Open Yeast Collection Synthesis Memo

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This follows on from the original approved description of the Open Yeast Collection found here: https://docs.google.com/document/d/1UkLIZ4wIxyGBoH7rU1uRutYq2LoCOaf2iU7ZrX99j_c/edit?usp=sharing

Parts and Design are available here.
https://docs.google.com/spreadsheets/d/1hhiKwaTJyWajH1fEUxZ_79DP4TRtlCBLvO6EtcqtxeY/edit?usp=sharing

Genbank files are available here
20200302: https://cp.sync.com/dl/b4a8a8120#9hiq2sqm-y8wzem55-m8fmh3xe-y5b62dhh

This first Open Yeast Collection is a foundational and enabling framework for contributing to the creation of an open, sustainable and equitable bioeconomy. More specifically OYC permits the building of plasmids from reusable and redistributable genetic elements for genetically modifying *Saccharomyces cerevisiae* (brewers yeast). The plasmids can be used for basic research (e.g. protein-protein interactions via yeast-2-hybrid) or for building metabolic pathways to create a wide variety of natural/commodity chemicals as well as fine and specialized chemicals such as pharmaceuticals. I envisage a wide variety of users from educators & students, community-based & academic researchers and bio-entrepreneurs.

Reference

A Highly Characterized Yeast Toolkit for Modular, Multipart Assembly.
Lee ME, DeLoache WC, Cervantes B, Dueber JE
ACS Synth Biol. 2015 Sep 18;4(9):975-86.
https://www.ncbi.nlm.nih.gov/pubmed/25871405

This first edition of the Open Yeast Collection contains the following genetic parts.
16 Yeast Promoters - strong, medium, weak and inducible (8,824bp)
16 CDS - genes for metabolic pathways - see below (24,880bp)
7 Yeast Terminators (2,092bp)
7 Left & 7 right assembly connectors - for multigene level two assembly (2,891bp)
3 5' & 3' Yeast homology regions - for integration into the yeast genome (2,710bp)
3 Yeast origins - for episomal maintenance and transfer of plasmids (2,385bp total)
8 Selection cassettes - for positive and negative selection options (10,646bp total)
3 Bacterial backbones - for parts assembly (8,097bp total)

Yeast Promoters

The Open Yeast Collection utilizes 16 yeast-specific promoters. A range of promoters have been provided to permit strong, medium, weak expression of the transcription unit. Also

included are a few inducible yeast promoters. This diversity provides flexibility for users to build their own metabolic pathways in yeast.

Yeast Terminators
The Open Yeast Collection utilizes 7 yeast-specific terminators. Transcription units can be insulated by placing a terminator at the 3' end of a gene.

Yeast Origins & Homology Regions
The Open Yeast Collection provides multiple strategies for maintaining one or more single or multi-gene plasmids within yeast cells. Two yeast origins - low copy and high copy - permit episomal maintenance of the plasmid. As an alternative to episomal maintenance 3 pairs of homology regions (URA3, LEU2 and HO) permit the stable integration of up to three mult-gene assemblies within the yeast genome at specific and well characterized locations. Also included is the oriT sequence to assist with trans-kingdom DNA transfer (e.g. *E.coli* to yeast).

Assembly Connectors
The 7 pairs of assembly connectors provided in the Open Yeast Collection are generic, agnostic to species, and can be used in almost any microbial or eukaryotic multi-gene assembly strategy. The assembly connectors provided in this collection facilitate assembly of a plasmid with up to 6 transcription units. More assembly connectors can be synthesized if a user plans to assemble more than 6 transcription units.

Selection Cassettes
The Open Yeast Collection provides a variety of options for positive and negative plasmid selection - including auxotrophic complementation (LEU2, URA3, HIS3 & TRP1) and antibiotic selection (Hygro, KanR, NAT & Zeo). These cassettes are full transcription units in themselves. In addition, a five additional yeast auxotrophic CDSs are provided: ADE2 complements adenine auxotrophy, LYS2 complements lysine auxotrophy, MET17 complements methionine auxotrophy, TRP1 complements tryptophan auxotrophy, and AUR1 confers aureobasidin resistance. These CDS provide greater flexibility for users of the Open Yeast Collection and can be used to create additional selection cassettes.

CDS

1) Additional Auxotrophic Yeast Genes
Five yeast auxotrophic CDSs (ADE2, LYS2, MET17, TRP1, and AUR1) are provided to extend the functionality of the Open Yeast Collection - see Selection Cassettes above for details.

2) Recombinases

The recombinase enzymes for both the Cre-LoxP recombinase and FLP-FRT recombinase systems are provided. These enzymes are generally useful for engineering recombination systems, not just in yeast.

3) Metabolic Pathway Enzymes

The generalized nature of the Open Yeast Collection permits the assembly of many metabolic pathways whose enzymes can be sourced from almost any species. Some pathways may involve many enzymes and others only a few. Many pathways start with substrates that are produced via existing native yeast pathways. Due to evolution, the early (core) enzymes in most pathways are common amongst many species whilst the terminal enzymes may be species-specific or limited to a few species. I see in future Open Yeast Collections the creation of many core enzyme parts.

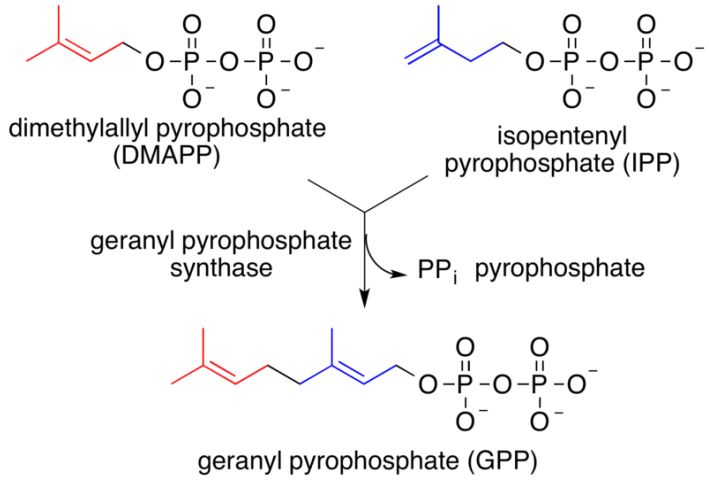
Terpene Biosynthesis Pathway

There are a wide variety of heterologous pathways that can be introduced into yeast. For the first release of the Open Yeast Collection I have chosen some enzymes in the ***terpene biosynthesis pathway***. The benefit of using this pathway is that the products have scents that can be detected without the use of expensive laboratory equipment such as HPLC - ideal for those wishing to learn about metabolic engineering in yeast.

Terpenes, also called isoprenoids, are a large and diverse class of organic compounds, produced mainly by a variety of plants and include carotenoids, quinones, lanosterol derivatives (e.g. steroids), natural rubber and many more (https://en.wikipedia.org/wiki/Terpene). Terpenoids (or isoprenoids) are modified terpenes as they contain additional functional groups, usually oxygen-containing.

The precursor to the terpene/isoprene units in biological systems is dimethylallyl pyrophosphate (DMAPP) and its isomer isopentenyl pyrophosphate (IPP). These molecules are *produced natively in yeast* isoprenoid biosynthesis.

Geranyl pyrophosphate (GPP) synthase produces geranyl pyrophosphate (GPP) from DMAPP and IPP. GPP is the universal C10 precursor of the monoterpenes.



Linalool synthase (LIS) produces (S)-linalool from GPP and water. Linalool is a naturally occurring terpene alcohol found in many flowers and spice plants.
https://en.wikipedia.org/wiki/Linalool

4S-limonene-synthase catalyzes the cyclization of geranyl diphosphate to (−)-(4S)-limonene. Limonene is a relatively stable colorless liquid aliphatic hydrocarbon classified as a cyclic monoterpene, and is a major component of the aromatic scents such as in the oil of citrus fruit peels.
https://en.wikipedia.org/wiki/Limonene

(−)-Limonene-3-hydroxylase (L3OH), using O2 and NADPH, catalyzes the allylic hydroxylation of (−)-(4S)-limonene at the 3 position to (−)-trans-isopiperitenol.

(R)-limonene synthase (EC 4.2.3.20) catalyzes the synthesis of (+)-(4R)-limonene from GPP. This enzyme occurs in Citrus, Carum (caraway) and Anethum (dill).

Geranylgeranyl diphosphate (GGPP) synthase catalyzes the synthesis of Geranylgeranyl diphosphate (GGPP) from farnesyl diphosphate and IPP.

Farnesyl pyrophosphate (FPP) synthase is an enzyme that synthesizes farnesyl pyrophosphate (FPP) from IPP and DMAPP. It is used by organisms in the biosynthesis of terpenes, terpenoids, and sterols.

(-)-isopiperitenol/(-)-carveol dehydrogenase is capable of utilizing (−)-trans-isopiperitenol in peppermint and (−)-trans-carveol in spearmint. Carveol is a natural unsaturated, monocyclic monoterpenoid alcohol that is a constituent of spearmint essential oil in the form of cis-(−)-carveol.

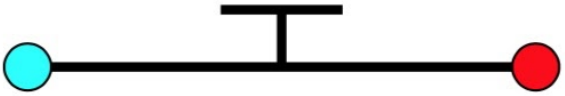
	Prefix	Part Type	Suffix	
●	*ATCC*	*Left Assembly Connector*	*GGAG*	●
●	*GGAG*	*Promoter/5'UTR*	*AATG*	●
●	*AATG*	*CDS*	*GCTT*	●
●	*GCTT*	*Terminator*	*CGCT*	●
●	*CGCT*	*Right Assembly Connector*	*AGAC*	●
●	*AGAC*	*Yeast Origin*	*CGAA*	●
●	*CGAA*	*Yeast Selection Marker*	*GCAA*	●
●	*GCAA*	*Backbone plasmid*	*ATCC*	●

Level One Bsal Parts

Left Assembly Connectors

Promoter/5'UTR

CDS/Gene



Terminators

Right Assembly Connectors

Yeast Selection Marker

Yeast Origin

Assembly Connectors for Multi-Gene Assembly

	Left BbsI sites		Right BbsI sites	
ACLstart-rev	<<CTGA			
ACLstart	CTGA>>			
ACL1	CCAA>>		<<CCAA	ACR1
ACL2	GATG>>		<<GATG	ACR2
ACL3	GTTC>>		<<GTTC	ACR3
ACL4	GGTA>>		<<GGTA	ACR4
ACL5	AAGT>>		<<AAGT	ACR5
			<<GCGA	ACRend
			GCGA>>	ACR6end-rev