-tagRFP, (C) araBAD-tagRFP, (D) rhaBAD-tagRFP, or negative controls (promoter only or tagRFP only; not shown) were propagated to stationary phase at 37°C in lysogeny broth (LB)-ampicillin in microtiter plates. The cultures were diluted 100-fold in duplicate and propagated to mid-log phase. Inducer—(A) IPTG, (B) tetracycline, (C) L-arabinose, (D) L-rhamnose—was added to half the micro-cultures, and fluorescence from each well (excitation 544 nm, emission 590 nm) was measured after 1, 3, 6, and 18 h. Values are plotted in green for the uninduced cultures and in red for the induced cultures.

High-throughput subcloning enables the combinatorial assembly of variant constructs. The filter-lift experiments described above show that the different promoter-reporter gene combinations vary in efficiency. The tagRFP reporter was the most reliable of the four. Induction of the various promoter-tagRFP cassettes was measured in microtiter plate culture assays in parallel with promoter-only and tagRFP-only controls. Colonies were picked in quadruplicate and propagated at 37°C in LB-ampicillin micro-cultures in 96-well microtiter plates. Saturated cultures were diluted in duplicate plates containing 100-fold in fresh LB-ampicillin and propagated at 37°C for another 4 h. Inducers appropriate for each promoter (IPTG, tetracycline, L-arabinose, or L-rhamnose) were added to each mid-log phase culture of one microtiter plate. Fluorescence from each well of both plates (excitation 544 nm, emission 590 nm) was measured after 1, 3, 6, and 18 h (Figure 4). The tac-lacO and tac-tetO promoters were strong and leaky under these conditions. Overexpression of tagRFP apparently reduced growth rates such that the signal from uninduced cells at later time points was greater from these expression vectors, on average, than that from induced cells. The araBAD and rhaBAD promoters were better repressed under non-inducing conditions, so induction led to increased signal, as expected.

Tip Snip cloning is most useful for the assembly of cloned parts into a construct without re-synthesizing or re-sequencing. It was demonstrated by combining four promoters with four reporter genes in parallel. The spin columns used in this study are also available in a 96-well format so the workflow could conceivably be scaled up to 48 × 48 pairs, although an alternative to the 15 × 100 mm Petri dishes used here to isolate individual clones would have to be devised. Such economies of scale could further reduce costs for investigators willing to adopt a cloning standard. It should also be possible to automate the set-up of restriction digests, ligations, and transformations so that the entire workflow, except for microbiological steps, could be executed by a liquid handler.

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Competing interests

A provisional patent application disclosing the work in the article was filed by the Emory University Office of Technology Transfer with the United States Patent and Trademark Office on February 22, 2017 and assigned Serial No. 62/462,210.

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