Supporting Information

Development of an Enzymatic Process for the Production of (*R*)-2-Butyl-2-ethyloxirane

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Contents

Media and buffers

LAEMELLI Electrode Buffer (RPN053)

Tris Base	29.0 g
Glycine	144.0 g
SDS	10.0 g

LB (Luria Bertani)

Tryptone (Bacto)	10 g/L
Yeast extract (Bacto)	5 g/L
NaCl	5 g/L

Adjust pH 7.4 pre-sterilisation

Terrific broth (TB)

Tryptone	12.0 g
Yeast Extract	24.0 g
KH_2PO_4	2.2 g
K_2HPO_4	9.4 g

GF01B

Yeast extract	8.252 g/L
$(NH_4)_2SO_4$,	8.252 g/L
$MgSO_4 \cdot 7H_2O$	6.752 g/L
$CaCl_2$	0.824 g/L
K_2SO_4	4.02 g/L
Thiamine hydrochloride	0.03 g/L
Dextrose monohydrate	0.7 g/L
Mineral salts solution 1	5.4 ml/L
Mineral salts solution 2	8.252 ml/L
H_3PO_4	2.46 ml/L
Polypropylene glycol antifoam	0.11 ml/L

Mineral salts solution 1

Disodium EDTA	100 g/L
$ZnSO_4 \cdot 7H_2O$	9 g/L
$MnCl \cdot 4H_2O$	4 g/L
H_3BO_3	2.7 g/L
$CuCl \cdot 2H_2O$	1.5 g/L
$NiCl \cdot 6H_2O$	0.18 g/L

$NaMoO_4 \cdot 2H_2O$ 0.192 g/L
in 1.5% potassium hydroxide solution.

Mineral salts solution 2
disodium EDTA 50 g/L
$FeSO_4 \cdot 7H_2O$ 20 g/L
in 0.1% potassium hydroxide solution.

GF01B Medium details

Material			%
Yeast Extract			0.825
Ammonium Sulphate			0.825
Magnesium Sulphate $\cdot 7\ H_2O$			0.675
Calcium Chloride			0.0825
Potassium Sulphate			0.402
Thiamine - HCl			0.003
Meritose			0.07
EX01A Mineral Salts solution 1	(mL)		0.54
EX02A Mineral Salts solution 2	(mL)		0.825
Orthophosphoric acid	(mL)		0.246
Add 0.11g/l Polypropylene glycol 2000		PPG	0.011

STERILISATION CONDITIONS 121^0C for 40 Minutes

Transformation and aliquoting of the EH glycerol stock

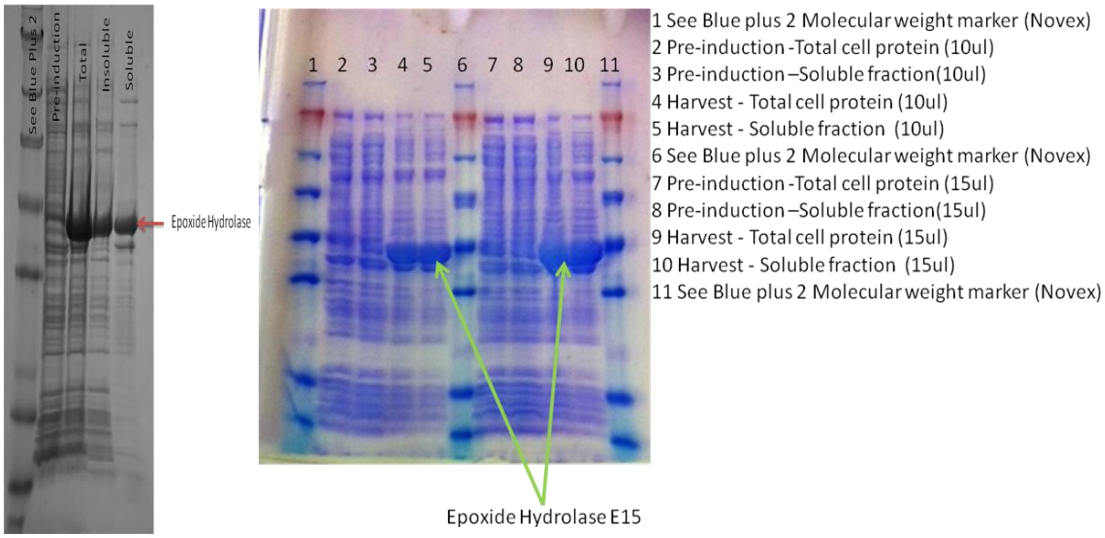
The following protocol was followed: plasmid (2 µl) was added into an aliquot of BL21(DE3) and left on ice for 15 min. Cells were heat shocked at 42°C in a water bath for 45 seconds then left on ice for 2 minutes. SOC media (150 µL) was then added and cells were shaken in a thermomixer at 37°C (1400 rpm) for 1 hour. Each culture was spread on a Luria Broth (LB) plate containing kanamycin (kan) 50 µg/mL and 1% glucose with a sterile spreader. Plates were incubated at 37°C overnight. A single colony was inoculated into LB medium (10 mL) with kanamycin (50 µg/mL) and after 5 hours at 37°C and 220 rpm in a Kuhner shaker the inoculum (750 µL) was added over 250 µL glycerol (80% w/v). The mixture was then frozen at -80°C for longer term storage.

Enzyme expression in shake flasks (2.5 L)

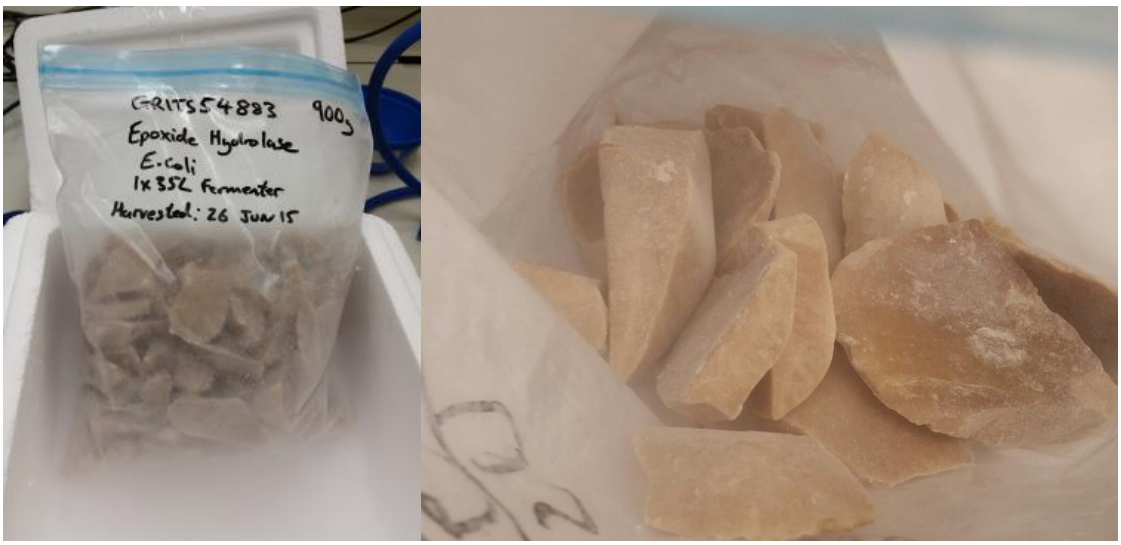
A 50 mL conical centrifuge tube containing LB (10 mL) and kanamycin (50 µg/mL) was inoculated with a single colony from EH5 cells [grown previously on an agar plate containing

kanamycin (50 µg/mL) and glucose (1% w/v)] and incubated for 5 h at 37°C with shaking (220 rpm). This pre-culture was used to inoculate a second stage seed, Terrific Broth (1 L) containing kanamycin (50 µg/mL) and incubated at 37°C (220 rpm) until an OD of 0.6–0.8 at 600 nm was reached. The culture was then induced by adding IPTG to a final concentration of 0.5 mM and the culture incubated overnight at 30°C at 220 rpm. Cells were pelleted by centrifugation (20 min, 4000 rpm at 4°C) frozen and stored at -80°C. At harvest the cell wet mass was 16.8 g/L.

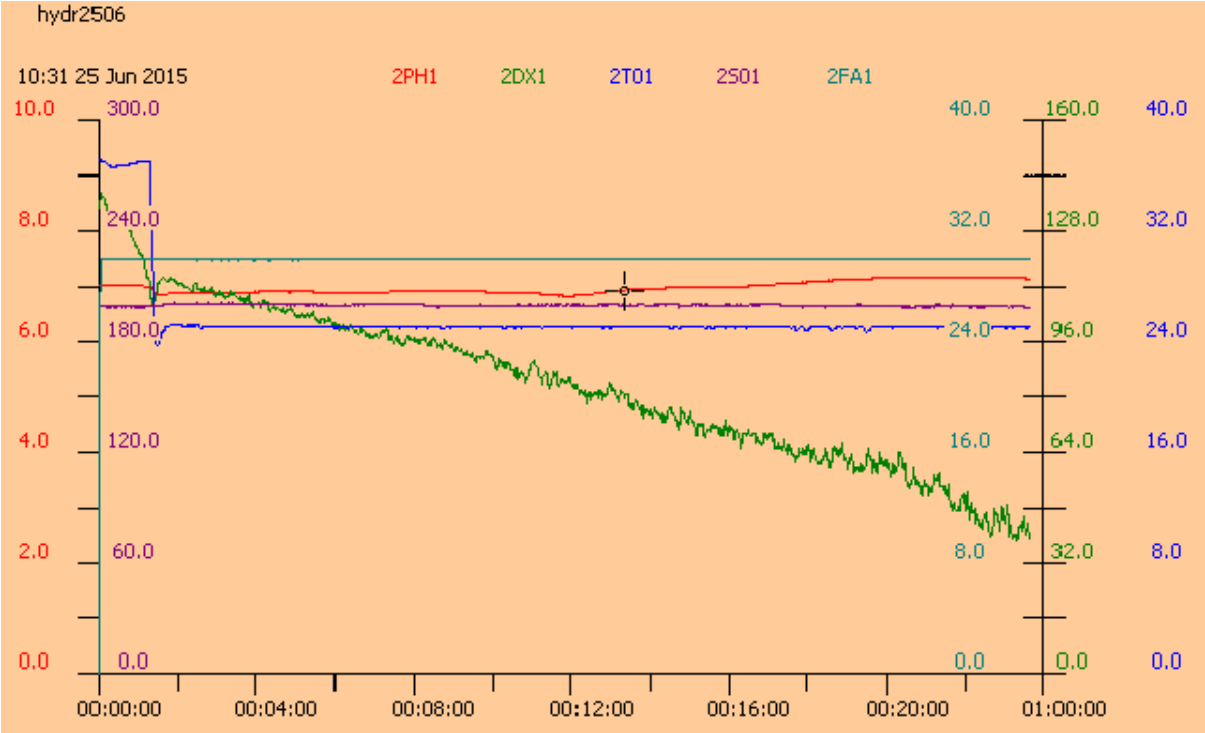
Epoxide Hydrolase (45kDa) SDS-PAGE from expression in 50L fermenter



Frozen pellets containing EH5 from expression in 50L fermenter



50L Fermenter Run Data



Fermenter data

2PH1 = pH

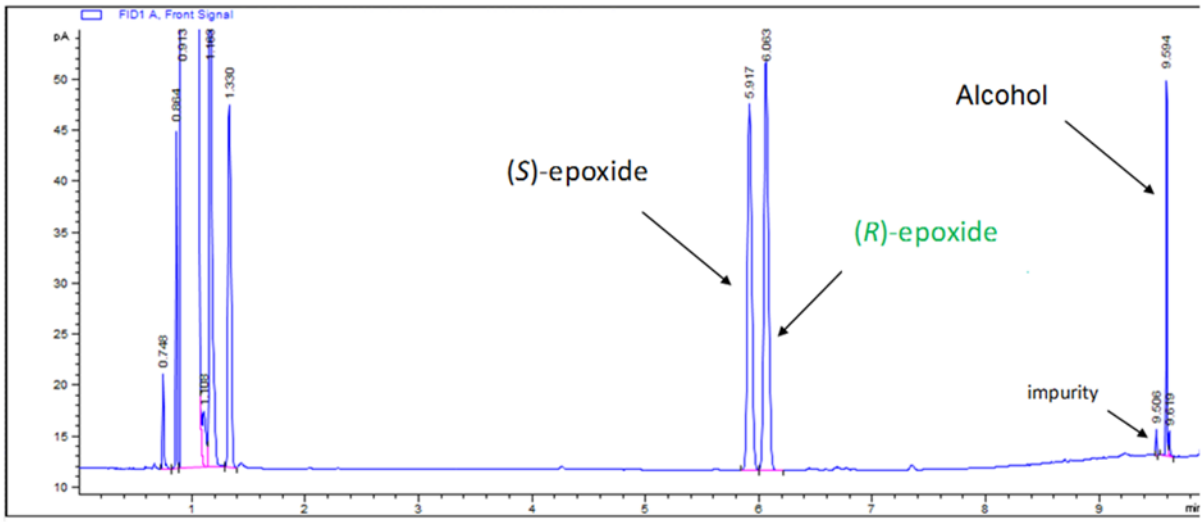
2DX1 = Dissolved oxygen

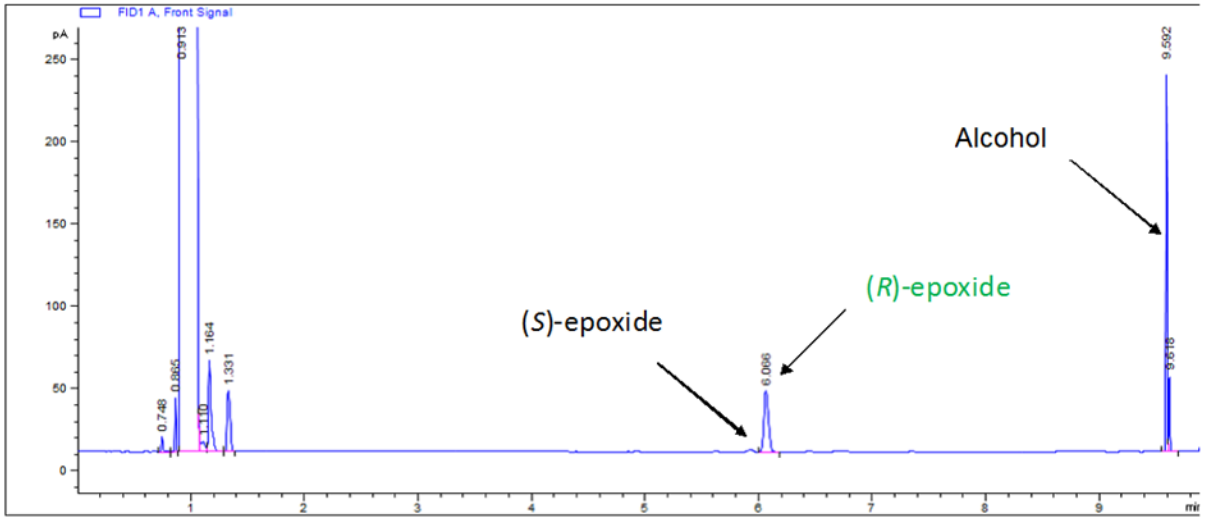
2FA1 = Air flow

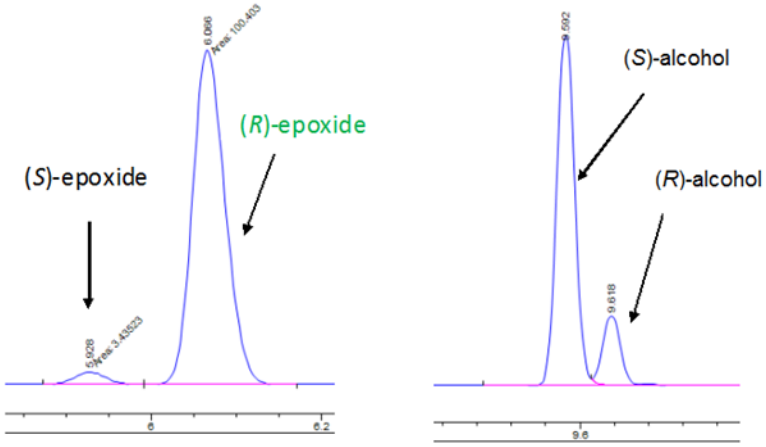
2TO1 = Temperature

2SO1 = Speed

Chiral GC Method with examples of chromatograms







Chiral GC conditions

Agilent 7890B

Oven

Equilibration Time :	0 min
Max Temperature :	250 °C
Maximum Temperature Override :	Disabled
Slow Fan :	Disabled
Cryo :	Off

Temperature

Setpoint :	On
(Initial) :	60 °C
Hold Time :	0 min
Post Run :	50 °C

Program

Rate (°C/min)	Value (°C)	Hold Time (min)
3	80	0
40	210	0

Front SS Inlet H2

Mode :	Split
Heater :	On 250 °C
Pressure :	On 1.4 bar
Total Flow :	On 218.82 mL/min
Septum Purge Flow :	On 3 mL/min
Gas Saver :	Off
Split Ratio :	50 :1
Split Flow :	211.59 mL/min

Column

Column Outlet Pressure : 0 bar

Column #1 Agilent 112-6632 :

CycloSil-B :

35 °C—260 °C (280 °C): 30 m x 250 µm x 0.25 µm :

In :	Front SS Inlet H2
Out :	Front Detector FID
(Initial) :	60 °C
Pressure :	1.4 bar
Flow :	4.2318 mL/min
Average Velocity :	90.308 cm/sec
Holdup Time :	0.55366 min

Lyophilisation recipe

Step	Temperature (°C)	Duration (min)	Vacuum (mTorr)
Thermal Treatment	-40	120	
Evacuation	-45		500
Drying			
Step 1	-40	180	100
Step 2	-20	900	100
Step 3	-20	300	100
Step 4	-15	900	100
Step 5	-15	180	100
Step 6	+4	180	100
Storage	-20		500
Total time		2760 (46 hours)	