SPyVET: to find regulators of EIL

1. Screen a library of IVET strains to find EIL (references 1-10)

2. Growth in absence of DAP? — *Moderate/High* → 4. Strain not suitable for SPyVET

Low

3. Prototrophic reversion frequency? — *Many* → 4. Strain not suitable for SPyVET

Few

5. Mutagenesis using transposons ISΩkm and Tn*5*

6. Identify and select prototrophic mutants (LacZ+)

7. *Alternative*: Detect and exclude transposon insertions near promoter marker using PCR

8. Sequence transposon insertion points

9. Both transposons in same gene — *Repressors only*

10. Only ISΩkm insertions in region — *Repressors & activators*

11. Clone gene into an expression vector

12. Clone genes nearest ISΩkm individually into an expression vector

13. Express candidate regulatory gene in TAF strain

14. Switch to auxotrophy (& LacZ-)? — *Yes* → 15. Potential **negative EIL regulator**

No

16. Express candidate regulatory gene in EIL strain

17. Switch to prototrophy (& LacZ+)? — *No* → 18. Not involved in EIL regulation

Yes → 19. Potential **positive EIL regulator**

20. Test putative functions of regulator & ectopic expression of EIL in the wild

21. Select TAF strain in which OE drives constitutive expression of an EIL positive regulator

22. Select TAF strain in which ISΩkm is located in a negative regulator gene

23. Use Cre to remove kanamycin resistance marker of ISΩkm

24. Mutagenesis using transposons ISΩkm andTn*5*

25. Select mutants in which auxotrophy is restored (EIL is silenced, LacZ-)

Additional rounds of mutagenesis