French lab resources for protein expression and purification – August, 2015 update

Vectors:

pET vectors	**antibiotic**	**tag(s)**	**cleavable?**	**features**
- pET28	Kan	N/C-6His	thrombin	
- pET22	Amp	C-6His	N	pelB leader
- pET26	Kan	C-6His	N	pelB leader
- pETDuet	Amp	N-6His, C-STag	N	two MCS's, pBR322 ori
- pRSFDuet	Kan	N-6His, C-STag	N	two MCS's, RSF ori
- pACYCDuet	Chlor	N-6His, C-STag	N	two MCS's, P15A ori
THT vectors				
- THT	Kan	N/C-6His	TEV	same MCS as pET28
- THST	Kan	N-Sumo, N/C-6His	TEV	same MCS as pET28
pDB vectors (tags for increased solubility or for alternative purification strategies)				
- pDB.His.MBP	Kan	N-MBP, N/C-6His	TEV	same MCS as pET28
- pDB.His.GST	Kan	N-GST, N/C-6His	TEV	same MCS as pET28
- pDB.His.TRX	Kan	N-TRX, N/C-6His	TEV	same MCS as pET28
- pDB.His.NusA	Kan	N-NusA, N/C-6His	TEV	same MCS as pET28
- pDB.His.Mistic	Kan	N-Mistic, N/C-6His	TEV	same MCS as pET28
pAES30-35 (secretory pathway – for periplasmic localization or membrane protein expression)				
- pAES30	Kan	none	N	dsbA signal sequence (SRP)
- pAES31	Kan	none	N	ompA signal sequence (Sec)
- pAES32	Kan	none	N	phoA signal sequence (Sec)
- pAES33	Kan	none	N	sufI signal sequence (Tat)
- pAES34	Kan	none	N	torA signal sequence (Tat)
- pAES35	Kan	none	N	torT signal sequence (SRP)
pQE vectors				
- pQE-9	Amp	N-6His	N	T5 promoter
- pQE-40	Amp	N-6His, N-DHFR	N	T5 promoter
- pQE-30/31/32	Amp	N-6His	N	T5 promoter
vectors for mammalian cells				
- pSecTag2	Amp/Zeo	N-IgK,C-myc,C-6His	N	secretion of expressed proteins
- pEGFP-N1	Kan/Neo	C-EGFP	N	GFP, NB – no start codon present
- pmCherry2-N1	Kan/Neo	C-mCherry2	N	mCherry2, NB – no start codon
- pmCherry2-C1	Kan/Neo	N-mCherry2	N	mCherry2, NB – no start codon
- pcDNA3-YFP	Amp	EYFP	N	YFP fluorescent protein
- mCerulean-N1	Kan	C-mCerulean	N	mCerulean fluorescent protein
- pBudCE4.1	Zeo	C-V5/6His, C-myc/6His	N	two MCS's
accessory plasmids				
- pBAD-σ32-I54N	Amp	NA	NA	overexpression of sigma32 factor (upregulates several chaperone proteins); induce with Arabinose (Ara)
- pDB1282	Amp	NA	NA	expresses isc operon to help with the reconstitution of iron-sulfur clusters; induce with Ara
- pG-KJE8 (chap1)	Chlor	NA	NA	dnaK, dnaJ, grpE and groES-groEL
- pGro7 (chap2)	Chlor	NA	NA	groES-groEL
- pKJE7 (chap3)	Chlor	NA	NA	dnaK-dnaJ-grpE
- pG-Tf2 (chap4)	Chlor	NA	NA	groES-groEL, tig
- pTf16 (chap5)	Chlor	NA	NA	tig

Cell lines for protein expression

Name	Antibiotic?	Properties
- BL21(DE3)	NA	standard protein expression strain; lon and ompT deficient
- HMS174(DE3)	Rif	recA derivative of Bl21 – may stabilize certain genes
- BLR(DE3)	Tet	recA derivative of Bl21 – may stabilize certain genes
- B834(DE3)	NA	NA
- Origami (DE3)	Kan, Tet	Trx and glut. Reductase mutatations; enhanced disulfide (works well with TRX fusion proteins; use Amp plasmid)
- Arctic Express(DE3)	Gen	Chaps Cpn60 and Cpn10; enables low temp growth (11-13 C)
- Rosetta(DE3)	Cam	enhances expression of proteins with rare codons
- Codon Plus(DE3) RIPL	Cam, Strep	enhances expression of proteins with rare codons
- C41(DE3)	NA	more tolerant to toxic proteins
- C41(DE3) pLysS*	Cam	more tolerant to toxic proteins
- C43(DE3)	NA	derived from C41 – additional mutation(s) to tolerate toxic proteins
- C43(DE3) pLysS*	Cam	derived from C41 – additional mutation(s) to tolerate toxic proteins (pLysS encodes T7 lysozyme which lowers background expression of genes under T7 promoter control)
- BL21 – Chap1	Chlor	BL21(DE3) line that contains Chap1 – induce with Ara and Tet
- BL21 – Chap2	Chlor	BL21(DE3) line that contains Chap2 – induce with Ara
- BL21 – Chap3	Chlor	BL21(DE3) line that contains Chap3 – induce with Ara
- BL21 – Chap4	Chlor	BL21(DE3) line that contains Chap4 – induce with Tet
- BL21 – Chap5	Chlor	BL21(DE3) line that contains Chap5 – induce with Ara
- BL21 – σ32	Amp	BL21(DE3) line that contains pBAD-σ32-I54N – induce with Ara

Variables/Additives for Lysis:

Variable/additive	range	typical	property
pH	6 – 9.5	7.5	pH, phosphate is usually best; also Tris, Hepes
temperature	0 – 30 C	0 – 4 C	temperature; usually done on ice
NaCl, KCl	100 mM – 1 M	300 mM	ionic strength
Sugars/polyols	5% - 25%	10%	osmalality/stabilizer; sucrose, trehalose, sorbitol
Detergents	0.1 – 1%	1%	hydrophillicity; use (Triton, CHAPS, Sarkosyl)
Glycerol	5 – 10 %	10%	stabilization
Amino Acids	100 – 300 mM	100 mM	stabilization/anti-aggregation, Arg, Pro, Gly, others
Proteins	0.1 – 10 mg/mL	1 mg/mL	stabilization/anti-aggregation; BSA often used
DTT	1 – 5 mM	2 mM	reducing agent
EDTA	1 – 10 mM	2 mM	chelator, reduces oxidation and proteolysis
PMSF	0.1 – 1.0 mM	0.5 mM	protease inhibitor
Ligand	0.1 – 10 mM	1 mM	may stabilize protein (eg. ATP, substrate, product)

Variables/Additives for Expression Conditions

Variable/Additive	range	typical	Notes
IPTG	0.05 – 1 mM	0.1 mM	induces protein expression
Antibiotics	25 – 200 mg/L	25 – 50 mg/L	selection of plasmid of interest – NB: after induction the plasmid will often be lost within 2 – 4 hours; addition of antibiotics pre-induction may help
Induction OD	0.4 – 10	0.6	to induce at a higher OD, specific media is required
Flask volume	250 – 1000 mL	1000 mL	for growth to higher OD, a lower volume is required
Shake rate	200 – 400 rpm	220 rpm	for growth to higher OD, a higher rate is required
Glucose	0 – 2%	1%	reduces background expression from T7 promoter
Temperature	10 – 37 C	37/18	under 'normal' conditions, cells will grow best at 37C; folding of proteins is often increased by growing at a lower temperature; for temp's below 18C, special cell lines are needed (eg. Arctic Express); NB: the temp should be reduced 0.5 – 1 hour prior to induction
Media	LB, TB, M9, others	LB or TB	the media have different properties; TB will allow culturing to higher cell density; M9 is a minimal/defined media with fewer components
Rifampicin	0 – 200 mg/L	100 mg/L	inhibits E. coli RNA polymerase; add ~30 minutes after induction with IPTG
pH	6.5 – 8.5	7.5	LB is not buffered (TB is); expression/solubility can be effected by pH changes; a buffer can be added to LB (or switch to TB or other) to compensate

Other things to consider:

- Different colonies from the same plate will often yield different results (likely due to plasmid copy number variations); it can often help to screen several plasmids from the same plate
- Ampicillin is quickly degraded in cell cultures; when possible use carbenicillin or add fresh Amp throughout the process
- High concentrations (~200 ug) of antibiotics may be needed to ensure that your plasmid is retained in the cells; this is particularly important for toxic proteins
- For toxic proteins, it is usually best to use a pLys strain and/or add glucose while growing and induce for short periods of time