Supplemental Table 1.

Strains and plasmids used in this study.		
Strain name	Relevant Genotype / Notes	Source
Staphylococcus aureus strains		
RN4220	Restriction-minus derivative of NCTC8325	[1]
JMB1100	USA300_LAC	Submitted
JMB1293	USA300_LAC *srrABΔ*	
JMB2579	USA300_LAC *srrABΔ*, Φ11*attB*::pLL39	This study
JMB2592	USA300_LAC *srrABΔ*, Φ11*attB*::pLL39_ _$srrA^{FLAG}$*srrB*	This study
JMB3946	USA300_LAC *srrABΔ*, Φ11*attB*::pLL39_$srrA^{FLAG\text{-}D53A}$*srrB*	This study
Escherichia coli strains		
PX5	Restriction-minus cloning.	Protein Express
BL21 (DE3) CodonPlus-RIL	Protein production.	Agilent Technologies
Saccharomyces cerevisiae strains		
FY2	*MAT*α, *ura3-52*	[2]
Plasmids		Source
pET24a		Novagen
pET24a_*nono*		This study
pCMVeGFP-C1		Clonetech
pCMVeGFP-C1_nono		This study
pLL39		[3]
pLL2787		[3]
pLL39_FLAG_*srrAB*		This study
pLL39_FLAG_$srrA_{D53E}$*srrB*		This study

Supplemental Table 2.

Primer	**Sequence**
pet24a-NONO-F	GACTGGTGGACAGCAAATGGGTCGCGGATCCcaaggaaacagaggaccccg
pet24a-NONO-R	CGGATCTCAGTGGTGGTGGTGGTGGTGCTCgaatctgcggcgtttgttgg
pET-YCC-F	GAGCACCGCCGCCGCAAGGAATGGTGCATGggttccgcgcacatttcc
pET-YCC-R	GCCGGGGGACTGTTGGGCGCCATCTCCTTGgtgagtttagtatacatgc
CMVeGFP-NONO-F	TGGACGAGCTGTACAAGTCCGGACTCAGATCTCGAGCTatgcaaggaaacagaggaccc
CMVeGFP-NONO-R	CGGGCCCGCGGTACCGTCGACTGCAGAATTCGAgaatctgcggcgtttgttgg
CMVeGFP-YCC-F	GGATAACCGTATTACCGCCATGCATTAGTTATggttccgcgcacatttcc
CMVeGFP-YCC-R	GAACTAATGACCCCGTAATTGATTACTATTAgtgagtttagtatacatgc
pLL39_yeast F	GCCCAATCACTAGTGAATTCCCGAAGCTTAGTTACGCTAGGGATAACAG
yeast_srrPro R	atatcttggatgtgtcattaaCTATATTACCCTGTTATCCC
yeast_srrPro F	GGGATAACAGGGTAATATAGttaatgacacatccaagatat
srrPro_Flag R	GGGATAACAGGGTAATATAGttaatgacacatccaagatat
srrPro_Flag F	gtgtgggaggtatgacctgtATGgactacaaagaccatga
Flag_srrAB R	ACGATAAGTATTTCGTTCGActtgtcatcgtcatccttgt
Flag_srrAB F	acaaggatgacgatgacaagTCGAACGAAATACTTATCGT
srrAB_pLL39 R	GGTAATAAAAAAGCTTGCATGCCTGCAGGttttattctggttttggtag
D53E SrrA F	ATTATGCTTGCATACTACTAGAATTAATGTTGCCTGAAATGGA
D53E SrrA R	TCCATTTCAGGCAACATTAATTCTAGTAGTATGCAAGCATAAT
srrABup5EcoRI	CCCGAATTCGGACTAGTTGAGGCTAAAGTGGTTAATGAACAGCG
srrABdwn3SalI	CCCGTCGACCTGTGCACCCCATATTCTATTGGACG

* *Note*: Oligonucleotide sequences that do not match the YCC sequences shown in Figure 1 contain the meganuclease sites. We recommend using the YCC sequences in Figure 1 unless you absolutely need to remove the YCC.

Supplemental Material. A Universal Cloning Method that is simple, efficient, and versatile.

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Yeast transformation (approximately 2 hrs of bench time). The Yeast transformation listed below is the protocol we used throughout but any high-efficiency yeast transformation should be suitable.

Prepare Cells

1| Back dilute a 50mL culture of YPD with 1 mL of overnight stationary phase yeast culture. Incubate at 30°C with shaking (150-250 rpm) for approximately 4-5 hours or until mid log phase of growth is achieved ($O.D._{600}$ = 1.0).

3| Transfer yeast to a sterile 50mL conical tube and pellet cells by centrifugation at 2,500 x g for 5 minutes. Wash cells with 25mL of sterile water and pellet again.

5| Decant supernatant and suspend cell pellet in 1mL of 0.1 M LiOAc and transfer to sterile 1.5mL microfuge tube.

6| Spin at 10,000 x g at room temperature for 15 seconds to pellet cells. Decant LiOAc supernatant and suspend cells in 400 μL of 0.1 M LiOAc.

8| Aliquot 50uL cells per transformation in a 1.5 mL microfuge tube. Keep cells on the benchtop at room temperature until use.
NOTE: cells can be refrigerated for up to a week with negligible effects on overall efficiency.

Prepare DNA

9| Combine ~0.5 -1.0 μg of each of the overlapping fragment into a sterile microcentrifuge tube. Bring to a total volume of 34 μl in water.

Prepare transformation mix

10| Prepare a master mix depending on the number of samples that will be transformed using the recipe shown below. 325 μL is used per transformation.

11| Dilute salmon sperm DNA to 2.0 mg/mL and denature by boiling for 5 minutes, then place on ice for an additional 5 minutes.

Transformaion Mix

240 μL 50% PEG 3350
36 μL 1M LiOAc
50 μL 2mg/mL carrier DNA
34- x μL sterile H_20 (x is the total volume of DNA fragments)

Perform Transformation

12| Pellet cells from step 8 by centrifugation at 16,000 x g for 15 sec. Decant the LiOAc.

13| Suspend cell pellet with the 34 ml of DNA mixture from Step 9.

14| Add 325 μl of transformation mix to each tube and vortex gently.

15| Incubate at 30°C for 30 minutes.

16| Mix by inverting, then heat shock at in a 42°C for precisely 30 minutes.

17| Spin cells for 15 sec as above and decant the supernatant.

19| Suspend the cell pellet in 100 μl sterile H_20 and spread on YMD agar plates Grow for 2-3 days at 30°C.

Plasmid Recovery.

Once yeast colonies have appeared on the plate, these can either be pooled by scraping cells off the plates in the presence of 1 ml H_2O and then isolate DNA directly from the pooled cells. Alternatively, individually colonies can be grown up overnight in liquid culture. There are numerous high-quality protocols to extract DNA from *S. cerevisiae* and any can be followed. In general, the quality of DNA will greatly affect the efficiency of the bacterial transformation. The "quick and dirty" yeast smash and grab protocol is sufficient after scraping and pooling all the yeast colonies off the plate, but this requires using high-efficiency electrocompetent cells for the bacterial transformation. This typically results in ~200 colonies per plate. If using less efficient competent cells, we recommend using a more elaborate DNA isolation method.

REAGENTS

- FY2 strain of *Saccharomyces cerevisiae*

-YPD / YPD Agar Plates

- Yeast Extract(Fisher Sci –BP1422)
- Peptone (Fisher Sci – BP1420)
- Dextrose (Fisher Sci – D16)
- Agar (US Biological – A0930)

- <u>YMD Plates</u>
  - Yeast Nitrogen Base (Becton & Dickinson – 291940)
  - Dextrose (see above)
  - Agar (see above)
- Sterile H_2O
- 1M LiOAc (Sigma – L6883)
- 50% PEG 3350 (Sigma – P3640)
- 2 mg/mL salmon sperm (carrier DNA)