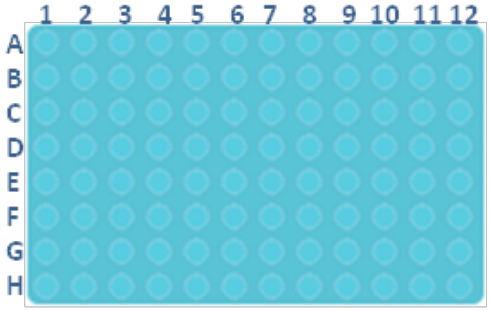
TABLES

Table S1. Representative short and explicit forms of well list definitions (for the plate geometry shown).

Short form	Explicit form
A2+4	A2,B2,C2,D2
A2~D2	A2,B2,C2,D2
9+4	A2,B2,C2,D2
A2x3	A2,A2,A2
A2+2x2	A2,B2,A2,B2
A2+2|2	A2,A2,B2,B2
A2-A6	A2,A3,A4,A5,A6



Note: Parentheses have not yet been introduced into PaR-PaR, as at this stage they might overly complicate the language. Currently, all well list definition expressions containing additional actions/multipliers are consistently evaluated. For example, A2+2x2 will result in A2,B2,A2,B2, and A2+2|2 will result in A2,A2,B2,B2. In both cases, PaR-PaR evaluates the initial expression (*e.g.*, A2+2) first, and then applies the modifier (*e.g.*, x2 or |2). For these two example cases, it is either 'repeat the well string', or 'repeat each well in the string'. PaR-PaR does not currently allow the chaining of modifiers.

Table S2. Predefined liquid transfer options.

	Aspirate from Liquid	
Dispense from	Level	Bottom
Air	LC_W_Lev_Air	LC_W_Bot_Air
Liquid Level	LC_W_Lev_Lev	LC_W_Bot_Lev
Liquid Bottom	LC_W_Lev_Bot	LC_W_Bot_Bot

Note: It is possible to configure PaR-PaR to use additional user-specified liquid classes. There are currently two ways of doing this: 1) PaR-PaR server-wide configuration (see the README file in the PaR-PaR source code root directory), and 2) script-specific configuration (refer to the online how-to user's guide for PaR-PaR).

METHODS

j5-designed PCR.

PCR reaction contents.

Component	Each 50 µl Reaction (µL)
Nuclease-free water	20.5

5X Phusion HF or GC Buffer	10
10 mM dNTPs	1
5 µM Forward Primer	5
5 µM Reverse Primer	5
Template DNA	5
DMSO	2.5
Phusion DNA Polymerase	1

PCR reaction thermocycle parameters.

Temperature (°C)	Time	Cycles
95	5 min	1
98	30 sec	1
98	10 sec	35
Gradient (see Supporting File “distribute_pcr.csv”)	30 sec	
72	5.5 min	
72	10 min	1
4	Hold	1

j5-designed PCR reaction setup PaR-PaR script. (See also Supplemental File “distribute_pcr.par”).

```
TABLE      Table_JBEI_1.ewt

"""""""
Distribute PCR Reactions j5 v1.1.7beta
Please cite: Hillson, N.J., Rosengarten, R.D., and Keasling J.D. (2012) j5 DNA Assembly Design Automation Software.
ACS Synthetic Biology 1 (1), 14-21. DOI: 10.1021/sb2000116
"""""""

#          # alias                              # name
PLATE      oligos_plate                         PL1
PLATE      templates_plate                      PL2
PLATE      assemblytoautomate_PCR_plate_1       PL4
PLATE      PCR_mix_tubes                        PL7

#          # name    # location          # method
COMPONENT  PCR_mix   PCR_mix_tubes:1     LC_W_Lev_Bot

#          # alias            # volume
VOLUME     template_volume    5
VOLUME     primer_volume      5
VOLUME     PCR_mix_volume     35

#          # recipe name
RECIPE     PCR_reactions
# PCR ID   # template         # template_volume # forward_primer # primer_volume # reverse_primer # primer_volume # PCR_mix # PCR_mix_volume
PCR_ID_0:  templates_plate:A1   template_volume  oligos_plate:C2   primer_volume  oligos_plate:D2   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_6:  templates_plate:C1   template_volume  oligos_plate:A1   primer_volume  oligos_plate:B1   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_3:  templates_plate:D1   template_volume  oligos_plate:G2   primer_volume  oligos_plate:F1   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_5:  templates_plate:F1   template_volume  oligos_plate:A3   primer_volume  oligos_plate:B2   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_10: templates_plate:F1   template_volume  oligos_plate:A2   primer_volume  oligos_plate:B2   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_1:  templates_plate:B1   template_volume  oligos_plate:E2   primer_volume  oligos_plate:D1   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_7:  templates_plate:B1   template_volume  oligos_plate:C1   primer_volume  oligos_plate:D1   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_2:  templates_plate:C1   template_volume  oligos_plate:A1   primer_volume  oligos_plate:F2   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_8:  templates_plate:D1   template_volume  oligos_plate:E1   primer_volume  oligos_plate:F1   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_4:  templates_plate:E1   template_volume  oligos_plate:H2   primer_volume  oligos_plate:H1   primer_volume   PCR_mix   PCR_mix_volume
PCR_ID_9:  templates_plate:E1   template_volume  oligos_plate:G1   primer_volume  oligos_plate:H1   primer_volume   PCR_mix   PCR_mix_volume

# make recipe(s)
# command  # recipe_name      # destination                                                  # method       # options
MAKE       PCR_reactions      assemblytoautomate_PCR_plate_1:A1,B1,A3,A5,B5,A7,B7,A9,B9,A11,B11     DEFAULT        MIX:10X8
```

Colony PCR.

PCR reaction contents.

Component	Each 30 µl Reaction (µL)
Nuclease-free water	13.8
5X Phusion HF or GC Buffer	6
10 mM dNTPs	1
0.5 µM Forward Primer	1.5
0.5 µM Reverse Primer	1.5
Template DNA	5
DMSO	0.9
Phusion DNA Polymerase	0.3

PCR reaction thermocycle parameters.

Temperature (°C)	Time	Cycles
98	90 sec	1
98	15 sec	34
PCR1 60; PCR2 58; PCR3 55	15 sec	
72	1 min	
72	10 min	1
4	Hold	1

Colony PCR PaR-PaR script. (See also Supplemental File “colony_pcr.par”).

```
TABLE Table_JBEI_4.ewt

"""
Colony PCR
"""

COMPONENT       Prm1_PCR_Mix            PL7:1+2 LC_W_Lev_Bot
COMPONENT       Prm2_PCR_Mix            PL7:4+2 LC_W_Lev_Bot
COMPONENT       Prm3_PCR_Mix            PL7:6+2 LC_W_Lev_Bot

PLATE           Templates               PL5

SPREAD          Prm1_PCR_Mix            PL4:A1+48       25      DEFAULT
SPREAD          Prm2_PCR_Mix            PL4:A7+48       25      DEFAULT

TRANSFER        Templates:A1-48         PL4:A1+48       5       DEFAULT         MIX:18x10
TRANSFER        Templates:A1-48         PL4:A7+48       5       DEFAULT         MIX:18x10

MESSAGE         Change Plate in PL4 position

SPREAD          Prm3_PCR_Mix            PL4:A1+48       25      DEFAULT

TRANSFER        Templates:A1-48         PL4:A1+48       5       DEFAULT         MIX:18x10
```

FIGURES

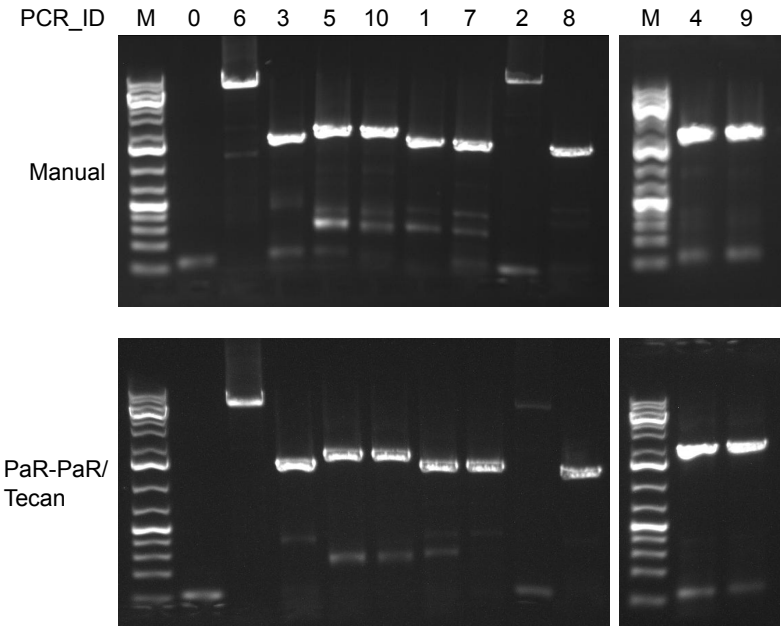


Figure S1. Agarose gel DNA electrophoresis comparison of PCR results for manually and robotically prepared j5-designed PCR reactions. 'M' indicates a Fermentas GeneRuler 1 kb Plus DNA ladder. Numbers indicate j5-output PCR ID numbers (see supplemental file "assembly.csv"). Lane labeling is consistent for the **(top)** manually and **(bottom)** PaR-PaR/Tecan Freedom Evo-prepared PCR reactions.

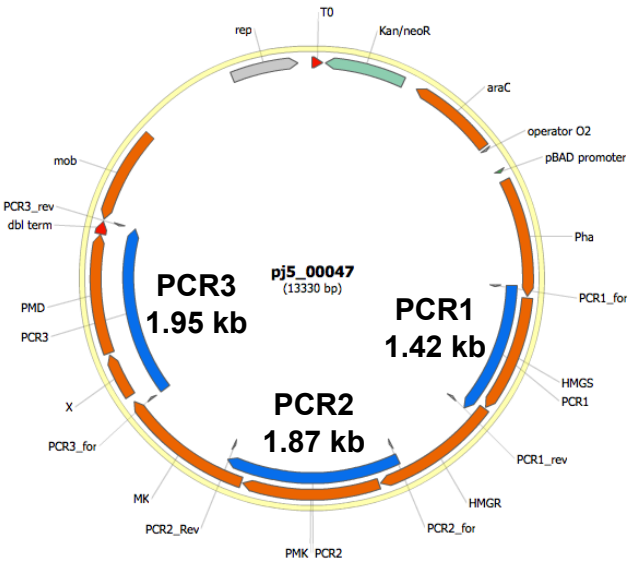


Figure S2. Plasmid pj5_00047 map. The expected products of PCR reactions PCR1-3 are indicated along with their lengths and corresponding DNA oligo primer sites. PCR1 spans the two assembly junctions between Pha/HMGS and HMGS/HMGR. PCR2 spans the two assembly junctions between HMGR/PMK and PMK/MK. PCR3 spans the three assembly junctions between MK/X, X/PMD, and PMD/dbl_term. VectorEditor software (*1*) was used to render the plasmid map.

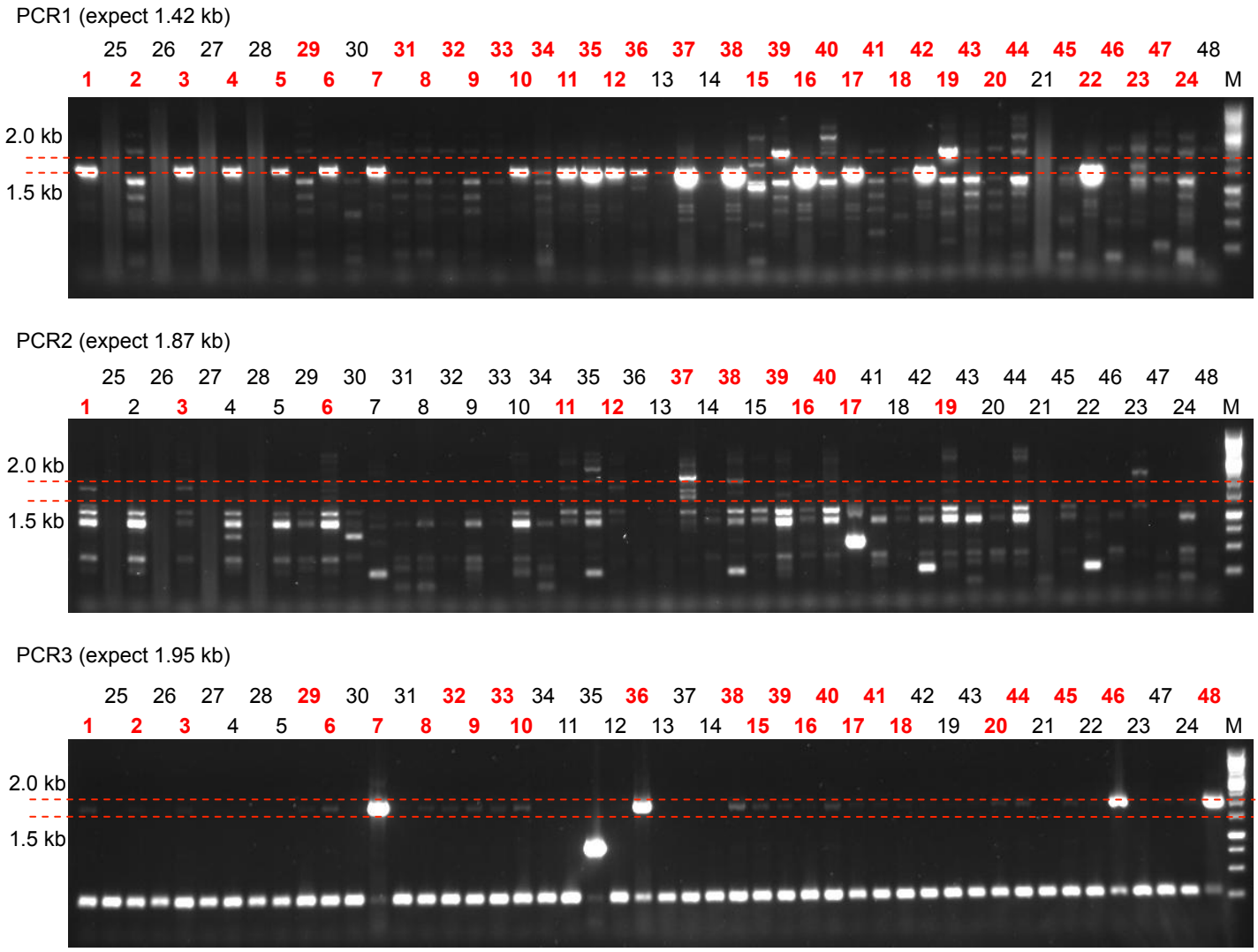


Figure S3. Agarose gel DNA electrophoresis of robotically prepared colony PCR reactions. 48 pj5_00047 DNA assembly reaction transformant colonies were screened with 3 PCR Reactions (PCR1-3, Figure S2) for correct assembly junctions. 'M' indicates a Fermentas GeneRuler 1 kb ladder. Dashed red lines indicate approximate migration distances for 2.0 and 1.5 kb DNA (as labeled). Numbers indicate colony numbers. Red numbers indicate lanes with DNA bands consistent with the expected product size. Colony 38 was subsequently determined to be a correct clone of the desired DNA sequence. **(Top)** PCR1. **(Middle)** PCR2. **(Bottom)** PCR3.

SUPPLEMENTAL FILES

File Name	Description
colony_pcr.par	colony PCR PaR-PaR script
colony_pcr.esc	colony PCR PaR-PaR script compiled for the Tecan Freedom Evo
assembly.csv	j5 DNA assembly design input file
plate_list.csv	j5 multi-well plate list input file
zipped_plates.zip	zipped j5 multi-well plates input file
distribute_pcr.csv	j5 distribute PCR reactions output file
distribute_pcr.par	j5 distribute PCR reactions PaR-PaR script output file
distribute_pcr.esc	j5 distribute PCR reactions PaR-PaR script compiled for the Tecan Freedom Evo
BreakfastDrinks.ewt	BreakfastDrinks Tecan Freedom Evo robotic work table file
liquid_classes.zip	zip file containing Tecan Freedom EVOware export output for the six liquid classes shown in Table S2

REFERENCES

1. Ham, T. S., Dmytriv, Z., Plahar, H., Chen, J., Hillson, N. J., and Keasling, J. D. (2012) Design, implementation and practice of JBEI-ICE: an open source biological part registry platform and tools, *Nucleic Acids Res*.