Lys counter-selection with αAAA

(α-AminoAdipic Acid (αAAA): US Biological, A1374-09: 5g $104)

NOTE 1: Many yeast labs use 5-FOA as a counter-selection agent to URA^+, but few use αAAA against LYS^+, usually because they've heard it doesn't work. Not so: it does, just not as well as FOA (*Anindita Basak, Personal Communication*). However when URA is unavailable, LYS is used in our lab.

NOTE 2: *S.cerevisiae* LYS2 encodes α-Aminoadipate reductase, LYS5 a phospho-pantetheinyl transferase that activates Lys2p (Chattoo *et al*, 1979a). Individual *lys2*Δ or *lys5*Δ strains are viable, Lys^- and resistant to α-AAA. Contrast with Lys^+ strains which convert αAAA to a toxic intermediate.

NOTE 3: When Lys^- strains are available they're usually *lys2*Δ (ORF = 4179bp). The pRS317 yeast shuttle vector (MKF118) complements LYS2 but the empty vector is already 8.3kb, complicating downstream cloning. The LYS5 ORF is much smaller (819bp) which facilitates cassette construction for PCR (eg: *geneX*Δ::Lys5.MX) (Ito-Harashima & McCusker, 2004)

1. α-AminoAdipic acid (αAAA) stock (4% in ddH_2O: will be used as 0.2% final)
Stock = 2g / 50ml (4%) in ddH_2O. Adjust pH to 6.0 with 5M KOH (see **NOTE 4**)
Filter sterilize (0.2μ) and store at 4°C. Use @ 0.2% final (50 ml stock / L)

NOTE 4: The pH-ing to 6.0 step is absolutely required to get the αAAA into solution. It may help to initially overshoot (to ≈ 8.0) and as more αAAA dissolves come closer to the desired 6.0. This will take hours: plan accordingly.

2. αAA media (per L: see **NOTE 5** – optionals indicated by a *)

YNB (**No NH_4SO_4**)	3.0 gm
Inositol (200 mM stock)	2.0 ml
Lys (100mM stock)	10.0 ml
* Ura (20mM stock)	10.0 ml
* His (100mM stock)	3.0 ml
* Trp (40mM stock)	10.0 ml
* Leu (100mM stock)	16.7 ml
* Met (100 mM stock)	14.76 ml
Agar	20.0 gm
Glucose	20.0 gm
ddH_2O	to 950 ml

Autoclave w/o αAAA. Cool to ≈50°C and add 50ml 0.2μ filter sterilized 4% αAAA / L

NOTE 5: On this media the αAAA will be used as a principle N-source by the yeast (hence the reason to use NH_4SO_4-free YNB). In LYS^+ strains this leads to the generation of a toxic intermediate of the lysine biosynthetic pathway while l*ys2* or *lys5* mutants grow unhindered. Be careful NOT to include any additional N- sources to the media: this includes the lab -6 or -4 amino acid powders (**which contain**

glutamate) or YNB with **NH_4SO_4**. As above use only those specific amino acids that supplement the auxotrophies of the strains used in the experiment – although not tested it is likely you can also supplement with Adenine.

3. αAAA is used as a counter selection agent (eg. to select for loss of a plasmid encoding a Lys2 or Lys5 locus in an otherwise *lys2*Δ or *lys5*Δ strain). This is preferably done by **Spotting**: follow the standard lab protocol (http://mckeogh.googlepages.com/protocols).

NOTE 6: It is ESSENTIAL to replica plate: LYS^+ strains will grow through on the first round of selection on an αAAA plate and can in fact be rescued if you replica plate back onto YPD. Two rounds of selection on αAAA media eliminates the majority of background – we have tried additional rounds but it doesn't help: those colonies that grew through on the second plate from an initial LYS^+ parent were now stable on αAAA, presumably because they had acquired a mutation at *LYS2* or *LYS5*.

NOTE 7: Those familiar with the FOA / URA system will not be overly impressed with αAAA / LYS counter-selection: the requirement for replica plating and relatively high backgrounds are significant issues. However it is a useful backup option if URA is otherwise unavailable.

REFERENCES

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