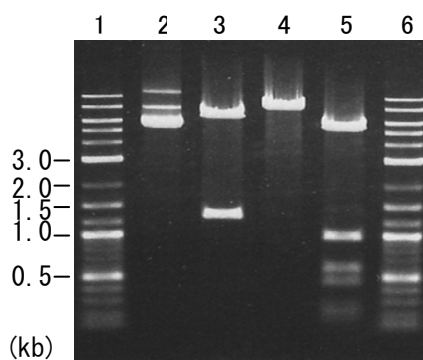


Plasmid name: pET21c_Tr-cel74a-His (RDB No. 8298)
Alternative name: pET21c_Tr021-His
Backbone plasmid: pET21c
Selection: ampicillin
Plasmid size: 7905 bp
CDS size: 2457 bp

1. Restriction enzyme digestion

Working #: BM0125

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 <i>EcoRI</i> digestion (6601 and 1304 bp)
4: plasmid DNA with <i>HindIII</i> digestion (7905 bp)
5: plasmid DNA with <i>SaI</i> digestion (5441, 978, 594, 469, 247 and 176 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

Plasmids name	pET21c_Tr-cel74a-His
RDB #	8298
Alternative name	pET21c_Tr021-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

Bug buster 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

Samples

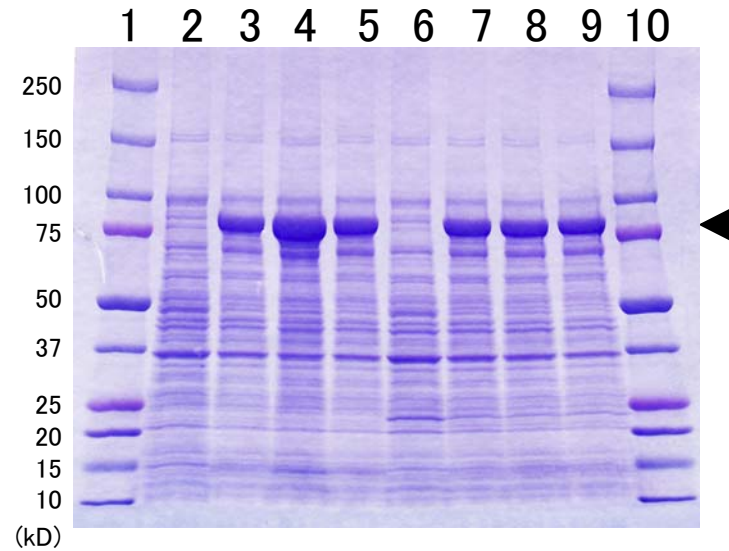
- 3 ul of Whole lysates
- 10 ul of Supernatants

Gel conc.: 5 to 20%

pET21c_Tr-cel74a-His (RDB # 8298)

Whole lysates and supernatants on SDS-PAGE

Samples: 3 ul of Whole lysate

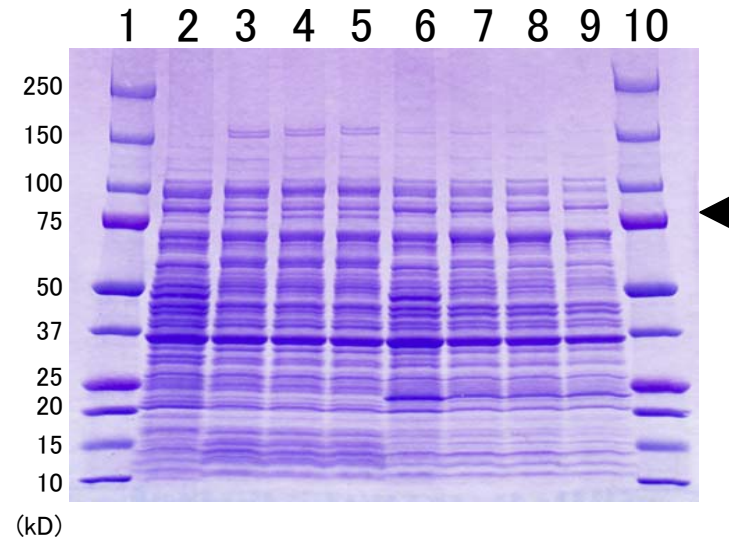


Lanes on SDS-PAGE

- 1: M.W. Marker
- 2: pET21c / BL21(DE3)
- 3: pET21c_Tr-cel74a-His clone 1 / BL21(DE3)
- 4: pET21c_Tr-cel74a-His clone 2 / BL21(DE3)
- 5: pET21c_Tr-cel74a-His clone 3 / BL21(DE3)
- 6: pET21c / Rosetta-gami B(DE3)
- 7: pET21c_Tr-cel74a-His clone 1 / Rosetta-gami B(DE3)
- 8: pET21c_Tr-cel74a-His clone 2 / Rosetta-gami B(DE3)
- 9: pET21c_Tr-cel74a-His clone 3 / Rosetta-gami B(DE3)
- 10: M.W. Marker

Culture media: LB media

Samples: 10 ul of Supernatant

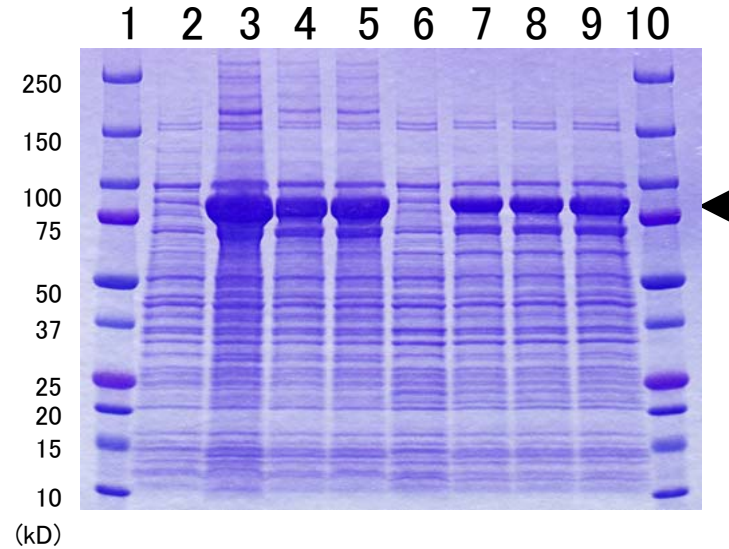


- M.W. Marker: 10 ul of Precision Plus Protein
Dual Color Standards
(Cat # 161-0374, BIO-RAD)
- Rosetta-gami B(DE3): (Cat # 71136, Novagen)

pET21c_Tr-cel74a-His (RDB # 8298)

Whole lysates and supernatants on SDS-PAGE

Samples: 3 ul of Whole lysate

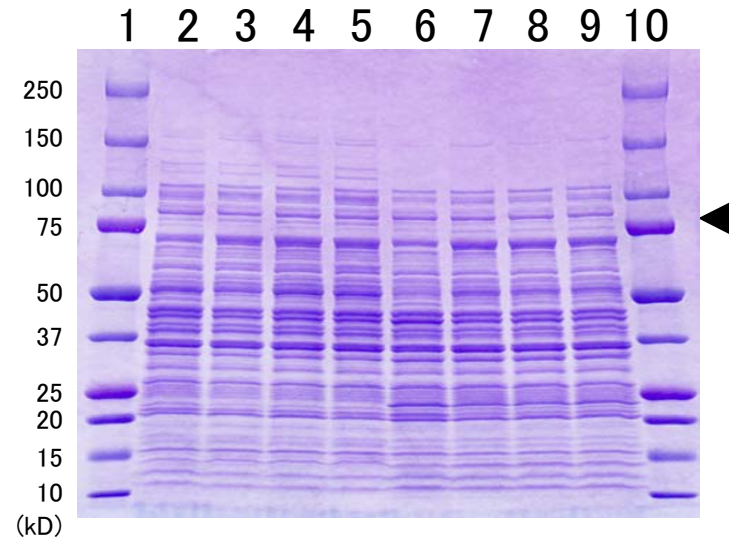


Lanes on SDS-PAGE

- 1: M.W. Marker
- 2: pET21c / BL21(DE3)
- 3: pET21c_Tr-cel74a-His clone 1 / BL21(DE3)
- 4: pET21c_Tr-cel74a-His clone 2 / BL21(DE3)
- 5: pET21c_Tr-cel74a-His clone 3 / BL21(DE3)
- 6: pET21c / Rosetta-gami B(DE3)
- 7: pET21c_Tr-cel74a-His clone 1 / Rosetta-gami B(DE3)
- 8: pET21c_Tr-cel74a-His clone 2 / Rosetta-gami B(DE3)
- 9: pET21c_Tr-cel74a-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)