

Homework 3

Design a producer strain hosting
a synthetic production pathway

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Goal

- To design a heterologous pathway which produces vanillin glucosidase in *Saccharomyces cerevisiae*.
- To achieve the goal, a heterologous pathway from *E. coli* is adapted into *Saccharomyces cerevisiae*.

Software and models used

- Framed in Anaconda (Python 3.6) environment
 - IBM ILOG CPLEX used as a solver for Framed
- XTMS: Pathway design in an eXTended Metabolic Space
 - Used for finding possible metabolic pathways
- Yeast-GEM model
 - Systems and Synthetic Biology at Chalmers University of Technology

Steps

- Choice of pathway
- Insertion into a yeast model
- Optimization

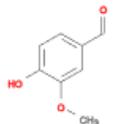
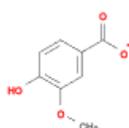
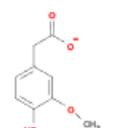
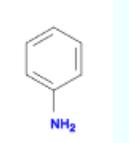
Steps

- **Choice of pathway**
- Insertion into a yeast model
- Optimization

XTMS: Pathway design in an eXTended Metabolic Space

- An online application which finds heterologous pathways for different compounds in E. coli
 - <http://xtms.issb.genopole.fr/>
- XTMS is used for finding possible pathways for adaption in yeast.

Choose the pathways

Compound	Structure	PATHWAYS			Type
		EMS diameter			
		14	12	10	
vanillin		<u>13</u>	<u>13</u>	<u>13</u>	Heterologous
vanillate					Endogenous
homovanillate		<u>1</u>	<u>1</u>	<u>1</u>	Heterologous
aniline		<u>2</u>	<u>2</u>	<u>2</u>	Heterologous

XTMS search for vanillin

Compound	Structure
vanillin	

Select pathway

Rank	Total score	Gene score	Steps	Putatives	Toxicity	Yield	Gibbs	Unfavorable	Pathway
1	332.252	0.253	2	0	-0.415	3.28e-01	-44.070	0	vanillin <- protocatechualdehyde <- <i>trans</i> -resveratrol
2	166.193	0.597	6	0	-0.842	1.65e-01	-19.333	1	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- ferulate <- <i>trans</i> -caffeate <- 4-coumarate <- L-tyrosine
3	166.161	0.585	6	0	-0.863	1.65e-01	-19.333	2	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- <i>trans</i> -caffeate <- 4-coumarate <- L-tyrosine
4	165.481	0.573	8	0	-0.978	1.65e-01	-13.810	2	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- <i>trans</i> -5-O-caffeoyl-D-quinate <- <i>trans</i> -5-O-(4-coumaroyl)-D-quinate <- 4-coumaroyl-CoA <- 4-coumarate <- L-tyrosine
5	165.343	0.452	8	0	-0.995	1.65e-01	-13.810	2	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- caffeoylshikimate <- 4-coumaroylshikimate <- 4-coumaroyl-CoA <- 4-coumarate <- L-tyrosine
6	4.925	0.606	6	0	-0.842	1.00e-03	-41.583	0	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- ferulate <- <i>trans</i> -caffeate <- 4-coumarate <- <i>trans</i> -cinnamate
7	4.893	0.594	6	0	-0.863	1.00e-03	-41.583	1	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- <i>trans</i> -caffeate <- 4-coumarate <- <i>trans</i> -cinnamate
8	3.575	0.579	8	0	-0.978	1.00e-03	-29.702	1	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- <i>trans</i> -5-O-caffeoyl-D-quinate <- <i>trans</i> -5-O-(4-coumaroyl)-D-quinate <- 4-coumaroyl-CoA <- 4-coumarate <- <i>trans</i> -cinnamate
9	3.437	0.459	8	0	-0.995	1.00e-03	-29.702	1	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- caffeoylshikimate <- 4-coumaroylshikimate <- 4-coumaroyl-CoA <- 4-coumarate <- <i>trans</i> -cinnamate
10	2.729	0.717	7	0	-0.847	1.00e-03	-18.564	1	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- ferulate <- <i>trans</i> -caffeate <- 4-coumarate <- (R+)-3-(4-hydroxyphenyl)lactate <- 4-hydroxyphenylpyruvate
11	2.702	0.707	7	0	-0.865	1.00e-03	-18.564	2	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- <i>trans</i> -caffeate <- 4-coumarate <- (R+)-3-(4-hydroxyphenyl)lactate <- 4-hydroxyphenylpyruvate
12	2.098	0.669	9	0	-0.966	1.00e-03	-13.923	2	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- <i>trans</i> -5-O-caffeoyl-D-quinate <- <i>trans</i> -5-O-(4-coumaroyl)-D-quinate <- 4-coumaroyl-CoA <- 4-coumarate <- (R+)-3-(4-hydroxyphenyl)lactate <- 4-hydroxyphenylpyruvate
13	1.975	0.562	9	0	-0.982	1.00e-03	-13.923	2	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- caffeoylshikimate <- 4-coumaroylshikimate <- 4-coumaroyl-CoA <- 4-coumarate <- (R+)-3-(4-hydroxyphenyl)lactate <- 4-hydroxyphenylpyruvate

Possible reactants

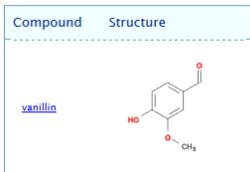
- Protocatechualdehyde
- *trans*-resveratrol
- 4-hydroxy-3-methoxyphenyl- β -hydroxypropionyl-CoA
- feruloyl-CoA
- Ferulate
- *trans*-caffeate
- 4-coumarate
- L-tyrosine
- caffeoyl-CoA
- *trans*-5-O-caffeoyl-D-quininate
- *trans*-5-O-(4-coumaroyl)-D-quininate
- 4-coumaroyl-CoA
- caffeoylshikimate
- 4-coumaroylshikimate
- *trans*-cinnamate
- *trans*-5-O-caffeoyl-D-quininate
- (R+)-3-(4-hydroxyphenyl)lactate
- 4-hydroxyphenylpyruvate

Reactants available in yeast

- L-tyrosine (s_1051, cytoplasm)
- 4-hydroxyphenylpyruvate (s_0204, cytoplasm)
- Pathways that do not have either of these reactants cannot be used unless the alternate reactants are produced using heterologous pathways.

The pathways possible in yeast

Home → Select compound → Select pathway → Pathway info Reaction info Downloads Statistics Help About



Select pathway

Rank	Total score	Gene score	Steps	Putatives	Toxicity	Yield	Gibbs	Unfavorable	Pathway
2	166.193	0.597	6	0	-0.842	1.65e-01	-19.333	1	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- ferulate <- <i>trans</i> -caffeate <- 4-coumarate <- L-tyrosine
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12	2.098	0.669	9	0	-0.966	1.00e-03	-13.923	2	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- <i>trans</i> -5-O-caffeoyl-D-quininate <- <i>trans</i> -5-O-(4-coumaroyl)-D-quininate <- 4-coumaroyl-CoA <- 4-coumarate <- (R+)-3-(4-hydroxyphenyl)lactate <- 4-hydroxyphenylpyruvate
13	1.975	0.562	9	0	-0.982	1.00e-03	-13.923	2	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- caffeoylshikimate <- 4-coumaroylshikimate <- 4-coumaroyl-CoA <- 4-coumarate <- (R+)-3-(4-hydroxyphenyl)lactate <- 4-hydroxyphenylpyruvate

Compound	Structure
vanillin	

Select pathway

Rank	Total score	Gene score	Steps	Putatives	Toxicity	Yield	Gibbs	Unfavorable	Pathway
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5	165.343	0.452	8	0	-0.995	1.65e-01	-13.810	2	vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- caffeoyl-CoA <- caffeoylshikimate <- 4-coumaroylshikimate <- 4-coumaroyl-CoA <- 4-coumarate <- L-tyrosine

Pathway info of Path 2

Home → Select compound → Select pathway → Pathway info → Reaction info → Downloads → Statistics → Help → About

Reactions

Reaction	ΔG	EC
RXN-9827_1_0_0	16.850	4.3.1.25
RXN-1103_1_0_0	-94.397	None
RXN-1104_1_0_0	-4.360	2.1.1.68
6.2.1.34- RXN_1_0_0	-13.960	6.2.1.34
RXN-10921_1_0_0	n.a.	4.2.1.101
4.1.2.41- RXN_1_0_0	-0.800	4.1.2.41

Metabolite Exchange

Precursors	Balance	Toxicity	Chassis pathways & reactions
S-adenosyl-L-methionine	-1.0	-1.18	S-adenosyl-L-methionine biosynthesis
L-tyrosine	-1.0	-0.66	TRANS-RXN-77

Consumed cofactors	Balance	Toxicity
ATP	-1.0	-0.31
coenzyme A	-1.0	-0.81
NADPH	-1.0	-1.16
oxygen	-1.0	1.42

Intermediates	Balance	Toxicity
4-coumarate	0.0	-1.07
4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA	0.0	-0.72
trans-caffeate	0.0	-0.74
ferulate	0.0	-0.81
feruloyl-CoA	0.0	-0.82

Intermediate cofactors	Balance	Toxicity
hydroxyl radical	0.0	1.84

Products	Balance	Toxicity
acetyl-CoA	1.0	-0.82
S-adenosyl-L-homocysteine	1.0	-1.25
vanillin	1.0	0.72

Pathway

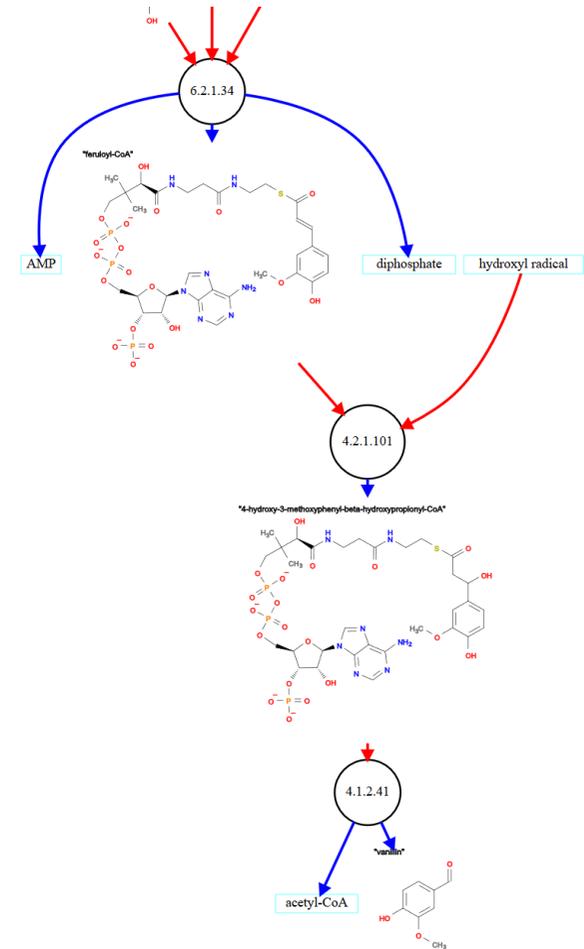
vanillin <- 4-hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA <- feruloyl-CoA <- ferulate <- trans-caffeate <- 4-coumarate <- L-tyrosine

Products	Balance	Toxicity
acetyl-CoA	1.0	-0.82
S-adenosyl-L-homocysteine	1.0	-1.25
vanillin	1.0	0.72

Produced cofactors	Balance	Toxicity
ammonia	1.0	0.46
AMP	1.0	-0.50
NADP+	1.0	-0.83
diphosphate	1.0	1.41
H+	1.0	nan

Pathway Yield

Maximum yield:	1.65e-01 mmol/gDW/h
Main bottlenecks:	S-ADENOSYLMETHIONINE, CO-A



Best gene options for the pathway

Top constructs

sbml sbol	Total score	RXN-9697	Score	RXN-1103	Score	RXN-1104	Score	6.2.1.34-RXN	Score	RXN-10921	Score	4.1.2.41-RXN	Score
<u>1</u>	0.597	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	sme:SM_b20515	1.565	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126
<u>2</u>	0.574	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	ret:RHE_PF00076	1.428	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126
<u>3</u>	0.567	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	sit:TM1040_1028	1.384	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126
<u>4</u>	0.557	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	bid:Bind_0707	1.324	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126
<u>5</u>	0.557	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	bid:Bind_3835	1.323	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126
<u>6</u>	0.547	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	eba:ebA670	1.265	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126
<u>7</u>	0.515	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	jan:Jann_2564	1.070	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126
<u>8</u>	0.509	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	pae:PA2735	1.036	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126
<u>9</u>	0.499	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	sit:TM1040_2227	0.978	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126
<u>10</u>	0.499	rsh:Rsph17029_3256	0.501	bj:bll8073	0.559	mca:MCA0278	0.977	rle:RL0889	0.368	abo:ABO_2150	0.464	dde:Dde_1990	0.126

Steps

- Choice of pathway
- **Insertion into a yeast model**
- Optimization

Modelling of the pathway

- Framed is used for modeling and analysis of the heterologous pathway in yeast.
- The yeast model is imported and a copy is made for the modified model

Init

```
In [1]: %matplotlib inline
import numpy
from framed import load_cbmodel, FBA, FVA, plot_flux_envelope, essential_genes, essential_reactions
```

Loading yeast model

```
In [2]: model = load_cbmodel('yeast_7.6_cobra.xml')
model_vanillin = model.copy()
```

RXN-9697_1_0_0:

L-tyrosine [\[cytoplasm\]](#) => 4-coumarate + ammonium [\[cytoplasm\]](#)

RXN-1103_1_0_0:

4-coumarate + H+ [\[cytoplasm\]](#) + oxygen [\[cytoplasm\]](#) + NADPH [\[cytoplasm\]](#) => trans-caffeate + H2O [\[cytoplasm\]](#) + NADP(+) [\[cytoplasm\]](#)

RXN-1104_1_0_0:

trans-caffeate + S-adenosyl-L-methionine [\[cytoplasm\]](#) => ferulate + S-adenosyl-L-homocysteine [\[cytoplasm\]](#) + H+ [\[cytoplasm\]](#)

6.2.1.34-RXN_1_0_0:

ferulate + coenzyme A [\[cytoplasm\]](#) + ATP [\[cytoplasm\]](#) => feruloyl-CoA + AMP [\[cytoplasm\]](#) + diphosphate [\[cytoplasm\]](#)

RXN-10921_1_0_0:

feruloyl-CoA + H2O [\[cytoplasm\]](#) => 4-hydroxy-3-methoxyphenyl- β -hydroxypropionyl-CoA

4.1.2.41-RXN_1_0_0:

4-hydroxy-3-methoxyphenyl- β -hydroxypropionyl-CoA => vanillin + acetyl-CoA

vanillin glucosyltransferase:

UDP-glucose + vanillin [\[cytoplasm\]](#) => UDP + vanillin glucoside [\[cytoplasm\]](#)

vanillin glucoside transport:

vanillin glucoside [\[cytoplasm\]](#) => vanillin glucoside [\[extracellular\]](#)

vanillin glucoside exchange:

:vanillin glucoside [\[extracellular\]](#) =>

Native metabolite identifiers:

s_0373 acetyl-CoA [\[cytoplasm\]](#)

s_0419 ammonium [\[cytoplasm\]](#)

s_0423 AMP [\[cytoplasm\]](#)

s_0434 ATP [\[cytoplasm\]](#)

s_0529 coenzyme A [\[cytoplasm\]](#)

s_0633 diphosphate [\[cytoplasm\]](#)

s_0794 H+ [\[cytoplasm\]](#)

s_0803 H2O [\[cytoplasm\]](#)

s_1051 L-tyrosine [\[cytoplasm\]](#)

s_1207 NADP(+) [\[cytoplasm\]](#)

s_1212 NADPH [\[cytoplasm\]](#)

s_1275 oxygen [\[cytoplasm\]](#)

s_1413 S-adenosyl-L-homocysteine [\[cytoplasm\]](#)

s_1416 S-adenosyl-L-methionine [\[cytoplasm\]](#)

s_1538 UDP [\[cytoplasm\]](#)

s_1543 UDP-D-glucose [\[cytoplasm\]](#)

Reactions are inserted into the model

```
In [3]: #add Vanillin glucoside synthesis pathway to the model
model_vanillin.add_reaction_from_str('r_9999: s_1051 <-> s_9999 + s_0419') #RXN-9697_1_0_0
model_vanillin.add_reaction_from_str('r_9998: s_0794 + s_1212 + s_1275 + s_9999 <-> s_0803 + s_1207 + s_9998') #RXN-1103_1_0_0
model_vanillin.add_reaction_from_str('r_9997: s_1416 + s_9998 <-> s_0794 + s_1413 + s_9997') #RXN-1104_1_0_0
model_vanillin.add_reaction_from_str('r_9996: s_0434 + s_0529 + s_9997 <-> s_0423 + s_0633 + s_9996') #6.2.1.34-RXN_1_0_0
model_vanillin.add_reaction_from_str('r_9995: s_9996 + s_0803 <-> s_9995') #RXN-10921_1_0_0
model_vanillin.add_reaction_from_str('r_9994: s_9995 <-> s_0373 + s_9994') #4.1.2.41-RXN_1_0_0
model_vanillin.add_reaction_from_str('r_9993: s_1543 + s_9994 <-> s_1538 + s_9993')
model_vanillin.add_reaction_from_str('r_9992: s_9993 <-> s_9992')
model_vanillin.add_reaction_from_str('r_9991: s_9992 <-> ')

model_vanillin.reactions.r_2111.set_objective(0) #Growth objective set to 0
model_vanillin.reactions.r_9991.set_objective(1)
```

```
In [4]: solution_vanillin=FBA(model_vanillin)

print('Vanillin glucoside production:', solution_vanillin.values['r_9991'])

Vanillin glucoside production: 0.3311258278145695
```

Steps

- Choice of pathway
- Insertion into a yeast model
- **Optimization**

Essential genes and reactions in vanillin production

```
In [7]: essential_gns_vanillin = essential_genes(model_vanillin, constraints={'r_9991': numpy.floor(numpy.power(10,9)*solution_vanillin)}  
print(essential_gns_vanillin)
```

```
< [ 'G_Q0045', 'G_Q0080', 'G_Q0085', 'G_Q0105', 'G_Q0130', 'G_Q0250', 'G_Q0275', 'G_YAL044C', 'G_YBL045C', 'G_YBL099W', 'G_YBR039W', 'G_YBR166C', 'G_YBR196C', 'G_YBR263W', 'G_YBR291C', 'G_YCR012W', 'G_YDL004W', 'G_YDL067C', 'G_YDR019C', 'G_YDR035W', 'G_YDR050C', 'G_YDR127W', 'G_YDR148C', 'G_YDR178W', 'G_YDR226W', 'G_YDR298C', 'G_YDR377W', 'G_YDR529C', 'G_YEL024W', 'G_YER043C', 'G_YER065C', 'G_YER091C', 'G_YFL018C', 'G_YFL030W', 'G_YFR033C', 'G_YGL148W', 'G_YGL187C', 'G_YGL191W', 'G_YGL202W', 'G_YGR183C', 'G_YGR208W', 'G_YGR240C', 'G_YGR244C', 'G_YHR001W_A', 'G_YHR051W', 'G_YHR137W', 'G_YHR208W', 'G_YIL125W', 'G_YJL121C', 'G_YJL166W', 'G_YJR095W', 'G_YJR105W', 'G_YJR121W', 'G_YJR148W', 'G_YKL016C', 'G_YKL060C', 'G_YKL067W', 'G_YKL085W', 'G_YKL141W', 'G_YKL152C', 'G_YLL041C', 'G_YLR038C', 'G_YLR058C', 'G_YLR174W', 'G_YLR295C', 'G_YLR304C', 'G_YLR395C', 'G_YML042W', 'G_YML081C_A', 'G_YMR189W', 'G_YMR205C', 'G_YMR256C', 'G_YMR267W', 'G_YNL241C', 'G_YOR065W', 'G_YOR095C', 'G_YOR142W', 'G_YOR184W', 'G_YPL078C', 'G_YPL262W', 'G_YPL271W', 'G_YPR021C', 'G_YPR060C', 'G_YPR191W'] >
```

```
In [8]: len(essential_gns_vanillin)
```

```
Out[8]: 84
```

```
In [12]: essential_rcs_vanillin = essential_reactions(model_vanillin, constraints={'r_9991': numpy.floor(numpy.power(10,9)*solution_vanillin)}  
print(essential_rcs_vanillin)
```

```
< [ 'r_0020', 'r_0039', 'r_0040', 'r_0065', 'r_0080', 'r_0091', 'r_0142', 'r_0144', 'r_0148', 'r_0156', 'r_0226', 'r_0252', 'r_0254', 'r_0278', 'r_0279', 'r_0279', 'r_0300', 'r_0366', 'r_0438', 'r_0439', 'r_0451', 'r_0452', 'r_0466', 'r_0467', 'r_0471', 'r_0486', 'r_0502', 'r_0503', 'r_0503', 'r_0505', 'r_0534', 'r_0569', 'r_0659', 'r_0662', 'r_0663', 'r_0664', 'r_0713', 'r_0726', 'r_0727', 'r_0811', 'r_0831', 'r_0832', 'r_0888', 'r_0889', 'r_0891', 'r_0892', 'r_0893', 'r_0917', 'r_0918', 'r_0939', 'r_0982', 'r_0984', 'r_0996', 'r_0997', 'r_1021', 'r_1022', 'r_1049', 'r_1050', 'r_1054', 'r_1110', 'r_1127', 'r_1166', 'r_1194', 'r_1245', 'r_1265', 'r_1277', 'r_1567', 'r_1596', 'r_1696', 'r_1697', 'r_1708', 'r_1714', 'r_1811', 'r_1898', 'r_1965', 'r_1978', 'r_1979', 'r_1992', 'r_2032', 'r_2045', 'r_2096', 'r_2100', 'r_9999', 'r_9998', 'r_9997', 'r_9996', 'r_9995', 'r_9994', 'r_9993', 'r_9992', 'r_9991'] >
```

```
In [13]: len(essential_rcs_vanillin)
```

```
Out[13]: 89
```

Questions