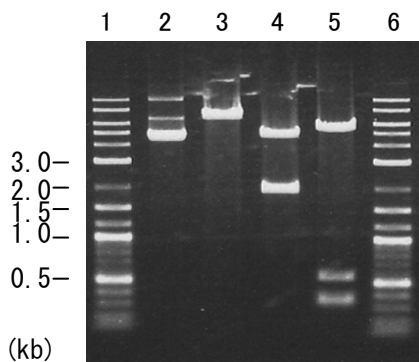


Plasmid name: pET21c_Tr-cel5b-His (RDB No. 8296)
Alternative name: pET21c_Tr019-His
Backbone plasmid: pET21c
Selection: ampicillin
Plasmid size: 6705 bp
CDS size: 1257 bp

1. Restriction enzyme digestion

Working #: BM0122

reaction volume: 10 ul
DNA: 300 ng of plasmid DNA
restriction enzyme: 10 to 15 units



Lane

-
- 1: Size marker
 - 2: uncut plasmid DNA
 - 3: plasmid DNA with *EcoRI* digestion
(6705 bp)
 - 4: plasmid DNA with *Sca I* digestion
(4750 and 1955 bp)
 - 5: plasmid DNA with *Sa/I* digestion
(5441, 558, 370 and 336 bp)
 - 6: Size marker
-

Electrophoresis: 1% agarose gel, 1x TAE buffer
Size marker: 2-Log DNA Ladder (250 ng/lane; Cat.# N3200L, NEB)

1-1-1
Culture

Materials

Plasmid name	pET21c_Tr-cel5b-His
RDB #	8296
Alternative name	pET21c_Tr019-His
Hosts (E. coli)	BL21(DE3) or Rosetta-gami B(DE3)
Media	LB media or Overnight Express

Rosetta-gami B(DE3): (Cat. # 71136, Novagen)

Overnight Express: Overnight Express instant LB Medium (Cat. # 71757, Novagen)

Culture conditions

Volume (ml)	10
Temperature (°C)	37
Duration (hr.)	20

Making of cell lysates

Materials

BugBuster 10X Protein Extraction Reagent (Cat. # 70921, Merck)

Complete, EDTA-Free (Cat # 1873580, Roche Applied Science)

Lysis buffer: 1X BugBuster, 1X Complete, 50 mM Sodium Acetate (pH 6.0)

Procedure

Cultured E. coli

↓ Add 1 ml of Lysis buffer and suspend well

Rotate for 20 min. at R.T.

↓ → Whole lysate

Centrifuge at 14,000 rpm (17800 g) for 20 min.

↓ → Supernatant

ppt

SDS-PAGE

Gel conc.: 5 to 20%

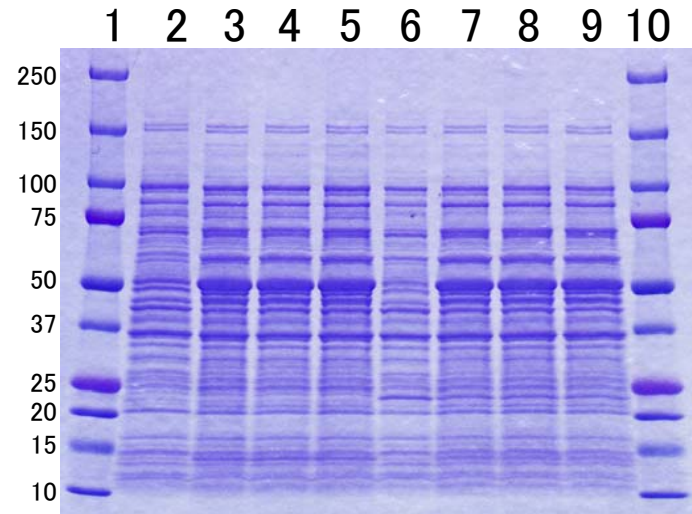
Samples

- 3 ul of Whole lysates
- 10 ul of Supernatants

pET21c_Tr-cel5b-His (RDB # 8296)

Whole lysates and supernatants on SDS-PAGE

Samples: 3 ul of Whole lysate

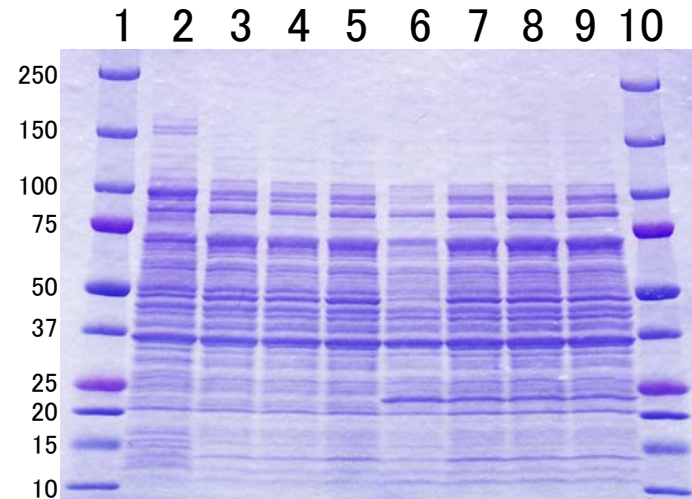


Lanes on SDS-PAGE

- 1: M.W. Marker
- 2: pET21c / BL21(DE3)
- 3: pET21c_Tr-cel5b-His clone 1 / BL21(DE3)
- 4: pET21c_Tr-cel5b-His clone 2 / BL21(DE3)
- 5: pET21c_Tr-cel5b-His clone 3 / BL21(DE3)
- 6: pET21c / Rosetta-gami B(DE3)
- 7: pET21c_Tr-cel5b-His clone 1 / Rosetta-gami B(DE3)
- 8: pET21c_Tr-cel5b-His clone 2 / Rosetta-gami B(DE3)
- 9: pET21c_Tr-cel5b-His clone 3 / Rosetta-gami B(DE3)
- 10: M.W. Marker

Culture media: LB media

Samples: 10 ul of Supernatant



M.W. Marker: 10 ul of Precision Plus Protein

Dual Color Standards

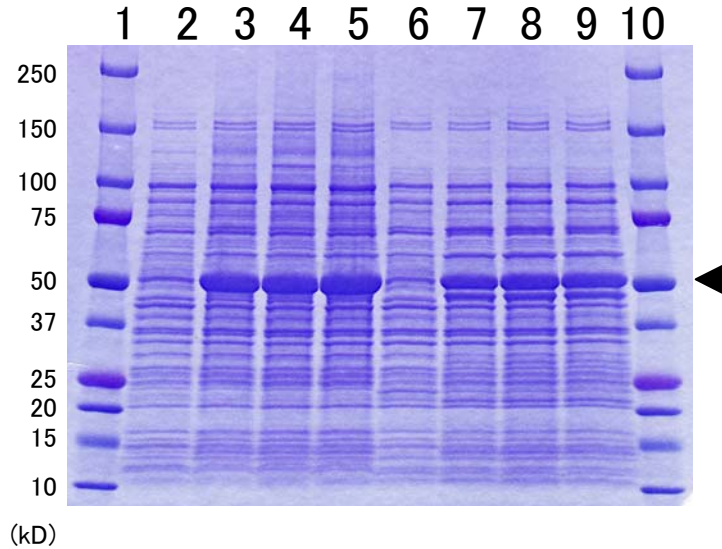
(Cat # 161-0374, BIO-RAD)

Rosetta-gami B(DE3): (Cat # 71136, Novagen)

pET21c_Tr-cel5b-His (RDB # 8296)

Whole lysates and supernatants on SDS-PAGE

Samples: 3 ul of Whole lysate

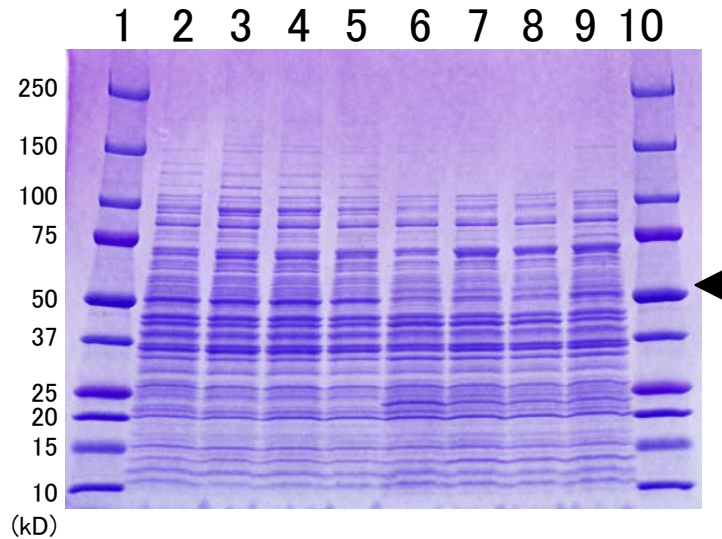


Lanes on SDS-PAGE

- 1: M.W. Marker
- 2: pET21c / BL21(DE3)
- 3: pET21c_Tr-cel5b-His clone 1 / BL21(DE3)
- 4: pET21c_Tr-cel5b-His clone 2 / BL21(DE3)
- 5: pET21c_Tr-cel5b-His clone 3 / BL21(DE3)
- 6: pET21c / Rosetta-gami B(DE3)
- 7: pET21c_Tr-cel5b-His clone 1 / Rosetta-gami B(DE3)
- 8: pET21c_Tr-cel5b-His clone 2 / Rosetta-gami B(DE3)
- 9: pET21c_Tr-cel5b-His clone 3 / Rosetta-gami B(DE3)
- 10: M.W. Marker

Culture media: Overnight Express

Samples: 10 ul of Supernatant



M.W. Marker: 10 ul of Precision Plus Protein
Dual Color Standards
(Cat # 161-0374, BIO-RAD)

Rosetta-gami B(DE3): (Cat # 71136, Novagen)

Overnight Express: Overnight express instant LB Medium
(Cat # 71757, Novagen)