Gene Engineering Division
RIKEN BioResource Center

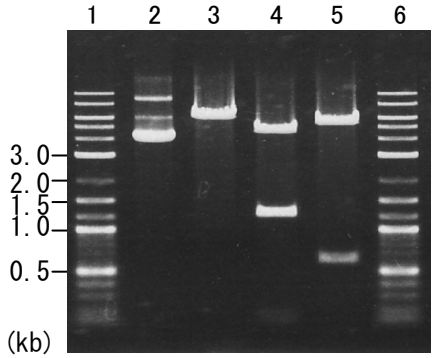
Plasmid name: pET21c_Tr-xyn2-His (RDB No. 8293)

Alternative name: pET21c_Tr010-His
Backbone plasmid: pET21c
Selection: ampicillin
Plasmid size: 6060 bp
CDS size: 612 bp

1. Restriction enzyme digestion

Working #: BM0090

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 *Eco*RI digestion (6060 bp)
4: plasmid DNA with *Sca* I digestion (4660, 1250 and 150 bp)
5: plasmid DNA with *Sa*l I digestion (5441 and 619 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-xyn2-His
RDB #	8293
Alternative name	pET21c_Tr010-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

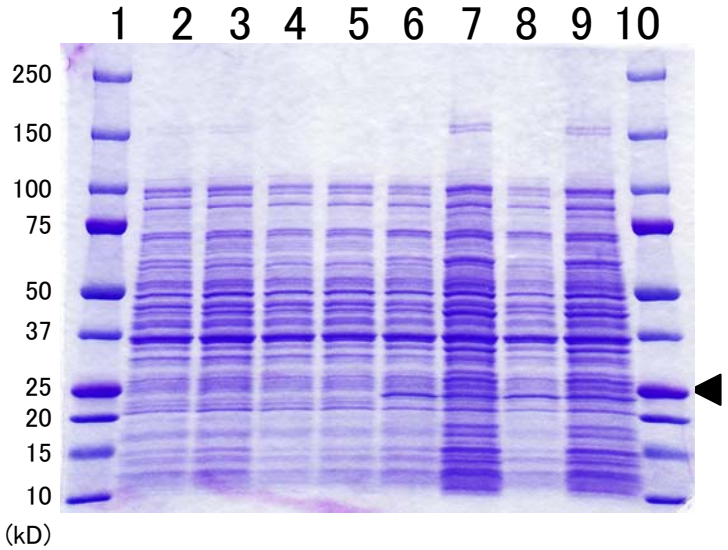
Gel conc.: 5 to 20%

Samples
・3 ul of Whole lysates
・10 ul of Supernatants

pET21c_Tr-xyn2-His (RDB # 8293)

Whole lysates and supernatants on SDS-PAGE

Samples: 3 ul of Whole lysate

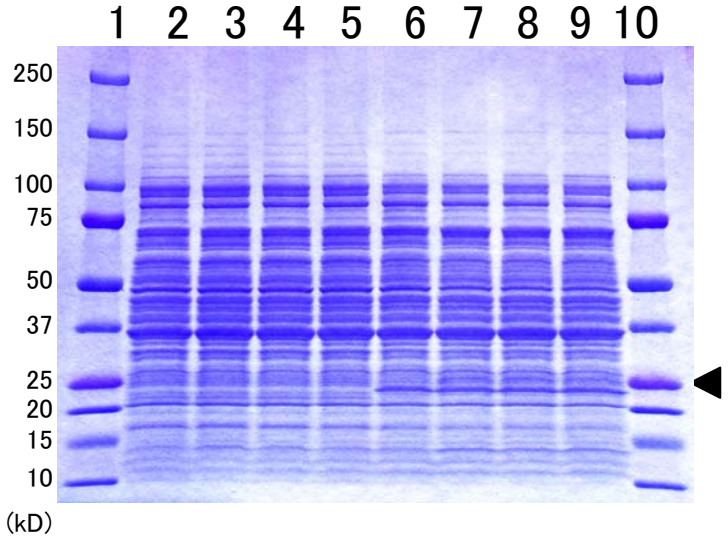


Lanes on SDS-PAGE

1: M.W. Marker
2: pET21c / **BL21(DE3)**
3: pET21c_Tr-xyn2-His clone 1 / **BL21(DE3)**
4: pET21c_Tr-xyn2-His clone 2 / **BL21(DE3)**
5: pET21c_Tr-xyn2-His clone 3 / **BL21(DE3)**
6: pET21c / **Rosetta-gami B(DE3)**
7: pET21c_Tr-xyn2-His clone 1 / **Rosetta-gami B(DE3)**
8: pET21c_Tr-xyn2-His clone 2 / **Rosetta-gami B(DE3)**
9: pET21c_Tr-xyn2-His clone 3 / **Rosetta-gami B(DE3)**
10: M.W. Marker

Culture media: LB media

Samples: 10 ul of Supenatant

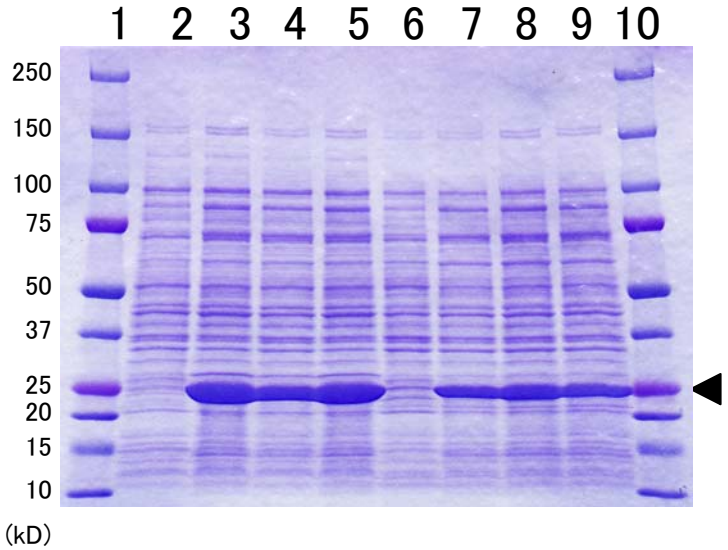


M.W. Marker: 10 ul of Precision Plus Protein
Dual Color Standards
(Cat # 161-0374, BIO-RAD)
Rosetta-gami B(DE3): (Cat # 71136, Novagen)

pET21c_Tr-xyn2-His (RDB # 8293)

Whole lysates and supernatants on SDS-PAGE

Samples: 3 ul of Whole lysate



Lanes on SDS-PAGE

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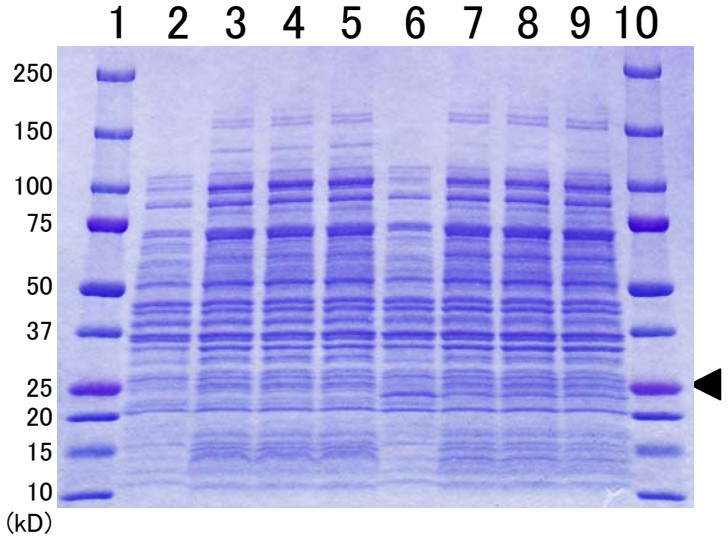
6: pET21c / **Rosetta-gami B(DE3)**
7: pET21c_Tr-xyn2-His clone 1 / **Rosetta-gami B(DE3)**
8: pET21c_Tr-xyn2-His clone 2 / **Rosetta-gami B(DE3)**
9: pET21c_Tr-xyn2-His clone 3 / **Rosetta-gami B(DE3)**
10: M.W. Marker

Culture media: Overnight Express

Samples: 10 ul of Supenatant



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