**Gene/Reaction Knockouts**

**Expectations**

**Learning Objectives**

Each student should be able to:

* Explain the purpose of a gene/reaction knockout
* Explain growth-coupled bioproduction.
* Explain the purpose of a production envelope plot.
* Explain the capabilities and limitations of OptKnock.
* Explain the capabilities and limitations of the Genetic Design Local Search (GDLS) tool.
* Explain the capabilities and limitations of OptGene.

**Prerequisites**

* Course Introduction
* Matlab Tutorial
* Flux Balance Analysis Overview
* *E.coli* Core Model
* Cobra Toolbox
* Robustness Analysis & Phenotype Phase Plane Analysis
* Flux Variability Analysis & Parsimonious Analysis

**Resources   
Required Reading**

1. Systems Biology: Constraint-based Reconstruction and Analysis, Bernhard O. Palsson, Cambridge University Press, 2015, Chapter 23
2. [Burgard, A. P., P. Pharkya, et al. (2003). "Optknock: a bilevel programming framework for identifying gene knockout strategies for microbial strain optimization." Biotechnology and bioengineering 84(6): 647-657.](http://www.ncbi.nlm.nih.gov/pubmed/14595777)
3. [Lun, D.S. et al. “Large-scale identification of genetic design strategies using local search,” Mol Syst Biol 5 (2009).](http://www.ncbi.nlm.nih.gov/pubmed/19690565)
4. [Patil, K., Rocha, I., Forster, J. & Nielsen, J. Evolutionary programming as a platform for in silico metabolic engineering. BMC Bioinformatics 6, 308 (2005).](http://www.ncbi.nlm.nih.gov/pubmed/16375763)

**Classroom Activities**

**Presentations**

* Lecture Presentation *(“Gene-Reaction Knockouts.pdf”)*
* Supporting Matlab Files *(“Knockout Matlab Files – 2021.zip”)*

**Laboratory**

* Lab #5 *(“Lab-5.docx”)*

**Reinforcement Activities**

**Examples**

* OptKnock
  1. [Burgard, A. P., P. Pharkya, et al. (2003). "Optknock: a bilevel programming framework for identifying gene knockout strategies for microbial strain optimization." Biotechnology and bioengineering 84(6): 647-657.](http://www.ncbi.nlm.nih.gov/pubmed/14595777)
  2. Schellenberger, J., R. Que, et al. (2011). "Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2.0." Nature protocols 6(9): pp. 1299, 1304, 1305.
* GDLS
  1. [Lun, D.S. et al. “Large-scale identification of genetic design strategies using local search,” Mol Syst Biol 5 (2009).](http://www.ncbi.nlm.nih.gov/pubmed/19690565)
  2. Schellenberger, J., R. Que, et al. (2011). "Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2.0." Nature protocols 6(9): pp. 1299, 1304, 1305.
* OptGene
  1. [Patil, K., Rocha, I., Forster, J. & Nielsen, J. Evolutionary programming as a platform for in silico metabolic engineering. BMC Bioinformatics 6, 308 (2005).](http://www.ncbi.nlm.nih.gov/pubmed/16375763)
  2. Schellenberger, J., R. Que, et al. (2011). "Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2.0." Nature protocols 6(9): pp. 1299.

**Assessment**

**Formative Assessment**

* Reflective Questions
  1. What is OptKnock?
  2. Why should the number of potential knockout reactions be limited?
  3. What type of reactions should not be included in an OptKnock search?
  4. How do you knockout a reaction using the Cobra Toolbox?
  5. What does it mean to couple the growth and metabolite production?
  6. What is a production envelope? What is a multiproduction envelope?
  7. How can a production envelope be created for all secreted metabolite?
  8. Why is there a trade-off between biomass growth and bioproduct production?
  9. What are some of the unintended consequences from optimized bioproduct production?
  10. How many knockouts can be identified by OptKnock?
  11. What are some of the key parameters needed for OptKnock?
  12. How can you simulate the engineered mutant cell using the knockouts identified by OptKnock?
  13. What are the limitations of OptKnock?
  14. What are some of the OptKnock supporting functions in the Cobra Toolbox?
  15. What is the purpose of GDLS?
  16. What is the difference between OptKnock, GDLS?
  17. What are some of the key parameters required by GDLS?
  18. What does it mean to reduce a model?
  19. How many iterations are required to complete the GDLS algorithm?
  20. How many knockouts can be identified by GDLS?
  21. What are the limitations of GDLS?
  22. Will OptKnock and GDLS give the same knockouts?
  23. What is the purpose of OptGene?
  24. What is the difference between OptKnock, GDLS, and OptGene?
  25. Explain how to use OptGene?
  26. How many knockouts can be identified by OptGene?
  27. What are the limitations of OptGene?
  28. What solvers are required by OptGene?
  29. Are there changes that need to be made to the OptGene function before it can be used?
  30. How does OptGene’s speed compare to OptKnock and GDLS?

**References**

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1. [Rocha, I., P. Maia, et al. (2010). "OptFlux: an open-source software platform for in silico metabolic engineering." BMC systems biology 4: 45.](http://www.ncbi.nlm.nih.gov/pubmed/20403172)
2. [Lun, D.S. et al. “Large-scale identification of genetic design strategies using local search,” Mol Syst Biol 5 (2009).](http://www.ncbi.nlm.nih.gov/pubmed/19690565)
3. [Patil, K., Rocha, I., Forster, J. & Nielsen, J. Evolutionary programming as a platform for in silico metabolic engineering. BMC Bioinformatics 6, 308 (2005).](http://www.ncbi.nlm.nih.gov/pubmed/16375763)
4. [Pharkya, P., A. P. Burgard, et al. (2004). "OptStrain: a computational framework for redesign of microbial production systems." Genome research 14(11): 2367-2376.](http://www.ncbi.nlm.nih.gov/pubmed/15520298)
5. [Burgard, A. P., P. Pharkya, et al. (2003). "Optknock: a bilevel programming framework for identifying gene knockout strategies for microbial strain optimization." Biotechnology and bioengineering 84(6): 647-657.](http://www.ncbi.nlm.nih.gov/pubmed/14595777)