Synthetic Lethal (or Bypass) Screen

General Info from Winston Lab

NOTE 1: Protocol uses the Snyder Library (Burns *et al* (1994) *Genes Dev* **8:**1087). If you are planning an FOA-based screen to look for synthetic interactions with a mutant it is best to replace the genomic locus with the mutant allele rather than have the shuffling plasmid and the mutant allele on plasmids covering a knockout. Also best to have the shuffling plasmid on a CEN / ARS low copy vector.

NOTE 2: Lab has two pools of the Snyder library #21 (L25; b2739, b2740) and #22 (L30; b2741, b2742), containing genomic inserts of approximately 8kB in a 2kB vector backbone. These should be dealt with separately. This protocol assumes you have already isolated plasmid DNA from each pool.

Variant of protocol used in: Keogh *et al* (2005) *Cell* **123:**593-605

1. Initial candidate screen

A. Digest Qiagen-quality DNA with NotI to completion. There is no need to gel purify, but you should extract and clean the DNA – gel analysis should show genomic inserts of approximately 8kB in a 2kB vector. <u>You will need 5µg of each pool digested per transformation</u>.

B. Grow 40mls of yeast strain of interest to $OD_{600} \approx 1$ ($\approx 10^7$ cells / ml). For high efficiency transformation it is important that the cells are in exponential growth. Wash cells twice in ddH_2O, resuspend in 100µl LiTE. Add the digested library DNA and 10µl of 10mg/ml previously boiled salmon sperm DNA (stocks at –20°C). Incubate 30 minutes at 30°C, then add 500µl PLATE and mix by inversion. Incubate 20 minutes at 30°C, then 60 minutes at 42°C. Flash spin the cells and resuspend in 1ml ddH2O.

C. From this 1ml transformed stock make a few serial dilutions of 50µl, spread these on –LEU plates and incubate 30°C. Store the remainder of the transformation at 4°C. Examine the plates at 2 – 4 days (remember it might take longer than normal as you're screening for integrants) and estimate the volume you need to spread on a –LEU **(+URA?)** plate to give ≈ 200 – 250 isolated colonies. Plate accordingly.

NOTE 1: If you're doing a bypass screen (trying to rescue Δ of an essential gene) you can plate at a much higher density as you will be looking for live cells on an empty plate, rather than smaller or missing colonies (as with an SS or SL screen).

NOTE 2: ≈ 30,000 colonies gives approximately 95% genomic coverage but 10,000 from each pool is enough to work with.

D. After the colonies have grown up replica plate to + FOA. Reincubate plates and look for FOA sensitive colonies or slow growers or whatever you're interested in. Streak the non-shuffled candidates onto YPD and after they've grown up replica them back to –LEU; only work with stable integrants.

2. Screening candidates (1st pass)

Old School option: Mate the candidates to a strain identical to that used for the initial screen but of an opposite mating type. Sporulate and tetrad dissect. Confirm that your mutant allele (MARKER) and the disruption (LEU) co-segregate. Remember you also have to track the shuffling (CEN/ARS, URA) plasmid.

Screw That: That looks like a lot of work, doesn't it. There is a faster (albeit dirtier) way. Isolate genomic DNA from your shuffled candidates. If doing a bypass screen confirm that the covering gene has really gone and not integrated into the genome: do a PCR with primers to the enzyme active site to make sure it's not present (see *Cell* paper, below). **Be most suspicious of the colonies growing as fast as WT – bypass is generally incomplete.** Dump the positives in this pass, Vectorette all the rest.

3. Vectorette PCR

See MCK **pH8.47**
Also see Current Protocols in Molecular Biology p**13.3.8** (supplement 51)

A. Harvest genomic DNA from each candidate (5ml confluent culture to 100µl DNA prep). Digest an aliquot with the restriction enzymes Rsa1 and HaeIII (strongly recommend using each of the enzymes individually). Each 20µl digestion will contain:

DNA (≈1µg)	5µl
x10 Rb^o	2µl
10mg/ml BSA	0.2µl
RE	1µl
ddH_2O	11.9µl

Digest 4hrs at 37°C. Store digests at –20°C until ready for PCR.

B. Prepare the anchor bubble primers: (stocks each were 40µM)

AncBub1 (**oligo # 696**; 53mer; was PAGE purified):
GAAGGAGAGGACGCTGTCTGTCGAAGGTAAGGAACGGACGAGAGAAGGGAGAG

AncBub2: (**oligo # 697**; 55mer; was PAGE purified):
GACTCTCCCTTCTCGAATCGTAACCGTTCGTACGAGAATCGCTGTCCTCTCCTTC

Assemble: in a 50µl volume make each AncBub primer to 4µM and add $MgCl_2$ to 2mM
PCR: 94°C 5', 50°C 5', 4°C 5' → move to ice bucket.

C. Anneal the anchor bubbles (per 30µl ligation):

Digested genomic DNA (from **3.A**)	5µl
x10 T4 Ligase b^o	2.5µl
10mM ATP	0.5µl
400U T4 Ligase	1µl
4µM annealed AncBub mix (from **3.B**)	1µl
ddH_2O	20µl

Anneal overnight at 14°C. Store digests at –20°C until ready for PCR.

D. Vectorette PCR screen (50µl mix per sample): use **Denville TaqPro**

Ligated genomic DNA (from **3.C**)	3µl
x10 Rb^o	5µl
2mM dNTP	5µl
$MgCl_2$	2µl
Oligo #32 2.5µM	5µl
Oligo #693 2.5µM	5µl
Denville TaqPro	0.5µl
ddH_2O	34.5µl

PCR reaction conditions (35 cycles)

T_1	94°C	45s
T_2	55°C	45s
T_3	72°C	90s
T_4	16°C	∞

E. Run vectorette PCR products on a 1.1% gel. Go with products that are single bands (although if multiple strong bands are present in a lane do them all individually). Excise with a blade and use the QiaexII kit to isolate the DNA (**NB.** This works with priducts <400bp). Elute DNA in 12µl. Send 6µl for automated sequencing with both oligos #32 and #693. You should get the same genomic location with the Rsa1 and HaeIII digestions of each clone.

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Transposon mutagenesis

The *bur1*Δ bypass screen was performed with the aid of a yeast genomic library containing 2 – 3kb of Sau3A partial cut yeast DNA interrupted by a mini-Tn3 (LEU2 / LacZ) transposon (Burns et al., 1994). 5µg of library DNA was cut with Not1 and transformed into the Bur1 shuffle strain YSB787 (*bur1*Δ::HIS3) (**Supplementary Table 2**), sufficient to give 19,000 Leu^+ colonies (estimated >90% genome coverage). Colonies were replica plated onto –HL +FOA to remove the pRS316-Bur1 plasmid and 90 viable colonies selected for further examination. To avoid $Bur1^+$ viable colonies formed by recombination of the pRS316-Bur1 plasmid we performed PCR analysis to assay the presence of the Bur1 active site and discarded the 35 positive clones. Genomic DNA was isolated from the 55 remaining clones and vectorette PCR performed to identify the transposon insertion site and thus the gene disrupted. All genes were confirmed by direct cross of each deletion with the Bur1 shuffle strain and isolating double knockout haploids by tetrad dissection. The strength of the bypass was scored by spot analysis.