

PCR Mutagenesis, Cloning, Expression, Fast Protein Purification Protocols and Crystallization of the Wild Type and Mutant Forms of Tryptophan Synthase

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jove.com/video/61839

Materials

Name	Company	Catalog Number	Comments
15 mL 10 kDa filter	MilliporeSigma	UFC901024	centrifugal filter unit
15 mL 100 kDa filter	MilliporeSigma	UFC910024	centrifugal filter unit
2 mL cryogenic vials	Corning	CLS430489	Cryogenic vials
2 mL microcentrifuge tubes	Fisher Scientific	05-408-141	microcentrifuge tubes
24-well Cryschem Plate	Hampton Research	HR3-158	24-well sitting drop plates
2-mercaptoethanol	Fisher Scientific	O3446I-100	Chemical
50 mL centrifuge conical tubes	Thermo Scientific	12-565-270	centrifuge conical tubes
AB15 ACCUMET Basic	Fisher Scientific	13-636-AB15	pH meter
Agarose	Fisher Scientific	BP1356-100	Agarose gel
ammonium sulfate	Fisher Scientific	A702-500	Chemical
Ampicillin	Fisher Scientific	BP1760-5	Antibiotic
Bacterial incubator	Fisher Scientific	S35836	incubator.
BamHI	New England Biolabs	R0136S	Restriction enzyme
bicine	Fisher Scientific	BP2646100	Chemical
Branson 450 Digital Sonifier	Branson	B450	Cell disruptor
Cesium chloride	Fisher Scientific	BP210-100	Chemical
Cesium hydroxide	Acros Organics	AC213601000	Chemical
Chloramphenicol	Fisher Scientific	BP904-100	Antibiotic
dimethyl sulfoxide	Fisher Scientific	D1391	Chemical
dithiothreitol	Fisher Scientific	BP172-5	Chemical
DNA Polymerase	Thermo Scientific	F530S	HF polymerase
dNTP Set	Invitrogen	10-297-018	dNTPs set
EcoRI	New England Biolabs	R0101S	Restriction enzyme
Ethylenediaminetetraacetic acid	Fisher Scientific	S311-100	Chemical
Excelsa E25R Orbital Shaker	Eppendorf New Brunswick	M1353-0004	Orbital incubator
GE AKTA Prime Plus	GE Healthcare	8149-30-0004	FPLC

Gel Extraction Kit	Invitrogen	K210012	DNA purification kit
Glycerol	Fisher Scientific	G33-500	Chemical
HindIII	New England Biolabs	R0104S	Restriction enzyme
His-Trap columns	GE Healthcare	GE17-5255-01	5 mL Hisrap column
imidazole	Fisher Scientific	O3196-500	Chemical
IPTG	Thermo Fisher Scientific	R0392	Inducer
Kanamycin	Fisher Scientific	BP906-5	Antibiotic
Kelvinator Series-100	Kelvinator	discontinued	Ultra low freezer
LB broth	Fisher Scientific	BP1426-500	Liquid broth
Luria Bertani agar	Fisher Scientific	BP1425-2	Solid broth
NaCl	Fisher Scientific	S271-500	Chemical
NcoI	New England Biolabs	R0193S	Restriction enzyme
Ni-NTA affinity beads	Thermo Fisher Scientific	R90115	Ni-NTA agarose beads
PEG 8000	Fisher Scientific	BP233-100	Chemical
phenylmethylsulfonyl fluoride	Fisher Scientific	44-865-0	Chemical
pyridoxal phosphate	Acros Organics	AC228170010	Chemical
S-200 HR	Cytiva	45-000-196	Size exclusion column
SacI	New England Biolabs	R0156S	Restriction enzyme
Sodium hydroxide	Fisher Scientific	S318-100	Chemical
Sorvall RC-5B centrifuge	Sorvall	8327-30-1004	Floor cetrifuge
Spermine	Acros Organics	AC132750010	Chemical
Superdex 200 prep grade	Cytiva	45-002-491	Size exclusion column
T4 DNA ligase	New England Biolabs	M0202S	DNA liagse
Tris	Fisher Scientific	BP152-500	Chemical
Ubl-specific protease 1	Thermo Scientific	12588018	SUMO Protease