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Rapid prototyping of microbial production strains for the biomanufacture of potential materials monomers

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ABSTRACT

Bio-based production of industrial chemicals using synthetic biology can provide alternative green routes from renewable resources, allowing for cleaner production processes. To efficiently produce chemicals on-demand through microbial strain engineering, biomanufacturing foundries have developed automated pipelines that are largely compound agnostic in their time to delivery. Here we benchmark the capabilities of a biomanufacturing pipeline to enable rapid prototyping of microbial cell factories for the production of chemically diverse industrially relevant material building blocks. Over 85 days the pipeline was able to produce 17 potential material monomers and key intermediates by combining 160 genetic parts into 115 unique biosynthetic pathways. To explore the scale-up potential of our prototype production strains, we optimized the enantioselective production of mandelic acid and hydroxymandelic acid, achieving gram-scale production in fed-batch fermenters. The high success rate in the rapid design and prototyping of microbiologically-produced material building blocks reveals the potential role of biofoundries in leading the transition to sustainable materials production.

1. Introduction

Synthetic biology has expanded our ability to engineer bio-based routes for the production of added-value chemicals, through rational genetic design and metabolic engineering (Choi et al., 2019; Machas et al., 2019; Smanski et al., 2016; Zhang et al., 2019). Progress in synthetic biology automation is leading to the establishment of biofoundry and biorefinery pipelines where the design, selection, robotic assembly and testing of engineered microbial strains is increasingly performed in an automated manner (Appleton et al., 2017; Carbonell et al., 2018a; Chao et al., 2017; Hillson et al., 2019). The current capabilities of biomanufacturing foundries have been demonstrated for several classes of compounds (Casini et al., 2018), including the application of a rapid Design-Build-Test-Learn (DBTL) pipeline for
iterative optimization of production strains (Carbonell et al., 2018a), and machine learning for the translational tuning of biosynthetic pathways (Jervis et al., 2019). Despite such successes in producing natural added-value chemicals, few bio-based alternatives to established chemical products have reached the market, and much effort has to be dedicated toward increasing bioproduction yield (Clomburg et al., 2017; Davy et al., 2017; Wehrs et al., 2019). The challenge is particularly compelling for compounds, including bio-based material building blocks, which are prevalent in modern daily life but are mainly derived from petrochemicals, with associated environmental and sustainability concerns. Bio-based production using synthetic biology could provide alternative green routes from renewable resources, allowing cleaner production processes by removing the need for harsh solvents and chemicals and by reducing associated waste (Ahmed et al., 2019; Anderson et al., 2018; Kawaguchi et al., 2017; Le Feuvre and Scrutton, 2018; Zhu et al., 2016). Further potential benefits from synthetic biology production approaches include rapid access to chemical diversity and the exploitation of enzymatic specificity, which can tackle chemistry that is difficult or not possible through organic synthesis, such as chiral specificity, regioselectivity or functionalization for improved properties (Wagner et al., 2019).

In this study, we applied a semi-automated synthetic biology pipeline to construct microbial strains capable of producing a range of materials monomers via fermentation of simple carbon sources. Our goal was to benchmark and demonstrate the capabilities of the DBTL approach to provide on-demand biosynthetic routes to chemical building blocks for materials (Fig. S1A). An important goal was to provide a competitive, widely applicable workflow, which is compound agnostic and can be applied to multiple targets in parallel with a short time to delivery, a requirement seldom achieved in synthetic chemistry production processes. The assessment presented here provides precise metrics on the ability of biomanufacturing foundries to rapidly deliver diverse bio-based monomers for sustainable materials applications. To that end, we applied a synthetic biology pipeline comprising state-of-the-art bioproduction pathway design tools, robotized strain engineering, and high-throughput product quantification. The pipeline proceeds iteratively through sequential steps, starting with the identification of a target compound of interest. Retrosynthetic design algorithms are used to explore known and putative biochemical routes connecting the target chemical to the metabolism of the host organism. DNA sequences encoding enzymes to catalyze each reaction in the pathway are then designed, synthesized and screened for activity. The build and test steps of the pipeline proceed through an automated platform for genetic pathway assembly, bacterial chiasis construction and culture, followed by automated high-throughput screening and quantification of key metabolites and target compounds.

The materials biomanufacturing pipeline was benchmarked based on multiple metrics, including target titers and time to delivery, using _Escherichia coli_ as a production strain. The purpose of selecting _E. coli_ was to perform rapid prototyping of the bio-based synthetic routes in a well-characterized host, which could later be transferred to other industrial strains for further optimization. We demonstrate how, for several of our targets, the application of our synthetic biology pipeline led to prototype production strains with target titers comparable to those reported for optimized _E. coli_ shake-flask cultures (Table 1). Over 85 days we were able to produce 17 potential materials monomers and key intermediates in _in vivo_. To explore the scale-up potential of our microbial production strains, we dedicated a second 65-day period to optimizing the production of mandelic acid and hydroxymandelic acid at high enantiopurity. We succeeded in producing 0.8–4.8 g/L of the (R)- and (S)-enantiomers of both targets from glucose or glyceral in fed-batch fermenter cultures. This study provides a proof-of-concept for the promising capabilities of synthetic biology pipelines for the production of materials monomers, and showcases how prototype producer strains can be optimized towards industrially relevant titers and economy-efficient production processes.

2. Results and discussion

2.1. Target selection

The first step required selection of the chemical targets for the benchmark set. Selection from the chemical space of industrially relevant monomers was based on targets that are either found in natural biosynthesis routes, or are biosynthetically accessible based on literature information and prospective bio-retrosynthetic research. In total, we selected a list of 25 targets encompassing 7 compound classes that were either materials monomers or advanced precursors, including variants of current materials monomers (Table 1). This chemical diversity includes a broad range of compounds that are not native to the _E. coli_ host (Fig. 1), and represents a number of challenges relevant to materials production: vinylbenzene targets were selected due to the chemical diversity accessible through biosynthetic routes and as less-energy intensive, sustainable alternatives to petrochemical equivalents; allylbenzenes form biodegradable polymers with novel routes to production and derivatization; mandelate lactides are used to prepare recyclable polymers, with chiral monomers accessible at high enantiomeric excess through enzymatic catalysis; butadiene is used to produce synthetic rubber, with biorefineries offering the potential to replace petrochemical cracking; isobutyl compounds are monomer targets accessible through clean biosynthesis routes; benzene dicarboxylates (phthalates) are high-volume material targets with prospects for regioselective biosynthesis; and tyrosol can be used for flame-retardant polymers, with fermentation offering simpler and less expensive purification than natural sources. Outside of the 7 primary compound classes, advanced precursor targets included the phenylacrylic acids (vinylbenzene intermediates), monoglycols (allylbenzene intermediates) and muconic acid (butadiene intermediate). These targets offer compelling reasons for pursuing alternative synthetic biology solutions to production; however, ongoing assessment and reflection on the anticipated implications of the chosen targets for responsible innovation and sustainability is important.

2.2. Design-Build-Test-Learn strategy

For each class of chemical target, synthetic metabolic pathways were selected following a semi-automated approach, which involved the use of retrosynthesis software tools such as RetroPath (Delépine et al., 2018), augmented with manual assessment of feasibility and identification of literature precedents. Enzyme candidates for each biosynthetic target were then designed and screened for activity. The build and test steps of the pipeline proceed through an automated platform for genetic pathway assembly, bacterial chiasis construction and culture, followed by automated high-throughput screening and quantification of key metabolites and target compounds.
Table 1
Summary of material monomer targets ordered by compound class. Targets are given by their common name, CAS number and three-letter code used in this study. Days to production: days from target selection to first in vivo production. Titers are listed as mean values (±standard deviation) for E. coli production strains grown at 30 °C for 24 h in TBP media supplemented with 0.4% glycerol. Mandelate titers are for 1-ml scale cultures prior to optimization and scale-up. Yields are given as mass of product divided by mass of substrate added. Prior published titers in shake-flask are listed, along with the corresponding reference.

| Target Code | CAS      | Compound class | Days to production | Titer (mg/L) ± SD | Titer (mM) ± SD | Yield (g/g) | Genes required | Pathways screened | Enzymes required | Pathway figure |
|-------------|----------|----------------|--------------------|-------------------|-----------------|-------------|----------------|------------------|------------------|---------------|
| CIN         | 140-10-3 | Phenylacrylic acids | 30                 | 669 ± 59         | 4.52 ± 0.17     | 1           | Fig. 3A        | 1                |                  |               |
| Coumaric acid | COU      | Phenylacrylic acids | 30                 | 405 ± 64         | 2.47 ± 0.09     | 3           | Fig. 3A        | 8                |                  |               |
| Ferulic acid | FER      | Phenylacrylic acids | 40                 | 495 ± 59         | 3.51 ± 0.12     | 1           | Fig. 3A        | 3                |                  |               |
| Urocanic acid | URO     | Phenylacrylic acids | 30                 | 319 ± 3         | 2.05 ± 0.06     | 2           | Fig. 3A        | 5                |                  |               |
| Styrene      | STY      | Vinylbenzenes     | 45                  | 26 ± 7           | 0.22 ± 0.01     | 3           | Fig. 3A        | 3                |                  |               |
| 4-Vinylphenol | 4VP     | Vinylbenzenes     | 45                  | 594 ± 24         | 3.36 ± 0.07     | 5           | Fig. 3A        | 10               |                  |               |
| 4-Vinylimidazole | 4VI    | Vinylbenzenes     | 54                  | 17 ± 0           | 0.18 ± 0.01     | 3           | Fig. 3A        | 7                |                  |               |
| 4-Vinylguaiacol | 4VG   | Vinylbenzenes     | 30                  | 28 ± 7           | 0.21 ± 0.06     | 3           | Fig. 3A        | 5                |                  |               |
| 4-Vinylimidazole | 4VI   | Vinylbenzenes     | 30                  | 102 ± 17         | 0.62 ± 0.18     | 8           | Fig. 3A        | 15               |                  |               |
| Chavicol     | CHV 501-92-8 | Allylbenzenes | 80                  | 23 ± 2           | 0.13 ± 0.04     | 6           | Fig. 3A        | 12               |                  |               |
| Eugenol      | EUG 97-53-0 | Allylbenzenes | 80                  | 28 ± 7           | 0.21 ± 0.06     | 6           | Fig. 3A        | 4                |                  |               |
| 4-Hydroxybenzoic acid | 4HB   | Vinylbenzenes     | 54                  | 17 ± 0           | 0.18 ± 0.01     | 3           | Fig. 3A        | 7                |                  |               |
| 4-Hydroxybenzoic acid | 4HB   | Vinylbenzenes     | 30                  | 102 ± 17         | 0.62 ± 0.18     | 8           | Fig. 3A        | 15               |                  |               |
| Coumarol     | CMO 20611-40-5 | Monolignols | 81                  | 23 ± 2           | 0.13 ± 0.04     | 6           | Fig. 3A        | 12               |                  |               |
| Coniferol    | CNO 90-31-2 | Monolignols | 80                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| Phthalic acid | PA       | Benzenedicarboxylates | 84                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| Isophthalic acid | IPA    | Benzenedicarboxylates | 70                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| Terephthalic acid | TPA    | Benzenedicarboxylates | 80                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| Isobutyric acid | IBA    | Isobutyl compounds | 84                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| 2-Oxoisovaleric acid | OIV    | Chassis metabolites | 83                  | 2.6 ± 0.7         | (>10xWT)§ | 0.02 | Fig. S8A   | 3                |                  |               |
| Phenylalanine | PHE      | Chassis metabolites | 82                  | 2352 ± 101       | (4xWT)§ | 12.98 | Fig. S2A   | 4                |                  |               |
| Tyrosine     | TYR 60-18-4 | Chassis metabolites | 82                  | 1283 ± 230       | (16xWT)§ | 7.77 | Fig. S2A   | 10               |                  |               |
| Phthalic acid | PA       | Benzenedicarboxylates | 84                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| Isophthalic acid | IPA    | Benzenedicarboxylates | 70                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| Terephthalic acid | TPA    | Benzenedicarboxylates | 80                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| Isobutyric acid | IBA    | Isobutyl compounds | 84                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| 2-Oxoisovaleric acid | OIV    | Chassis metabolites | 83                  | 2.6 ± 0.7         | (>10xWT)§ | 0.02 | Fig. S8A   | 3                |                  |               |
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| Terephthalic acid | TPA    | Benzenedicarboxylates | 80                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| Isobutyric acid | IBA    | Isobutyl compounds | 84                  | 11 ± 16          | 0.22 ± 0.01     | 3           | Fig. 3A        | 5                |                  |               |
| 2-Oxoisovaleric acid | OIV    | Chassis metabolites | 83                  | 2.6 ± 0.7         | (>10xWT)§ | 0.02 | Fig. S8A   | 3                |                  |               |

*Titers for targets with substrate feeding (3 mM of relevant phenylacrylic acid substrate). † Pathways for these targets were not built.

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different targets shared common intermediates (Fig. S1B). An optimal design of experiments approach was used to combine the selected genes for each pathway/module into a library of expression constructs, with genes arranged in different permutations and with regulatory elements of different strengths (Carbonell et al., 2018a). This approach allowed rapid prototyping of expression construct designs from screening a limited subset of potential combinatorial space, as well as providing robustness against assembly failures given the time constraints. Moreover, while it was not feasible to ‘close the loop’ of the DBTL cycle within the strict 85-day time limit of this study, our statistical design of experiments was implemented in such a way that we not only identified well-producing prototype strains, but also were able to identify the underlying design rules that can be used to accelerate the next stage of optimization, as illustrated below for the example of mandelic acid production (section 2.9).

Once pathways and enzymes were selected, the construction and screening of target-producing strains followed a semi-automated DBTL pipeline described previously (Carbonell et al., 2018a), but with a number of additional optimizations. The combinatorial library of expression constructs was assembled through an automated approach using liquid handling robots following in silico generated worklists (Supplementary Information - Build). The resulting DNA constructs were screened first for the correct size by capillary electrophoresis, then subjected to next-generation sequencing to confirm correct assembly (Currin et al., 2019). During the timeframe of this project our first-pass assembly efficiency (screening 4–6 clones per assembly) was 29%, with 115 of the 400 planned constructs successfully assembled (Table S2). The sequence-verified expression constructs were then introduced into E. coli host strains for monitoring of chemical target production by mass spectrometry (MS; 11 bespoke methods established to quantify 40 target analytes; Table S3). To further boost the titers of certain target compounds, 9 mutant bacterial strains were engineered to overproduce...
pathway precursors (tyrosine, phenylalanine and 2-oxoisovalerate; Table S4) through a combination of targeted gene knockouts and overexpression of native genes including feedback-resistant mutations. In total, the whole process took 85 working days to perform in parallel for all the 25 material target projects (Fig. 2).

### 2.3. Chassis engineering for phenylalanine/tyrosine overproduction

A majority of the materials monomer targets are accessible through pathways that branch off from the native shikimate pathway for aromatic amino acid biosynthesis (Fig. S2A). To enhance titers for these
targets we undertook chassis engineering of *E. coli* strains (DH5α and MG1655) to boost production of phenylalanine and tyrosine (Rodriguez et al., 2014). First, we deleted the tyrR gene (a transcriptional regulator of aromatic amino acid biosynthesis) from the host cell genome, followed by deletion of either the pheA or tyrA genes (chorismate mutase/prephenate dehydratase (CMPDH) for phenylalanine and tyrosine biosynthesis, respectively). For pathways with aromatic aldehyde intermediates we also deleted the fadA gene (phenylacetaldehyde dehydrogenase). These knockout strains were then used as hosts for plasmids carrying a number of genes to channel carbon into the shikimate pathway. Transketolase (tktA gene) and phosphoenolpyruvate synthase (ppsA) both boost availability of shikimate pathway substrates, whereas 3-deoxy-D-arabinoheptulosonate 7-phosphate synthase (DAHPS; aroF, aroG) and CMPDH (pheA, tyrA) exist as amino acid specific isoenzymes that are feedback regulated. We incorporated mutations into our DAHPS and CMPDH genes to relieve feedback inhibition (denoted with a * (Kikuchi et al., 1997; Lütke-Eversloh and Stephanopoulos, 2005; Nels et al., 1992; Weaver and Herrmann, 1990)).

To boost tyrosine production, we designed a plasmid library containing the tktA, ppsA, aroG* and tyrA* genes. However, the tktA gene consistently failed to assemble into these constructs; therefore, we screened a panel of ten 3-gene plasmids without this gene (Fig. S2B, Table S2). In the ΔtyrR, ΔpheA Δdouble knockout strain one plasmid clearly outperformed the rest (SBC005753), producing 1.28 g/L of tyrosine, representing a 16-fold improvement over the wildtype strain with no plasmid (0.08 g/L). To boost phenylalanine production, we designed a separate plasmid library containing tktA, ppsA, aroG* and pheA* genes, and noticed similar difficulties in assembling tktA into our constructs, however in this instance we identified two complete 4-gene plasmids (SBC008290 and SBC008376, Table S2). The two plasmids were screened in the ΔtyrR, ΔtyrA double knockout strain (Fig. S2C), producing 2.35 g/L and 1.96 g/L of phenylalanine respectively, representing a 3.9- and 3.2-fold improvement over the wildtype strain without plasmid (0.61 g/L). These engineered host strains, transformed with amino acid boost plasmids (Fig. S2D), were used as described with target production plasmids to measure final target titers. For mandelic acid targets, later optimization work was done with double knockout strains in which the amino acid boost constructs were integrated into the genome, to relieve the burden on cells to maintain multiple plasmids in the presence of multiple antibiotics.

### 2.4. Production of phenylacrylic acid targets

Phenylacrylic acids are intermediates along the pathways to our vinylbenzene and allylbenzene targets (Fig. 3A), but are also themselves interesting materials monomer targets. Cinnamic acid can be polymerized through its double bond to produce acrylic resins with high thermal stability (Imada et al., 2019), or it can be co-polymerized with styrene or methyl acrylate (Terao et al., 2019). In contrast, coumaric acid and ferulic acid can be polymerized through their carboxylate and hydroxyl moieties to form polyester materials that are cytocompatible for biomedical applications (Thi et al., 2008). Given these potential materials uses of phenylacrylic acids, and since these compounds are intermediates towards several other monomer targets, we expressed the gene(s) for their biosynthesis from separate plasmid modules.

Phenylalanine ammonia-lyase (AtPAL1, from *Arabidopsis thaliana*) and tyrosine ammonia-lyase (FjTAL, from *Flavobacterium johnsoniae*) were chosen on the basis of our prior work with these enzymes (Carbonell et al., 2018a), and were expressed from *trc* or lacUV5 promoters (Fig. 3B). Both promoters yielded similar final titers of product, so we selected the *Ptrc* plasmids for both ammonia-lyase genes. The SBC007575 (APAL1) plasmid yielded 0.67 g/L (4.5 mM) of cinnamic acid when expressed in wildtype DH5α cells, or 1.2 g/L (8.1 mM) when media was supplemented with 3 mM phenylalanine; whereas SBC007575 (FjTAL) yielded 0.34 g/L (2.1 mM) coumaric acid, or 1.02 g/L (6.2 mM) when supplemented with 3 mM tyrosine. *E. coli* strains have been reported to produce 650 mg/L cinnamic acid (Bang et al., 2018) and 974 mg/L coumaric acid (Kang et al., 2012); however, these titers are for shake-flask cultures fed considerable quantities of glucose, so our 1-ml scale cultures compared favorably. For urocanic acid production, we selected 5 histidine ammonia-lyase (HAL) homologs for gene synthesis (Table S1) and screened their activity in wildtype DH5α cells (Fig. 3B). All five HAL plasmids were functional, although SBC007202 (HAL from *Burkholderia lata*) was the most productive, yielding 0.48 g/L (3.5 mM) urocanic acid without media supplementation and 1.3 g/L (9.4 mM) with 3 mM histidine. We did not find prior literature reports for optimization of urocanic acid production in *E. coli*.

We attempted to construct a 3-gene pathway to ferulic acid using genes available in house (Fig. S3A). This pathway progresses from tyrosine to L-DOPA (tyrosinase, TYR), then through caffeic acid (TAL from *Herpetosiphon aurantiacus*) to ferulic acid (caffeate O-methyl transferase, COMT). We designed a 12-member DoE library of ferulic acid plasmids and successfully constructed 11 (Table S2). These plasmids were tested in wildtype DH5α cells and although they produced coumaric acid and L-DOPA, there was little detectable caffeic acid and no ferulic acid (Fig. S3B). We subsequently tested the *in vitro* activity of our TYR and COMT enzymes, alongside a panel of homologs (Fig. S3D; Table S1), and found that our COMT (MsCOMT1, *Mentha spicata*) had poor activity with both L-DOPA and caffeic acid as substrates. Another issue with this pathway is likely to come from competition for tyrosine between TYR and TAL, generating coumaric acid which tyrosinase cannot process. In redesigning this pathway, we would seek to create a sequential route (Fig. S3A), by replacing TYR with a coumarate 3-hydroxylase (Berner et al., 2006) and expressing this alongside HaTAL and the best performing COMT enzyme (PcCOMT1, *Papulus kitakamiensis*).

#### 2.5. Production of vinylbenzene targets

Poly styrene is one of the most widely produced plastics, commonly used for protective packaging, food packaging and disposable cups/cutlery. It is produced by free-radical catalyzed polymerization of styrene through its vinyl moiety. 4-Vinylphenol (4VP) and 4-vinylguaiacol (4VG) are styrene derivatives that polymerize in a similar manner, provided the hydroxyl moieties are first protected (Barclay et al., 1998; Takeshima et al., 2017). Poly(4-vinylphenol) is used in electronics applications due to its dielectric and photosensitive properties, whilst poly(4-vinylguaiacol) is antimicrobial and potentially biodegradable (Hatakeyama et al., 1977). 4-vinylimidazole (4VI) is another styrene analog that can form polymers with potentially novel properties, such as the absorbance of heavy metal ions (Rivas et al., 1998). Styrene and its derivatives are produced industrially from the unsustainable use of petrochemical feedstocks (Cavani and Trifirò, 1995). The decarboxylation of *trans*-phenylacrylic acids offers an alternative production process from a renewable resource, as these compounds are precursors in the biosynthesis of lignin by plants (Boerjan et al., 2003).

We planned to produce vinylbenzene targets through a 2-step pathway (Fig. 3A). Amino acid substrates would first be converted to *trans*-phenylacrylic acids by ammonia-lyases, followed by decarboxylation by ferulic acid decarboxylase (FDC). FDC requires a prenylated flavin cofactor (PrFMN), and so to support enhanced production of this cofactor the enzyme UbiX was also included. We expressed the gene(s) for biosynthesis of phenylacrylic acids from separate plasmids (section 2.4) to the FDC and UbiX genes. Replication origins and antibiotic resistance were selected to ensure compatibility of ammonia-lyase plasmids with amino acid overexpression plasmids, as well as with vinylbenzene and allylbenzene constructs (Table S2). We were able to construct most of these plasmids immediately from genes already in house.

To produce the vinylbenzene targets, from *trans*-phenylacrylic acid precursors, we modified a plasmid carrying AnFDC (*Aspergillus niger*)
and PaUbIx (Pseudomonas aeruginosa), kindly provided by a colleague (Prof. David Leys). We tested this plasmid (SBC007639) in wildtype DH5α cells grown in media supplemented with 3 mM of phenylacrylic acid precursors (Fig. 3C). We grew these cultures in gas-tight glass vessels and overlaid culture media with 50% v/v isooctane to capture the volatile products. With this set-up we successfully produced all four targets in vivo: 336 mg/L styrene; 71 mg/L 4VP; 504 mg/L 4VG; and 55 mg/L 4VI. Finally, to express full biosynthetic pathways we dual-transformed wildtype and double knockout strains of DH5α with ammonia-lyase and FDC plasmids (Fig. 3D). We measured our highest titer of styrene (318 mg/L) in the ΔtyrR, ΔtyrA strain and highest 4VP (26 mg/L) in the ΔtyrR, ΔpheLA strain (Fig. 3E). The highest literature titers for E. coli production of vinylbenzenes are from bioreactor cultures fed high quantities of glucose with gas-stripping of products;
Fig. 4. (A) Pathway from coumarate to coumarol and chavicol. Enzyme abbreviations: 4CL (4-coumarate-CoA ligase); CCR (cinnamoyl-CoA reductase); CAD (cinnamyl alcohol dehydrogenase); CFAT (coniferyl alcohol acyltransferase); and EGS (eugenol synthase). (B) Screening enzyme candidates in cell lysates with 3 mM substrate. No enzyme candidates were selected from the paired 4CL + CCR screen fed coumarate. PsCAD (Pseudomonas strain HR199) was selected to convert coumaraldehyde to coumarol. PhCFAT (Petunia hybrida) and OcEGS1 (Ocimum basilicum) were selected for converting coumarol to chavicol. (C) Screening coumarol pathway constructs in DH5α cells fed 3 mM coumarate. After 24 h, SBC009918 was the most productive plasmid. (D) Screening chavicol pathway constructs in DH5α cells fed 3 mM coumarol. After 24 h, SBC009876 was the most productive plasmid. (E) Best performing plasmid constructs. SBC007589 was screened in Fig. 3. MtCCR1 (CCR from Medicago truncatula), Gm4CL3 (4CL from Glycine max). (F) In vivo production of coumarate and coumarol. DH5α (ΔtyrR ΔpheLA) cultures with the indicated plasmids were assayed after 24 h. (G) In vivo production of coumarol and chavicol. DH5α (ΔtyrR ΔpheLA) cultures with the indicated plasmids were fed 3 mM coumarate or coumarol and assayed after 24 h.
however, for shake-flask cultures, titers of 836 mg/L styrene, 355 mg/L 4VP and 64 mg/L 4VG have been reported (Kang et al., 2012; McKenna et al., 2015). Titers for our prototype production strains compare favorably to the literature, especially since they were obtained at 1-ml scale with just 0.4% glycerol. Our highest titer of 4VI without uronic acid feeding was 17 mg/L in wildtype DH5α (Fig. 3E). To our knowledge this is the first time 4VI has been produced in E. coli. With further time we would next combine ammonia-lyase genes with FDC and UbiX into full pathway constructs, these plasmids could then be co-transformed into our double knockout strains along with amino acid boost plasmids (Fig. S2D). Vinylbenzenes are quite toxic to E. coli cells (styrene toxicity threshold is ~300 mg/L for E. coli (McKenna et al., 2015)), so further improvements in production might require tolerance engineering or in situ product removal, for example by gas-stripping from bioreactor cultures.

2.6. Production of allylbenzene and monolignol targets

Lignin is a polymer component of plant cell walls, conferring mechanical strength to plant tissues and facilitating water-transport. Monolignols are the monomer precursors in lignin biosynthesis, and are crosslinked into a complex branched polymer structure (Boerjan et al., 2003). The three common monolignols are coumaryl alcohol (coumarol), coniferyl alcohol (coniferol) and sinapyl alcohol; and there is significant interest in using these monomers to produce synthetic lignin mimics for materials applications (Ganewatta et al., 2019; Önnerud et al., 2002). Monolignols are difficult to purify from plant materials, and so microbial production of these valuable compounds has been investigated (Chen et al., 2017; Jansen et al., 2014). As well as their role in lignin biosynthesis, monolignols are also precursors in the biosynthesis of the allylbenzenes chavicol and eugenol, volatile compounds used as flavors and fragrances. Allylbenzenes resemble vinylbenzenes, with terminal double bonds for polymerization; however, most materials use seek to polymerize through functionalization of the phenyl hydroxyl group, to preserve the allyl moiety for its antimicrobial properties (Kaufman, 2015). For example, eugenyl methacrylate and ethoxyeugenyl methacrylate monomers are simple to prepare (Rojo et al., 2006), and have been polymerized into oil-absorbent microspheres (Deng et al., 2015). Whilst chavicol-based benzoxazine monomers can be polymerized into thermoset resins with adjustable thermo-mechanical properties through controlled crosslinking (Dumas et al., 2016). Allylbenzenes have been produced from plant genes expressed in E. coli, but only from feeding of their respective monolignol precursors (Kim et al., 2014). Therefore, we selected chavicol and eugenol, along with their precursors coumarol and coniferol, as targets for production.

Coumaric acid and ferulic acid can be produced in E. coli through pathways already described (section 2.4). These trans-phenylacrylic acids can then be activated as CoA thioesters by 4-coumarate-CoA ligase (4CL), and reduced twice by cinnamoyl-CoA reductase (CCR) and cinnamyl-alcohol dehydrogenase (CAD) to yield coumarol or coniferol (Figs. 4A and S4A). Allylbenzenes can then be reached through acetylation by coniferyl alcohol acetyltransferase (CFAT) and reduction by eugenol synthase (EGS). We selected 3–6 enzyme homologs for each step in this pathway (Table S1) for in vitro enzyme screening (Figs. 4B and S4B). We could not identify functional 4CL and CCR candidates in a paired assay, presumably due to instability of the aldehyde products. For the remaining steps, PsCAD (Pseudomonas sp. strain HR199), PhCFAT (Petunia hybrida) and ObEGS (Octimum basilicum) were the best performing candidates for both the chavicol and eugenol targets. A library of plasmid constructs was designed to express PsCAD with Gm4CL3 (Glycine max), which we knew to be functional from previous studies (Carbonell et al., 2018a), and all six CCR gene candidates. From this library we successfully constructed 12 plasmids (Table S2), and screened E. coli DH5α transformants for monolignol production with feeding of phenylacrylic acid substrates (Figs. 4C and S4C). The best performing plasmid for coumarol (SBC009918) produced relatively little coniferol, and the best plasmid for coniferol (SBC009968) likewise produced little coumarol. These plasmids differ in the source of their genes for CCR (MtCCR1, Medicago truncatula for coumarol; PhCCR1 P. hybrida for coniferol), indicating that these enzymes discriminate between the two corresponding CoA thioesters (Figs. 4A and S4A). A second plasmid library was designed to express PhCFAT and ObEGS1, to convert monolignols into allylbenzene targets. We constructed 5 plasmid variants (Table S2) and tested DH5α transformants for production of allylbenzene targets with monolignol feeding (Figs. 4D and S4D). For this library, a single plasmid (SBC009876) performed best at producing both chavicol and eugenol.

Combining the coumarol pathway plasmid (Fig. 4E; SBC009918) with the TAL plasmid (Fig. 3D; SBC007589) should allow for production of coumarol from tyrosine. The DH5α (ΔtyrR ΔpheLA) double knockout strain was transformed with the TAL plasmid alone, or in combination with the coumarol plasmid (Fig. 4F). In this strain, the TAL plasmid produced 405 mg/L of coumaric acid after 24 h culture alone, or in combination with the coumarol plasmid (Fig. 4F). The coumarol plasmid produced 405 mg/L of coumaric acid after 24 h culture in TBP media with 0.4% glycerol. This was a modest improvement over the 342 mg/L observed for this plasmid in wildtype DH5α cells (Fig. 3B). The double knockout strain with both the TAL and coumarol plasmids, produced 25 mg/L coumarol (Fig. 4F). To expand the pathway to chavicol, the double knockout strain was transformed with the coumarol and CFAT/EGS (SBC009876) plasmids, both together and singularly (Fig. 4G). The coumarol plasmid transformants yielded 97 mg/L of coumarol in media supplemented with 3 mM coumaric acid, however most of the coumaric acid substrate remained unconverted. Cells transformed with just the CFAT/EGS plasmid yielded 168 mg/L chlorvalic acid in media supplemented with 3 mM coumarol. In this case, whilst the substrate was not detectable after 24h, chlorvalic titers were below the theoretical maximum of 403 mg/L (3 mM). It is possible that the substrate was converted to other side products by these cells, or alternatively the volatile chlorvalic product may have been lost from the media. When double knockout cells were transformed with both the coumarol and CFAT/EGS plasmids, 28 mg/L of chavicol was produced from the coumaric acid substrate (Fig. 4G).

Similar experiments were conducted for the coniferol/eugenol targets (Fig. S4E); however, in this case no pathway to ferulic acid was available to connect to primary metabolism. Double knockout cells transformed with the coniferol plasmid (Fig. S4F; SBC009968) yielded 23 mg/L coniferol from 3 mM ferulic acid substrate, whilst those transformed with the CFAT/EGS plasmid yielded 28 mg/L eugenol from 3 mM coniferol. Combining both plasmids in the double knockout strain gave our highest titer of 102 mg/L eugenol after 24 h culture, but with a significant amount of the ferulic acid substrate left unconverted (Fig. S4E). To our knowledge this is the first time chavicol and eugenol have been produced microbially from phenylacrylic acid substrates. Coumarol and coniferol have been produced in engineered E. coli cells before, yielding 502 mg/L and 125 mg/L respectively in shake-flask cultures (Chen, 2017). Given further time we would seek to consolidate the genes for coumarol/coniferol and chavicol/eugenol biosynthesis into single plasmid constructs, allowing production of these targets directly from tyrosine produced by the E. coli host. We would then further transform these cells with our tyrosine boost plasmid (Fig. S2D; SBC005753) to channel carbon flux towards production of the targets. Similarities in structure between the allylbenzenes and styrene suggest that these targets may also be toxic and volatile, therefore further improvements in yield might be achieved through including an organic overlay or gas-stripping from bioreactor fermentations.

2.7. Additional material monomer targets

Butadiene is an important commodity chemical as a monomer for the production of synthetic rubber. The FDC enzyme has documented activity for converting sorbic acid into 1,3-pentadiene (Alekhu et al., 2018), so we considered the potential for this enzyme to produce...
butadiene from muconic acid. For this purpose, we planned to construct a muconic acid pathway to support the potential in vivo production of butadiene. Muconic acid is also a materials target, which can be used as monomer for producing bio-based polyacrylate mimics (Quintens et al., 2019) and is a valuable platform chemical for the production of nylon, polyurethane and PET monomers. Muconic acid production by E. coli has been thoroughly explored, with a titer of 3.1 g/L reported for a ‘metabolic funnel’ pathway which converts the metabolites 3-dehydroshikimate (3DS) and chorismate into protocatechuic acid, then onto cis,cis-isomer of muconic acid (Thompson et al., 2018). Since it would be difficult to improve upon this highly optimized pathway (Fig. S5A), we constructed a 5-gene plasmid encoding the same set of enzymes (Fig. S5B). This pathway was tested in a range of wildtype and knockout host strains (Fig. S5C), with a highest observed titer of 31 mg/L of muconic acid (138 mg/L with 3DS substrate feeding). Although this was significantly less than the titer reported by Thompson et al., that study was conducted in shake-flasks with optimized media supplemented with 20 g/L glucose. We in vitro tested a panel of five different FDC homologs in combination with five UbIX homologs, for their ability to convert cis,cis- or trans,trans-muconic acid into butadiene, but failed to detect this product. We also failed to observe butadiene production from muconic acid fed to E. coli cells carrying the functional FDC/UbiX plasmid (SBC007639), despite these cells producing 1,3-pentadiene from sorbic acid. Future projects to produce butadiene might consider screening a wider library of FDC homologs or undertaking mutagenesis of the active site to alter the substrate tolerance of this enzyme.

Tyrosol is an antioxidant compound found naturally in olive oil, that can be polymerized into flame-retardant plastics (Bouldin et al., 2017). Tyrosol can be synthesized in E. coli by expressing phenylacetaldehyde synthase (PAAS) to convert tyrosine into 4-hydroxyphenylacetaldehyde (HPAA), which is then reduced by native aldehyde dehydrogenases (ADHs) into tyrosol (Chung et al., 2017). Alternatively, tyrosine decarboxylase (TDC) can produce tyramine, which is acted upon by monooamine oxidase (MAO) enzyme to produce HPAA (Satoh et al., 2012). We aimed to improve on these studies by combining both routes in a 4-gene pathway (Fig. S6A), including an exogenous ADH gene. We in vitro screened 6 enzyme homologs each for the PAAS, TDC and ADH enzymes, and 3 for MAO (Fig. S6B; Table S1). Whilst we discovered enzymes with good activity for all steps, the best performing homologs were those previously identified (Chung et al., 2017; Satoh et al., 2012). For this reason, and due to time constraints, we did not continue work on tyrosol.

The benzene dicarboxylate terephthalic acid (TPA) is a monomer for polyethylene terephthalate (PET), the most widely produced plastic in the world, used to make clothing and food/drink containers. Isophthalic acid (IPA) and phthalic acid (PA) are isomers of TPA, and also commodity chemicals used to make plastics, often as copolymers with TPA. Acid (IPA) and phthalic acid (PA) are isomers of TPA, and also commodity chemicals used to make plastics, often as copolymers with TPA. Polyurethane and PET monomers. Muconic acid production by E. coli has been thoroughly explored, with a titer of 3.1 g/L reported for a ‘metabolic funnel’ pathway which converts the metabolites 3-dehydroshikimate (3DS) and chorismate into protocatechuic acid, then onto cis,cis-isomer of muconic acid (Thompson et al., 2018). Since it would be difficult to improve upon this highly optimized pathway (Fig. S5A), we constructed a 5-gene plasmid encoding the same set of enzymes (Fig. S5B). This pathway was tested in a range of wildtype and knockout host strains (Fig. S5C), with a highest observed titer of 31 mg/L of muconic acid (138 mg/L with 3DS substrate feeding). Although this was significantly less than the titer reported by Thompson et al., that study was conducted in shake-flasks with optimized media supplemented with 20 g/L glucose. We in vitro tested a panel of five different FDC homologs in combination with five UbIX homologs, for their ability to convert cis,cis- or trans,trans-muconic acid into butadiene, but failed to detect this product. We also failed to observe butadiene production from muconic acid fed to E. coli cells carrying the functional FDC/UbiX plasmid (SBC007639), despite these cells producing 1,3-pentadiene from sorbic acid. Future projects to produce butadiene might consider screening a wider library of FDC homologs or undertaking mutagenesis of the active site to alter the substrate tolerance of this enzyme.

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Screening of these plasmids in wildtype DH5α cells revealed a range of RMA titers from 22 to 251 mg/L, with generally no detectable SMA (Fig. 5C). However, it was notable that all plasmids also produced significant quantities of phenylglyoxylate (42–195 mg/L), the intermediate between SMA and RMA, perhaps due to the reversibility or product inhibition of the RgDMD enzyme. As an alternative route to RMA we designed a library of plasmids to express SyHMAS with PpMR (mandelate racemase from Pseudomonas putida). We successfully constructed 11 plasmids and screened them in wildtype DH5α (Fig. 5D; Table S2), observing a fairly uniform ratio of 65% RMA to 35% SMA, with the most productive plasmid (SBC009527) yielding 220/126 mg/L RMA/SMA. Whilst this plasmid produced the most MA overall (346 mg/L in total), chiral resolution of these enantiomers would be necessary to yield a useful product, and again the presence of...
phenylglyoxylate highlights inefficiencies in the pathway.

The pathways to_RMA pass first through SMA, suggesting that our SyHMAS enzyme is more productive when expressed from plasmids other than pBBR22 (ColE1 origin, _tet_ promoter, _chb_). We therefore subcloned SyHMAS into a different plasmid backbone, pBBB5a (BBR1 origin, _lacUV5_ promoter, _amp_). The original SyHMAS plasmid (SBC007172) was screened alongside the new plasmid (SBC010238) in a mutant DH5α strain (Δ_ΔtyrR_, Δ_αpyrR_), with or without co-transformation with a phenylalanine boost plasmid (SBC008376). There was a clear increase in SMA titer when comparing the original SyHMAS plasmid to the new plasmid (33 vs. 141 mg/L), and our highest SMA titer (170 mg/L) was achieved when the new plasmid was expressed in a host with enhanced carbon flux towards the _PP_ substrate.

### 2.9. Optimisation of mandelic acid production

From our list of 25 material monomers and precursors we successfully constructed _E. coli_ producer strains for 17 targets over an 85-day time limit (Table 1). This showcases the power of the biofoundry approach to rapidly prototype microbial production strains for materials monomer targets. Having developed these strains we anticipate that further significant improvements in target titers can be achieved through a focused optimization of the genetic construct design, further engineering of host strains, and fine-tuning of fermentation conditions for scale-up to bioreactor cultures. To explore this potential, we undertook a further time-limited project (65 days) to optimize and scale-up production of the (S)- and (R)-_enantiomers of MA. We had already observed a significant increase in SMA titer when SyHMAS was subcloned from the pBBE22 vector into pBBB5a (Fig. S5E); this prompted us to investigate other expression vectors from the BglBrick collection (pBB vectors [Lee et al., 2011]). Design of experiments was used to select a library of 12 plasmids, sampling different combinations of replication origins (S = SC101, _A_ = p15A, _B_ = BBR1, _E_ = ColE1), inducible promoters (1 = Pirc, 2 = Pet, 5 = PlacUV5, 8 = Pbad), and antibiotic resistance genes (a = ampR, _k_ = kanR, _c_ = camR). We successfully constructed 11 members of this library and screened them, along with our 2 original plasmids, in wildtype DH5α cultures (Fig. 6A). After 24 h, MA titers for these plasmids ranged from 8 to 100 mg/L, with the highest titer observed for pBbA1a-SyHMAS. This experimental data was used to model the effects of the different vector variables to predict MA titers for other vectors that were not tested. To validate the model, we constructed a second library of 6 plasmids, predicted to encompass high, medium and low MA producers. Screening of this second library showed good correlation between predicted and experimental titers (_R²_ = 0.88, _p_-value ≤ 0.006), and identified pBBB1a-SyHMAS (121 mg/L MA) as the optimal plasmid construct. In an attempt to further boost titers, we investigated the effects of gene dosage. We cloned one, two or three copies of SyHMAS (using different redundant codons to minimize sequence homology) into a single expression vector and cultured _E. coli_ transformants to measure MA and HMA titers (Figs. S9A and S9B). Whilst we observed no significant effect on MA production, HMA titers increased linearly for each additional copy of SyHMAS present (2.05-fold for two copies, 3.15-fold for three copies after 72 h).

The native substrate for HMAS is hydroxyphenylpyruvate (HP), which is converted to 4-hydroxymandelic acid (HMA). We anticipated that significant amounts of HMA will be produced as a side-product in our MA cultures, limiting productivity. However, HMA can be considered a material monomer target in its own right, which could be dimerized into hydroxymandelide for ring-opening polymerization. We developed new mass spectrometry methods, first to quantify both mandelic acid and hydroxymandelic acid in our cultures, then to determine relative proportions of (R)- and (S)-_enantiomers for these targets. The specificity of HMAS for HPP over phenylpyruvate (PP) can be influenced by active site mutagenesis, as can the enantioselectivity of product formation (Pratter et al., 2013; Reifenrath and Boles, 2018).

We undertook mutagenesis of our SyHMAS enzyme to determine whether we could replace our 3-step RMA pathway with a single enzyme, and to select mutants that favor production of MA over HMA. We prepared a homology model of SyHMAS in SWISS-MODEL (Waterhouse et al., 2018), using the crystal structure of AoHMAS bound to HMA (Brownlee et al., 2008), and selected residues S204, I219 and I338 for mutagenesis (Fig. 6B). Each residue was randomized separately (by PCR using the NNK codon) and the library transformed into _E. coli_ DH5α. Automated colony picking selected 96 colonies for each single mutation (3x coverage of NNK codon variants), and MA and HMA titers were quantified in 1-ml cultures after 24 h. Cultures with MA titers higher than wildtype HMAS were then selected for DNA sequencing and chiral analysis of the MA and HMA products (Figs. 6C and S10). Chiral analysis for the wildtype enzyme showed that whilst the HMA produced was > 99.9% SHMA, the MA produced contained ~7% RMA. The S204V mutation had the greatest effect on MA titers, enhancing these 3.1-fold to 206 mg/L. The S204V mutation also inverted the enantioselectivity of SyHMAS, yielding 92.9% RMA and > 99.9% HMA, an effect that was more pronounced than described for other HMAS homologs (Pratter, 2013; Reifenrath, 2018). In contrast, the I219V mutation enhanced titers 2.2-fold over wildtype whilst retaining the selectivity for SMA & SHMA. The I338V mutation slightly improved the SMA/RMA ratio (96%/4%), but reduced overall MA titer compared to I219V (106 mg/L vs. 145 mg/L). We subcloned the S204V and I219V mutants from the pBBB5a vector into both pBB6e1 and pBB8e1, the best expression vectors identified from the library screen (Fig. 6A), and confirmed that pBBB1a supported the highest titers of MA and HMA (Figs. S9C and S9D).

In parallel to optimization of our expression constructs, we sought to improve our host strain and identify optimal fermentation conditions for bioreactor scale-up. We already constructed gene knockout strains to support phenylalanine (Δ_ΔtyrR_, Δ_αpyrR_) and tyrosine (Δ_ΔtyrR_, _αphleLA_) _overproduction_, along with ancillary plasmids to channel metabolic flux to these targets (SBC008290 and SBC005753 respectively). We further engineered these strains using recombineering with CRISPR editing to integrate the plasmid constructs into the genomes at the _lacZ_ locus. This allowed us to use these engineered host strains (identified as DH5α(Phe+) and DH5α (Tyr+)) with just one plasmid (for SyHMAS) and antibiotic. To optimize fermentation conditions (temperature, stirrer speed, _O₂_ feed-rate, etc.) we ran pilot fermenter cultures using our original pBBB5a-SyHMAS plasmid in wildtype DH5α cells. We also conducted 1-ml deepwell block cultures to screen different media compositions and carbon sources (Fig. S11A). Once we had constructed our engineered host strains we further screened media requirements for constructs that produced predominantly (S)- and (R)-_enantiomers of MA and HMA (Figs. S11B and S11C). From these screens we identified our optimal strains and media for production of all four targets: SMA strain [DH5α(Phe+) with pBBB1a-SyHMAS (I219V) in SOB-glycerol]; RMA strain [DH5α (Phe+) with pBBB1a-SyHMAS (S204V) in TBP-glycerol]; SHMA strain [DH5α (Tyr+) with pBBB1a-SyHMAS (I219V) in M9-glycerol]; RHMA strain [DH5α (Tyr+) with pBBB1a-SyHMAS (S204V) in M9-glucose]. The four strains were grown in fermenter cultures in triplicate, with media samples taken at 24/48/72 h for quantification of MA (Fig. 6D), HMA (Fig. 6E) and chiral analysis (Fig. 6F). In addition, triplicate bioreactor cultures of the SMA and RMA strains were sampled after 72 h for genome and plasmid resequencing. No single-nucleotide variants were detectable above background noise, indicating that our producer strains were genetically stable over the duration of the fermentation. Our final scaled-up cultures yielded 0.97 ± 0.13 g/L MA (89% S-enantiomer), 0.80 ± 0.17 g/L MA (98% R-enantiomer), 4.78 ± 0.80 g/L HMA (> 94% S-enantiomer) and 0.90 ± 0.39 g/L HMA (99% R-enantiomer), respectively. Previously, titers of 1.02 g/L SMA and 0.88 g/L RMA were reported for _E. coli_ shake-flask cultures (Sun et al., 2011), whereas HMA was produced at 15.8 g/L in fed batch fermentation (Li et al., 2016) but with no indication of enantiopurity.
3. Conclusions

We have demonstrated the rapid prototyping capabilities of an automated synthetic biology pipeline for the production of materials monomer targets. We successfully produced 17 chemically diverse key materials building blocks out of 25 selected targets, in some cases with titers close to those reported for optimized E. coli fermentations. This was achieved over an 85-day timeframe and without subsequent optimization. Overall, we estimate that the personnel and consumable costs amounted to approximately £15,000 (US$18,500) per successful target compound, corresponding to an average of 360 personnel hours per compound. A second 65-day period was used for optimization and scale-up of strains capable of producing mandelic acid and hydroxymandelic acid. These strains produced 0.8–4.8 g/L of targets with high enantiopurity in fed-batch bioreactor cultures. This rigorous and comprehensive benchmarking study showcases the ability of biofoundries to provide quick access to a diverse range of materials monomers, and demonstrates how prototype microbial production strains can be rapidly scaled-up and optimized to achieve gram-scale fermentations. Through further iterations of the Design-Build-Test-Learn cycle, together with subsequent synthetic biology-based chassis engineering approaches for increasing fluxes, regulating enzyme expression and engineering enhanced enzyme performance; microbial production strains can be optimized to meet the technical specifications for industrial biomanufacturing. The rapid prototyping capacity demonstrated here establishes the foundation for a comprehensive biomanufacturing pipeline for the sustainable microbial production of diverse materials monomers.

Declarations of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymben.2020.04.008.
Author contributions

R.B., E.T. and N.S.S. conceived the initial study design and supervised all aspects of the work. C.J.R., A.J.J., M.D., C.J., R.S., P.C., R.B., E.T. and N.S.S. selected the target compounds. C.J.R., A.J.J., M.D., C.J., A.C., N.S. and S.T. performed the design work. A.J.J., M.D., C.J.R., N.S. and S.T. performed the Build work. C.Y., K.A.H., R.S., N.S. and C.Y. contributed to the manuscript. K.J.M., R.J.T., P.B., N.J.T., M.D., and N.S. supervised all aspects of the work. C.J.R., A.J.J., M.D., K.A.H., C.Y., P.C., N.S. and C.Y. revised the manuscript. All authors contributed to the operational management of the project. K.J.M., R.J.T., P.B., N.J.T., M.D., and N.S. contributed to discussions. C.J.R. and P.C. drafted the manuscript. A.J.J., M.D., A.C., N.S., K.A.H., and C.Y. contributed to the manuscript. R.B., E.T. and N.S.S. revised the manuscript. All authors approved the final manuscript.

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