Summary of parts submitted:

Part	Type	Name	Description
BBa_K1808000	Basic	DBR2	coding region of the DBR2 gene
BBa_K1808001	Basic	ALDH1	coding region of the ALDH1 gene
BBa_K1808002	Basic	ADH1	coding region of the ADH1 gene
BBa_K1808003	Basic	DBR2-His	coding region of the DBR2 gene with a 6x His-Tag
BBa_K1808004	Basic	ALDH1-His	coding region of the ALDH1 gene with a 6x His-Tag
BBa_K1808005	Basic	ADH1-His	coding region of the ADH1 gene with a 6x His-Tag
BBa_K1808006	Basic	CYB5-His	coding region of the CYB5 gene with a 6x His-Tag
BBa_K1808007	Basic	CYP71AV1-His	coding region of the CYP71AV1 gene with a 6x His-Tag
BBa_K1808008	Basic	ADS-His	coding region of the ADS gene with a 6x His-Tag
BBa_K1808009	Basic	CPR-His	coding region of the CPR gene with a 6x His-Tag
BBa_S05303	Construction Intermediate	RBS (BBa_0032) + ALDH1	Ribosome Binding Site (RBS) (BBa_0032) ligated to the coding region of the ALDH1 gene with a 6x His-Tag
BBa_S05304	Construction Intermediate	RBS (BBa_0034) + DBR2	RBS (BBa_0034) ligated the coding region of the DBR2 gene
BBa_S05305	Construction Intermediate	Promoter (BBa_K823005) + RBS (BBa_0034) + DBR2	Promoter (BBa_K823005) ligated to RBS ligated to coding region of the DBR2 gene
BBa_S05306	Construction Intermediate	RBS (BBa_0032) + ALDH1 + Terminator (B0015)	RBS ligated to coding region of the ALDH1 gene with a 6x His-Tag ligated to a terminator (B0015)
BBa_K1808010	Composite	Promoter (BBa_K823005) + RBS (BBa_0032) + ALDH1 + Terminator (B0015)	ALDH1 generator
BBa_K1808011	Composite	PTAC (BBa_K864400) + RBS (BBa_0032) + ALDH1 + T	ALDH1 regulated by the TAC promoter (BBa_K864400)
BBa_K1808012	Composite	B569α (Promoter ((BBa_K823005)) + RBS ((BBa_0034)) + DBR2 + RBS ((BBa_0032)) + ALDH1 + Terminator ((B0015)))	ALDH1 and DBR2 generator "Alpha"

Basic Parts:

DBR2: BBa_K1808000

Source: http://www.ncbi.nlm.nih.gov/nuccore/197310859

This is the coding region of the DBR2 gene from Artemisia annua with added BioBrick suffix and prefix in a pSB1C3 plasmid backbone. The DBR2 gene encodes the enzyme artemisinic aldehyde delta11(13)-reductase, which catalyses the conversion of artemisinic aldehyde to (11R)-dihydroartemisinic aldehyde with NADPH as the electron donor substrate. The product can be acted upon by the Artemisia annua aldehyde dehydrogenase 1 enzyme (see part BBa_K1808001) to give rise to dihydroartemisinic acid, which can be isolated and converted to the anti-malarial artemisinin. This part is codon optimised for BioBrick 3A assembly and expression in E. coli cells (see BBa_K1808003 for DBR2 coding region with a 6x His-Tag).

ALDH1 : BBa_K1808001

Source: http://www.ncbi.nlm.nih.gov/nuccore/JQ609276.1

This is the coding region of the ALDH1 gene from Artemisia annua with added biobrick suffix and prefix in a pSB1C3 plasmid backbone. The ALDH1 gene encodes the enzyme aldehyde dehydrogenase 1, which catalyses the conversion of (11R)-dihydroartemisinic aldehyde to dihydroartemisinic acid as well as the conversion of artemisinic aldehyde to artemisinic acid with NADP+ as the substrate. Artemisinic acid and dihydroartemisinic acid can be extracted from E. coli cells through lysis and chemically converted to the anti-malarial artemisinin. This part is codon optimised for BioBrick 3A assembly and expression in E. coli cells (see BBa_K1808004 for ALDH1 coding region with a 6x His-Tag).

ADH1: BBa_K1808002

Source: http://www.ncbi.nlm.nih.gov/nuccore/JQ582842.1

This is the coding region of the ADH1 gene from Artemisia annua with added BioBrick suffix and prefix in a pSB1C3 plasmid backbone. The ADH1 gene encodes the enzyme alcohol dehydrogenase 1, which catalyses the conversion of artemisinic alcohol to artemisinic aldehyde with NAD+ as the electron donor substrate. The product can be acted upon by the Artemisia annua DBR2 enzyme (see part BBa_K1808000) to give rise to dihydroartemisinic acid, which can be isolated and converted to the anti-malarial artemisinin. This part is codon optimised for BioBrick 3A assembly and expression in E. coli cells (see BBa_K1808005 for ADH1 coding region with a 6x His-Tag).

CYB5: BBa_K1808006

Source: http://www.ncbi.nlm.nih.gov/nuccore/JQ582841.1

This is the coding region of the CYB5 gene from Artemisia annua with added BioBrick suffix and prefix in a pSB1C3 plasmid backbone and a 6x His-tag. CYP71AV1, CPR1 and CYB5 oxidize amorphadiene to artemisinic alcohol. This part is codon optimised for BioBrick 3A assembly and expression in E. coli cells.

CYP71AV1: BBa_K1808007

Source: http://www.ncbi.nlm.nih.gov/nuccore/JF951731.1

This is the coding region of the CYP71AV1 gene from Artemisia annua with added biobrick suffix, prefix and a 6x His-tag in a pSB1C3 plasmid backbone. The CYP71AV1 gene encodes the enzyme amorphadiene oxidase, which catalyses the conversion of amorpha-4,11-diene to artemisinic alcohol, subsequent conversion of the alcohol to artemisinic aldehyde and the subsequent and final conversion of the artemisinic aldehyde to artemisinic acid with NADP+ as the substrate. The amorphadiene oxidase enzyme is a member of the cytochrome p450 family of hemoproteins and is the rate limiting enzyme in the synthesis pathway for artemisinic acid developed by Amyris. This part is codon optimised for BioBrick 3A assembly and expression in E. coli cells as well as protein purification.

ADS: BBa_K1808008

Source: http://www.ncbi.nlm.nih.gov/nuccore/JF951730.1

This is the coding region of the ADS gene from Artemisia annua with added BioBrick suffix, prefix and a 6x His-Tag in a pSB1C3 plasmid backbone. The ADS gene encodes for amorphadiene synthase which catalyses the conversion of farnesyl diphosphate to amorpha-4,11-diene. This part is codon optimised for BioBrick 3A assembly and expression in E. coli cells as well as protein purification.

CPR: BBa_K1808009

Source: http://www.ncbi.nlm.nih.gov/nuccore/JF951732.1

This is the coding region of the CPR gene from Artemisia annua with added BioBrick suffix, prefix and a 6x His-Tag in a pSB1C3 plasmid backbone. CYP71AV1, CPR1 and CYB5 oxidize amorphadiene to artemisinic alcohol. This part is codon optimised for BioBrick 3A assembly and expression in E. coli cells as well as protein purification.

Construction Intermediates:

BBa_S05303

This part is composed of RBS BBa_B0032 ligated to the ALDH1 coding region (with a 6x His-Tag) (BBa_K1808004).

BBa_S05304

This part is composed of RBS BBa_B0034 ligated to the DBR2 coding region (BBa_K1808000).

BBa_S05305

This part is composed of promoter BBa_K823005 ligated to RBS BBa_B0034 ligated to the DBR2 coding region (BBa_K1808000).

BBa_S05306

This part is composed of RBS (BBa_0032) ligated to the coding region of the ALDH1 gene with a 6x His-Tag (BBa_K1808004) ligated to a terminator (B0015).

Composite Parts:

ALDH1 Generator: BBa_K1808010

The ALDH1 Generator is composed of the following parts: Promoter (BBa_K823005) + RBS (BBa_0032) + ALDH1 with 6x His-Tag (BBa_K1808004)+ Terminator (B0015). The ALDH1 Generator was designed to overexpress aldehyde dehydrogenase 1 in E.coli. Aldehyde dehydrogenase 1, catalyses the conversion of (11R)-dihydroartemisinic aldehyde to dihydroartemisinic acid as well as the conversion of artemisinic aldehyde to artemisinic acid. Artemisinic acid and dihydroartemisinic acid can be extracted from E. coli cells through lysis and chemically converted to the anti-malarial artemisinin. See results for more information on the characterisation and validation of this part.

ALDH1 regulated by the TAC promoter (BBa_K864400): BBa_K1808011

This ALDH1 generator is composed of the following parts: PTAC (BBa_K864400) + RBS (BBa_0032) + ALDH1 with 6x His-Tag (BBa_K1808004)+ Terminator (B0015). This ALDH1 Generator was designed to overexpress aldehyde dehydrogenase 1 in E.coli in the presence of IPTG and in the absence of tryptophan. Aldehyde dehydrogenase 1, catalyses the conversion of (11R)-dihydroartemisinic aldehyde to dihydroartemisinic acid as well as the conversion of artemisinic aldehyde to artemisinic acid. Artemisinic acid and dihydroartemisinic acid can be extracted from E. coli cells through lysis and chemically converted to the anti-malarial artemisinin.

DBR2 and ALDH1 generator "Alpha": BBa_K1808012

The alpha construct is composed of the following parts: Promoter (BBa_K823005) + RBS (BBa_B0034) + the DBR2 coding region (BBa_K1808000) + RBS (BBa_0032) + ALDH1 with 6x His-Tag (BBa_K1808004)+ Terminator (B0015). The alpha construct was designed to overexpress aldehyde dehydrogenase 1 and artemisinic aldehyde delta11(13)-reductase. Artemisinic aldehyde delta11(13)-reductase catalyses the conversion of artemisinic aldehyde to (11R)-dihydroartemisinic aldehyde. Aldehyde dehydrogenase 1, catalyses the conversion of (11R)-dihydroartemisinic aldehyde to dihydroartemisinic acid. See project for further information.

References:

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Paddon, C, & Keasling, J 2014, 'Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development', *Nature Reviews Microbiology*, 12, 5, pp. 355-367

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