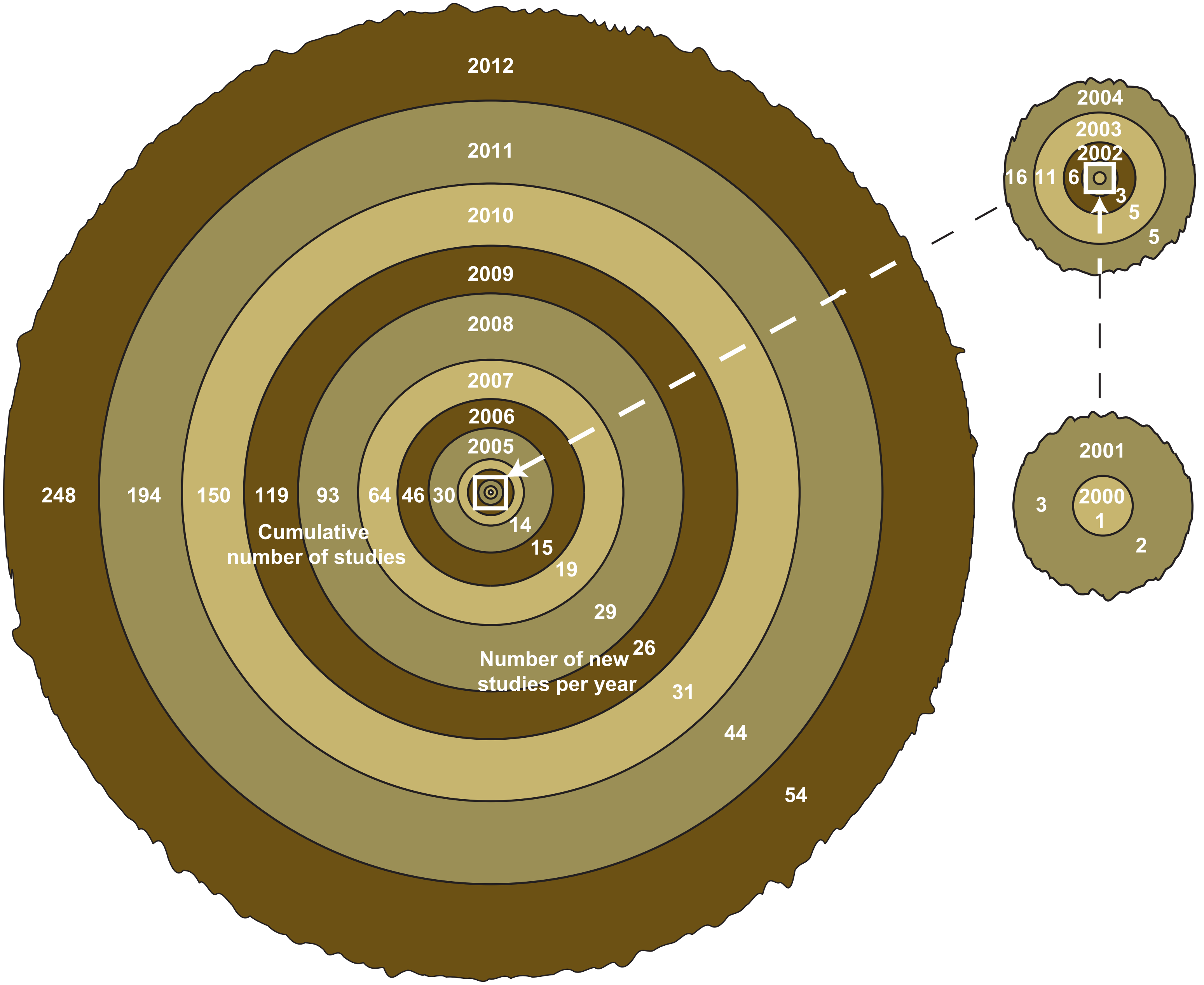
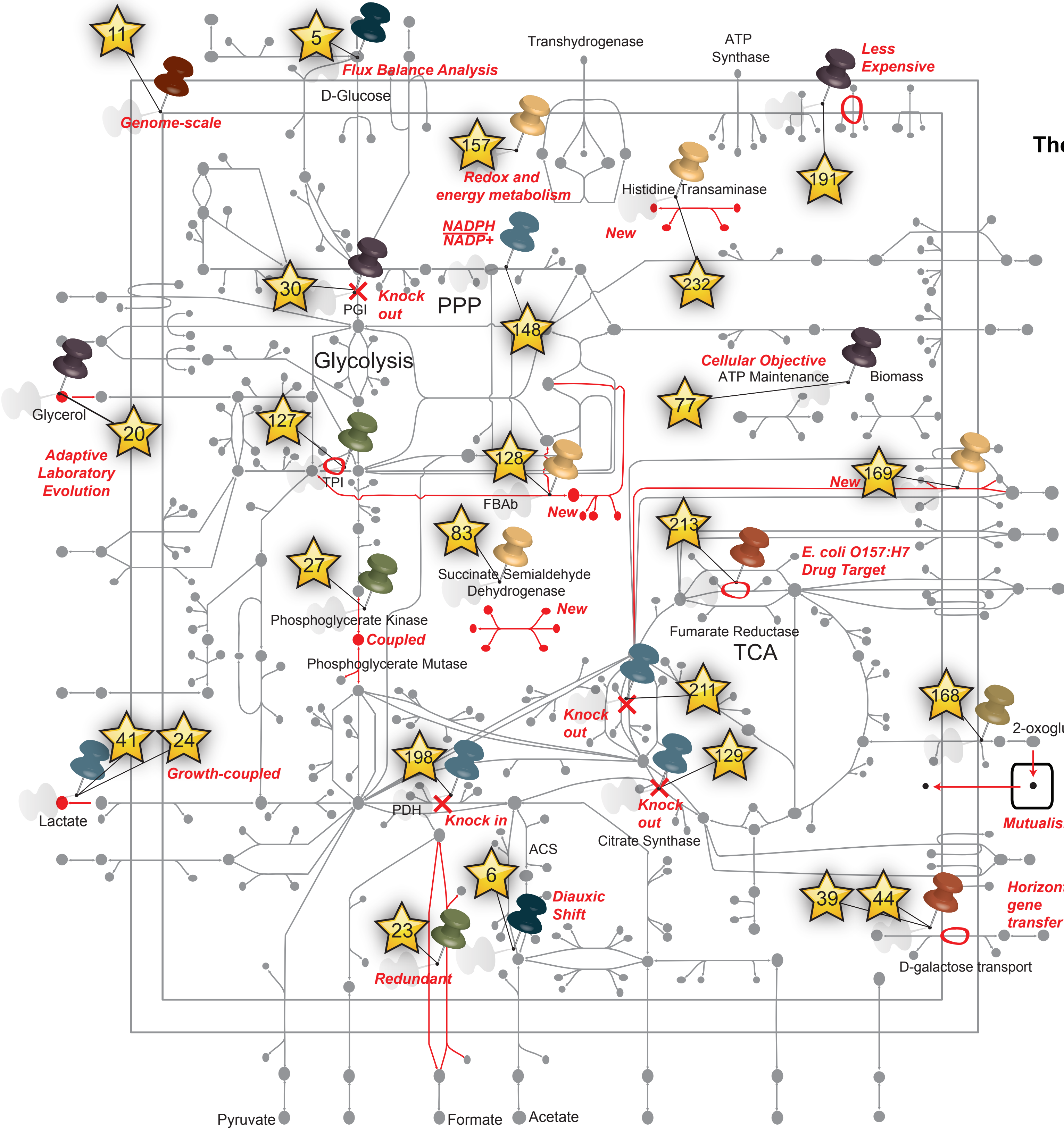
Supplementary Figure 1: The overall usage of the *E. coli* metabolic GEM over time. The cumulative number and new number of studies that have utilized the *E. coli* GEM of metabolism are shown as a function of year. The inserts show the early use of the *E. coli* GEM.

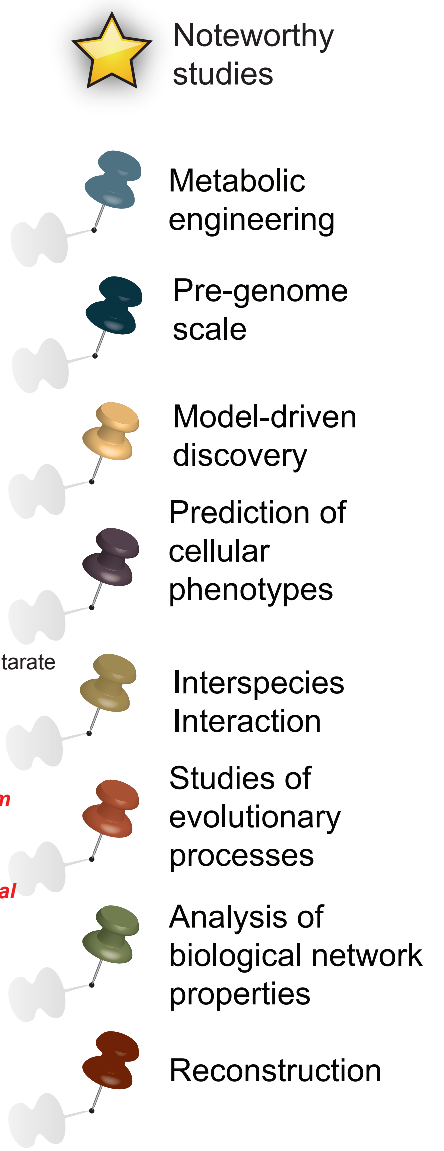

2012
2011
2010
2009
2008
2007
2006
2005
248
194
150
119
93
64
46
30
Cumulative
number of studies
14
15
19
29
26
Number of new
studies per year
31
44
54
2004
2003
2002
16
11
6
3
5
5
2001
2000
3
1
2

Supplementary Figure 2: The *E. coli* ‘road map.’ Studies that made a significant contribution to the *E. coli* knowledgebase are marked on a central carbohydrate map of the *E. coli* metabolic network in panel 1. Pins are color-coded according to the category of the study. The citations for each study (Supplementary Table 1) are encompassed in a star next to the pin. The location of the pin on the metabolic map signifies a feature of the *E. coli* knowledgebase that was contributed to by the study. A short description of the significant contribution of each study is given in panel 2.

The E. coli Applications Road Map



Legend



5

Flux balance analysis (FBA) - the birth of constraint-based modelling (CBM).

6

The authors demonstrate FBA specifically, and CBM in general, can quantitatively describe the metabolic physiology of *E. coli* observed experimentally at the systems level.

11

iJE660 - the first genome-scale metabolic reconstruction of *E. coli*.

20

30

The metabolic flux distribution of the cell tends to seek optimality when grown on a suboptimal carbon source or after exposure to a genetic perturbation following ALE.

23

FVA - a method to explore alternate optimal flux distributions calculated by FBA. Biologically, alternate optimal flux distributions are defined as equivalent pathways or redundant pathways in the metabolic network that contribute to robustness in the context of optimal growth.

24

OptKnock - an algorithm for the design of growth-coupled overproduction strains.

27

FCA - The method identifies reactions that are directly, partially, and fully coupled. This analysis method has found a remarkable number of uses.

39

44

Bacterial evolution occurs primarily by horizontal gene transfer.

41

The hypothesis that metabolite over-production can be stoichiometrically coupled to biomass generation is supported by 11 strains that are designed using OptKnock and evolved to predicted production profiles.

77

While no single objective function is valid under all growth conditions for wild-type *E. coli*, the maximization of ATP or biomass yield is appears most accurate for nutrient-limited chemostat cultures.

83

Prediction and validation of the role of ynel (sad) as the NAD+/NADP+ -dependent succinate semialdehyde dehydrogenase using a custom gap-filling algorithm.

127

The study introduces an algorithm to explore the higher order epistatic interactions of the *E. coli* genome.

128

Discovered the uncharacterized activities of pfkA and fbaA.

129

Highest reported flavanone production levels acheived. The study highlights the importance of constraint-based modeling to recognize optimal combinations of gene deletions.

148

Improved flavenoid production indicates that optimal heterologous enzymatic efficiency may be a function of not only the availabe NADPH cofactors, but also the specific intracellular redox ratio.

157

The authors integrated transcript-, flux- and metabolome data onto the GEM to gain insight into redox and energy cofactor control on metabolism.

168

The stoichiometric metabolic model is used to accurately model, guide, experimental validation, and explore the conditions for cooperative behavior of co-colture growth.

169

The integrated regulatory and metabolic model more accurately predict phenotypic behavior of metabolic and transcription factor mutants.

191

FBA^me - the respiro-fermentation physiology is constrained by the available space on the plasma membrane.

198

The metabolic engineering workflow - pathway engineering followed by in vivo optimization.

211

A demonstration of proper use of the model to guide metabolic enginering strategies towards improved naringenin production.

213

The pan genome of the species *E. coli* is reconstructed and explored to gain biological insight into the evolution of *E. coli*.

232

The genes responsible for the orphan reactions of asparagine oxo-acid transaminase and histidine transaminase were predicted and experimentally confirmed.

Citations

	Author	**Year**
5	Varma	1994
6	Varma	1994
11	Edwards	2000
20	Ibarra	2002
23	Mahadevan	2003
24	Burgard	2003
27	Burgard	2004
30	Fong	2004
41	Fong	2005
39	Pal	2005
44	Pal	2005
77	Schuetz	2007
83	Fuhrer	2007
127	Suthers	2009
128	Nakahigashi	2009
129	Fowler	2009
148	Chemler	2010
157	Holm	2010
168	Wintermute	2010
169	Barua	2010
191	Zhuang	2011
198	Yim	2011
211	Xu	2011
213	Baumler	2011
232	Yamada	2012