

Supporting Information

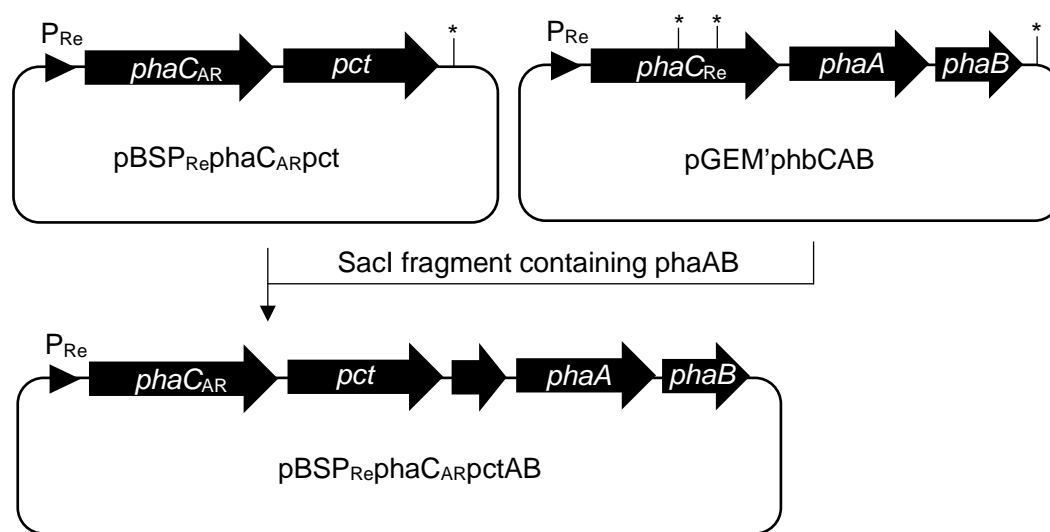
for

**Biosynthesis of random-homo block copolymer poly[glycolate-*ran*-3-hydroxybutyrate (3HB)]-*b*-poly(3HB) using sequence-regulating chimeric polyhydroxyalkanoate synthase in *Escherichia coli***

Shuzo Arai<sup>1</sup>, Sayaka Sakakibara<sup>1</sup>, Robin Mareschal<sup>2,3</sup>, Toshihiko Ooi<sup>4</sup>, Manfred Zinn<sup>3</sup>  
and Ken'ichiro Matsumoto<sup>4,\*</sup>

<sup>1</sup>Graduate School of Chemical Sciences and Engineering, Hokkaido University, Sapporo, Japan, <sup>2</sup>Department of Engineering, Hokkaido University, Sapporo, Japan, <sup>3</sup>Institute of Life Technologies, University of Applied Sciences and Arts Western Switzerland (HES-SO Valais-Wallis), Sion, Switzerland, and <sup>4</sup>Division of Applied Chemistry, Faculty of Engineering, Hokkaido University, Sapporo, Japan.

Scheme S1



\* *SacI* site

Table S1. P(GL-*co*-3HB) production in *E. coli* harboring pBSP<sub>Re</sub>phaC<sub>AR</sub>pctAB grown on glucose and GL

GL	Cell dry weight (g/L)	Polymer production (g/L)	GL (mol%)	3HB (mol%)
0	5.2	1.5	0	100
5	4.2	0.58	8	92
10	4.7	1.2	7	93

The glucose concentration was 10 g/L.

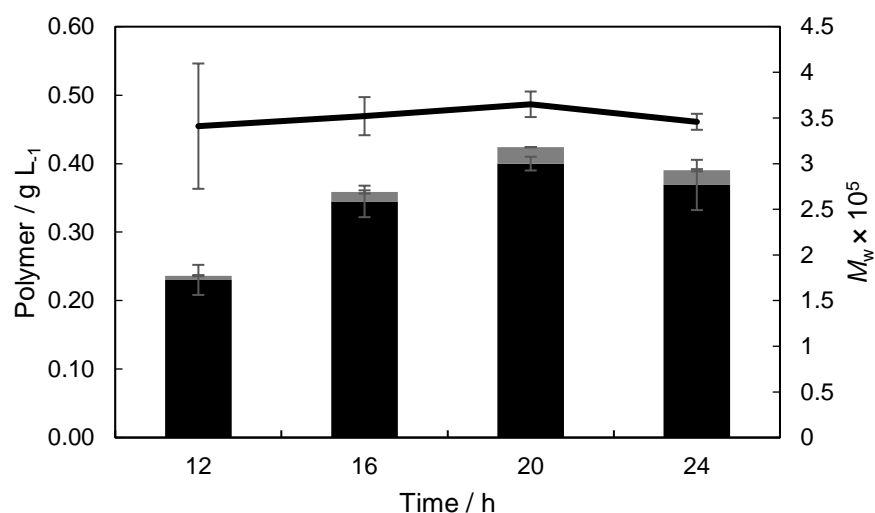
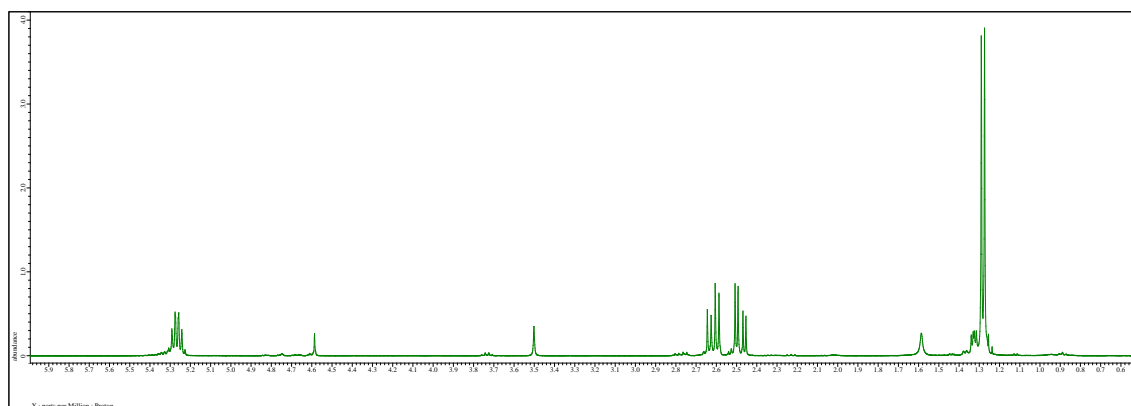
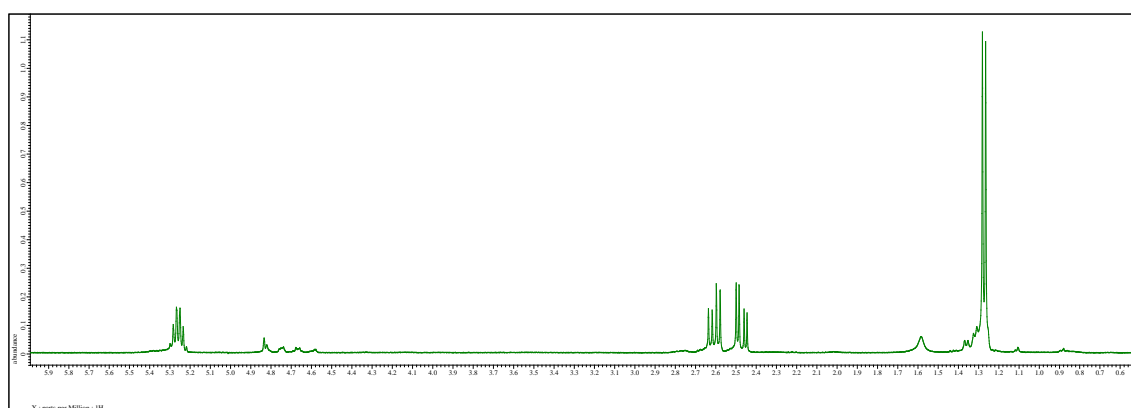


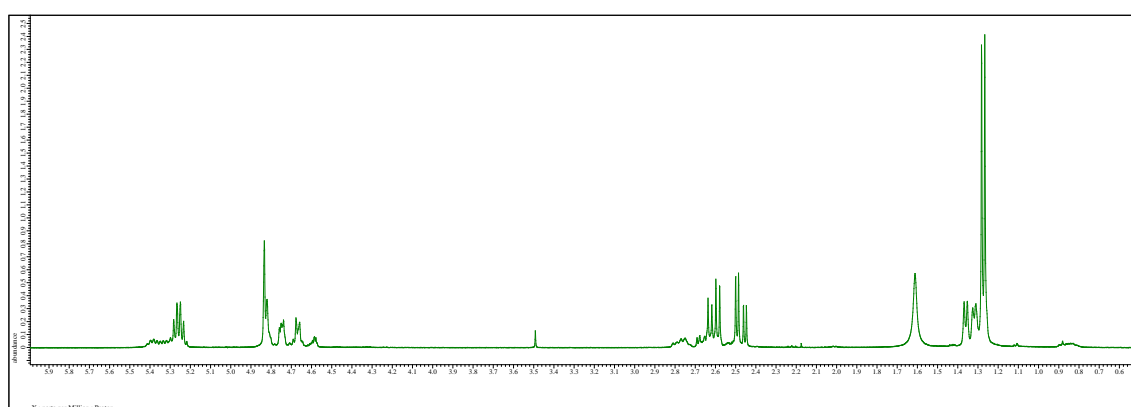
Figure S1. The time-course of P(GL-*co*-3HB) production by PhaC1<sub>Ps</sub>STQK. The polymer was produced with the supplementation of 4 g/L sodium GL. GL and 3HB were supplemented at 0 h. Black bar, 3HB; gray bar, GL. The line graph indicates the weight average molecular weight. The data are the average  $\pm$  standard deviations of three trials. Doublet data is marked at 20 h.



(A): P(GL-*co*-3HB) synthesized by PhaC<sub>1PsSTQK</sub> at 24 h



(B): P(GL-*co*-3HB) synthesized by PhaC<sub>AR</sub> at 24 h produced with the initial addition of GL and 3HB



(C): P(GL-*co*-3HB) synthesized by PhaC<sub>AR</sub> at 24 h produced with the 3HB addition at 12 h

Figure S2. <sup>1</sup>H NMR analysis of P(GL-*co*-3HB)s.

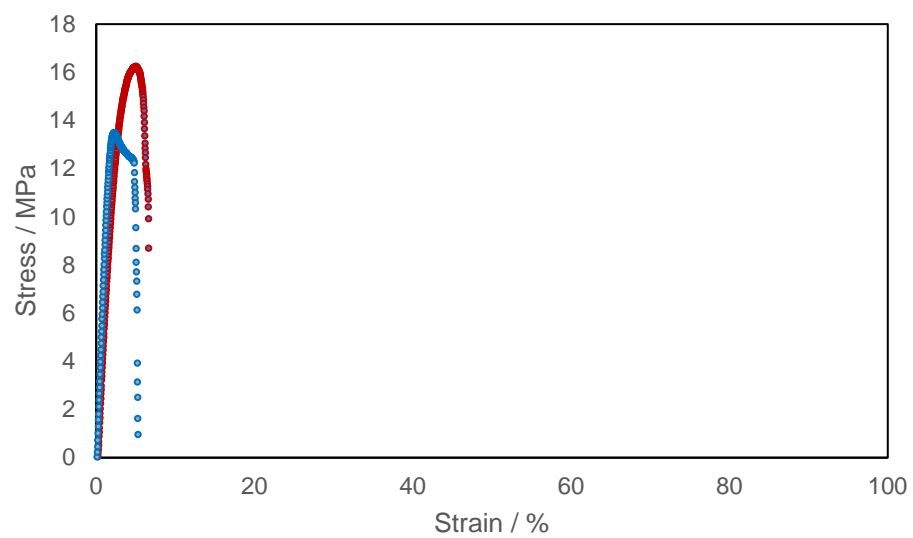
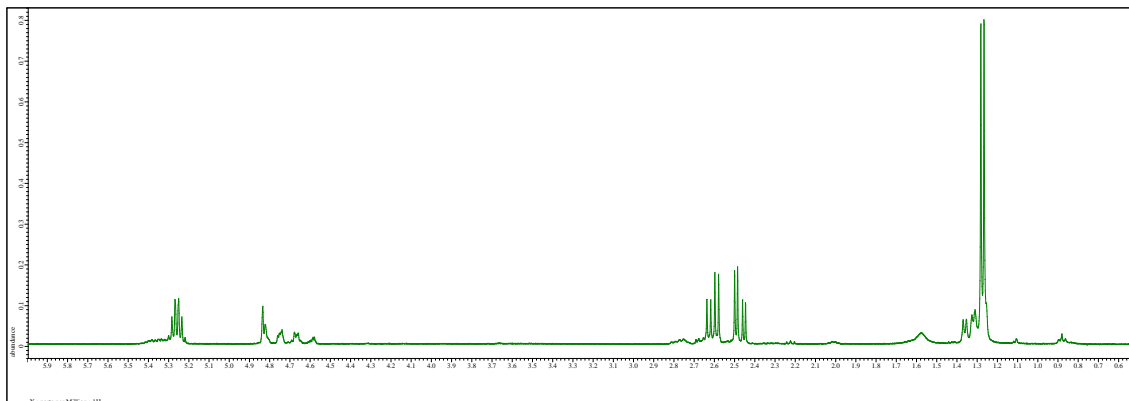
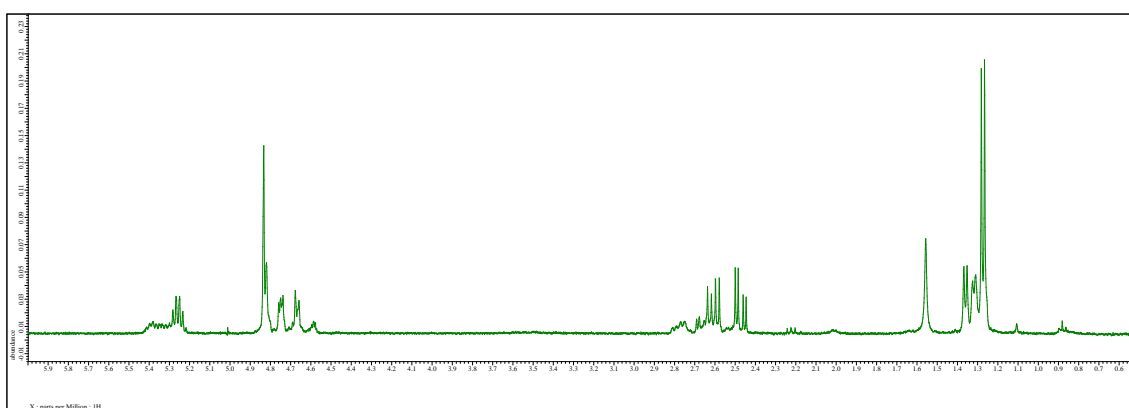


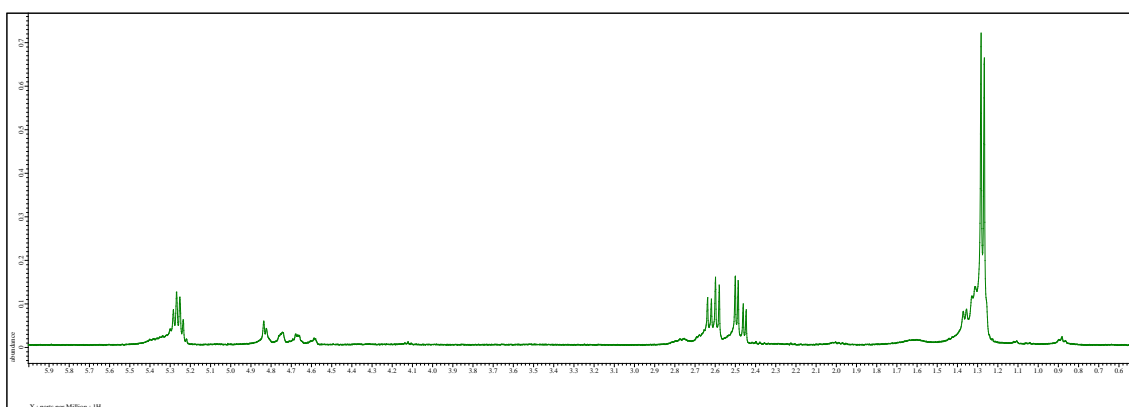
Figure S3. Stress/strain test of solvent-cast films of P(3HB) (blue) and P(GL-*ran*-3HB)-*b*-P(3HB) containing 20 mol% total GL units (red).



Original P(GL-*co*-3HB) synthesized by PhaCAR



Precipitated fraction of P(GL-*co*-3HB) synthesized by PhaCAR



Soluble fraction of P(GL-*co*-3HB) synthesized by PhaCAR

Figure S4.  $^1\text{H}$  NMR analysis of solvent-fractionated P(GL-*co*-3HB) synthesized by PhaCAR. The chloroform solution of the original polymer was partially precipitated by adding methanol.