PANOS GROUP

PLAYBOOK 1.5

Contents

Introduction

This book contains recipes and protocols used by the Panos Group. This is intended as a handbook for use in the laboratory on a daily basis. If the reader identifies a useful recipe or protocol of common interest that is not in the book, please email suggestions or edits to matthew.green@nottingham.ac.uk.

Buffer Recipes

N.B.

- % taken as Biological definition: 1g in 100ml is 1% solution (commonly g/ml). This is based on the approximation that 1ml of H_2O is 1g therefore this is an approximate w/w percentage. This logic breaks down at higher concentrations.
- MiliQ water should be used to make all buffers.

General Buffers

Stock Solutions

Buffer	Stock Recipe
Tris	1 M (**121.14 g/L**) and generally pH set to 7.5 with HCl
NaCl	5 M (**292.2 g/L**)
EDTA	0.1 M (**37.224 g/L**) pH 8.0
Urea	8 M (**480.8 g/L**)
DTT	1 M (**154.25 g/L**)
$MgCl_2$	1 M (**203.3 g/L** or **4.07 g** in 20 ml)
Imidazole	2 M (**136.16 g/L**)
PMSF	0.1 M (**17.4 g/L** in ethanol)
IPTG	1 M (**238.3 g/L**)
Guanidinium chloride	8 M (**764.24 g/L**)
APS	10% (**0.5 g** in 5 ml water)
SDS	10% (**10 g** in 100 ml water)

Phosphate Buffer Recipes

For 0.1 M Potassium Phosphate:

pH	Volume of 1 M K_2HPO_4 (mL)	Volume of 1 M KH_2PO_4 (mL)
5.8	8.5	91.5
6.0	13.2	86.8
6.2	19.2	80.8
6.4	27.8	72.2
6.6	38.1	61.9
6.8	49.7	50.3
7.0	61.5	38.5
7.2	71.7	28.3
7.4	80.2	19.8
7.6	86.6	13.4
7.8	90.8	9.2
8.0	94.0	6.0

Table 1: Make up to 1L with milliQ water.

For 0.1 M Sodium Phosphate:

pH	Volume of 1 M Na_2HPO_4 (mL)	Volume of 1 M NaH_2PO_4 (mL)
5.8	7.9	92.1
6.0	12.0	88.0
6.2	17.8	82.2
6.4	25.5	74.5
6.6	35.2	64.8
6.8	46.3	53.7
7.0	57.7	42.3
7.2	68.4	31.6
7.4	77.4	22.6
7.6	84.5	15.5
7.8	89.6	10.4
8.0	93.2	6.8

Table 2: Make up to 1L with milliQ water.

Antibiotics

Antibiotic	Stock Recipe
Ampicillin	50 mg/ml in 50% ethanol
Carbenicillin	50 mg/ml in 50% ethanol
Chloraphenicol	34 mg/ml in 100% ethanol
Kanamycin	30 mg/ml in water
Tetracyclin	12.5 mg/ml in 100% ethanol

Table 3: These are the antibiotic stock solutions used in the lab. These stocks are used at 1 in 1000 in LB and LA. Previously tetracyclin was 2.5 mg/ml used at 1 in 200 but we are trialling the higher stock solution.

Nickel Affinity Buffers

- Nickel Stripping Buffer: 50 mM Tris pH 7.5, 0.5 M NaCl, 50 mM EDTA
- Nickel Recharging Buffer: 0.1 M $NiSO_4$ (**26.285 g/L** or **6.571 g** in 250 ml)

Agarose Gels

Select the appropriate percentage of agarose according to the length of DNA you have (see 4). Then weigh out the agarose and dilute into 1x TAE buffer.

Fragment Size (bp)	% Agarose	g per 50ml
<400	1.2%	0.6g
<1000	1%	0.5g
>5000	0.7%	0.35g
High Res	1.5%	0.75g

Table 4: Optimal percentage of agarose used to resolve DNA

50x TAE

Component	Amount Required
Tris	242g
Acetic Acid	57.1g/54.4ml
EDTA	18.6g
H_2O	make up to 1l

Table 5: This is the recipe for 50x TAE. The final working concentration is 0.04 M Tris and 0.001 M EDTA.

1. Mix Tris with stir bar to dissolve in about 600 mL of ddH2O.
2. Add the EDTA and Acetic Acid.
3. Bring final volume to 1 L with ddH2O.
4. Store at room temperature.

6x Agarose Loading Buffer

This buffer requires an EDTA stock at 0.5M pH 8.0.

Component	Final Concentration (6x)	Amount Required
Glycerol	50%	[1]
EDTA	0.1%	54μL
Bromophenol Blue	0.25%	25mg
Xylene Cyanol	0.25%	25mg
H_2O		make up to 10ml

Table 6: Recipe for agarose loading buffer. NB. You can add Bromophenol Blue, Xylene Cyanol or both to avoid the dyes masking EtBr signal. Bromophenol Blue Runs at 500-400bp and Xylene Cyanol runs at 10000-4000bp.

[1] Add 5 ml of 100%, 7.5 ml of 50% or 7.14 ml 70% glycerol stock solution

SDS-PAGE Gels

To run a SDS-PAGE gel you require running buffer (to fill the tank), resolving buffer (various percentages depending on the resolution required), stacker (always 4% and serves to concentrate samples before resolving) and loading buffer (which denatures the protein). You will also normally require a lane of protein markers which we buy remade from NEB (stored at -20^{o}C). It is recommended that you read exactly how SDS PAGE works in more detail elsewhere.

SDS-PAGE 10x Running Buffer

Dissolve 30.0 g of Tris base, 144.0 g of glycine, and 10.0 g of SDS in 1000 ml of H2O. The pH of the buffer should be 8.3 and no pH adjustment is required. Store the running buffer at room temperature and dilute to 1X before use.

SDS-PAGE Resolving Buffer

Component	8%	10%	12%	15%	17.5%	18%
30% Acrylamide	26.7 ml	33.3 ml	40.0 ml	50.0 ml	58.3 ml	60.0 ml
1.5M Tris HCL pH8.8	25.0 ml	25.0 ml	25.0 ml	25.0 ml	25 ml	25.0 ml
10% SDS	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml	1.0 ml
H_2O	46.2 ml	39.6 ml	34.0 ml	22.9 ml	14.6 ml	12.9 ml

Store at room temperature in a 100ml Duran wrapped in foil to protect from light.

SDS-PAGE 4% Stacker

Component	Amount to add
30% Acrylamide	8 ml
0.5M Tris HCL pH 6.8	15 ml
10% SDS	0.6 ml
H_2O	36.4 mll

Gradient Gels

Typically our gradient gels are 5% to 20% although other ranges can be used. Make up the following solutions and put them in the gradient maker with a flea in the outer chamber. Mixing while pouring is critical as it improves the graduation and flow. The solutions need to be place the correct way around depending on the filling direction as we fill single gels held in the Bio-Rad apparatus top down while the multi caster fills bottom up. Also the mixer must be placed as high as possible to create flow (for example on a large upturned icebox).

Component	5%	20%
30% Acrylamide	4.175 ml	16.65 ml
1.5M Tris HCL pH8.8	6.25 ml	6.25 ml
10% SDS	250 μl	250 μl
H_2O	14.325 ml	0.0 ml
100% Glycerol	0.0 ml	1.83 ml

SDS-PAGE Loading Buffer

This buffer requires an EDTA stock at 0.5M pH 8.0 To make 5ml:

Component	4x Concentration	Add
100%Glycerol	40%	2 ml
1M Tris HCl pH 6.8	200 mM	1ml
Bromophenol Blue	0.2%	Approximate
EDTA	50 mM	0.5ml
DTT	400mM	0.309 g
H_2O		up to 5ml
SDS	8%	0.4 g

Mix all components vigorously before added SDS. Once SDS is added reduce mixing to prevent frothing.

Preparing a Gel

1. Thoroughly was glass and combs with water and 70% isopropanol
2. Mix 5 ml resolving solution, 20 μL 10% APS and 5 μL TEMED per gel in a universal
3. Fill assembled gel (to correct height) and check for leaks
4. Add 50 μL 100% isopropanol tot eh top of the gel to create a smooth top
5. When dry ass stacker (prepare 2 mL per gel with the same amount of APS and TEMED as previously added)
6. Gels are generally ran at 180V. 50 mA (per gel) for 60 min but this can change depending on the experiment.

5x TBE

To make 1l of 5X TBE buffer add **54 g** Tris base, **27.5 g** Boric acid and **20 ml** 0.5M EDTA (pH 8.0) to 700ml H_2O and mix. Then bring final volume to 1000 ml.

10x TBE

0.9 M Tris base (**108 g/L**), 0.9 M Boric acid (**55 g/L**), 20 mM EDTA (**7.444 g/L**)[2]

[2] Use 10x TBE for the stock when using it regularly as this buffer can precipitate over time.

Destain

You can use water to destain but 20% v/v methanol, 10% v/v glacial acetic acid works much faster

Preparing Dialysis Tubing

1. Cut dialysis tubing to desired length
2. Heat tubing to 80-100 C for 20 mins in 500 mL of wash solution (2% w/v $NaHCO_3$, 1 mM EDTA pH 8)[3]
   in a 1 L beaker with stirring. Ensure tubing is fully immersed.
3. Wash tubing thoroughly inside and out with dH2O
4. Store tubing in a duran in 20% EtOH.

[3] Wash solution recipe (500 mL): Sodium bicarbonate ($NaHCO_3$), 10 g; EDTA pH 8.0 stock solution (0.1 M), 5 mL; dH2O, 495 mL

DNA Handling

Ethanol Precipitation

1. Add 1/10th v/v 3M NaAc pH5.2 (1.23g/5ml) to the purified plasmid DNA
2. Add double the volume of ethanol (i.e. 66% v/v ethanol) and leave on ice for 2 hours (or overnight)
3. Spin at high speed for 30 min
4. Carefully pipette off the supernatant without displacing the pellet
5. Wash the pellet by applying 70% ethanol and again spin (maintain tube orientation as not to displace the pellet) for a few minutes
6. Carefully pipette off the ethanol and leave to air dry
7. Resuspend the pellet in any volume to concentrate the DNA

Radioactive Substrate Labelling

General Substrate Labelling

The oligo is 100 uM (therefore 100umoles/L or 100nmoles per mL or 100 pmoles per μL)

10 fold stock dilution then add 1 μL (10pMoles) to the 20μL reaction

Also add:

- 2μL 10X PNK buffer
- 1μL γ32P ATP (set concentration)
- 1μL T4 PNK enzyme
- 15μL dH2O
- 20μL total

1. Incubate for 2 hours at 37^{o}C (in heat block)

2. After 2 hours turn the temperature up to 75^{o}C to kill the reaction and leave for30 min
3. Cool reaction on ice for 5 min
4. Use a small gel filtration column (in radioactive lab) pack by spinning at 3000 rpm for 1 min
5. Discard flow through and use a separate eppendorf
6. Add 30 μL to the 20 μL reaction (as the optimal volume for the column is 50 μL. Put onto column and spin for 2 min 3000 rpm

   Add another 50μl H2O to make the total volume 100μL

The final concentration is 10p moles in 100μL (assume 100% recovery) 0.1pmol/μL which is 0.1μM

Making Annealed Substrates e.g. M13 Oligo Probe

1. From a 100μM stock of oligo do a 1in100 dilution then add 2 μl of this dilution
2. Also add 2 μl γ23P ATP, 1 μl T4 PNK, 2 μl T4 PNK buffer (10x) and 13 μL H2O.
3. Incubate at 37 for 2 hours
4. Heat deactivate at 75 ^{o} C for 30 min (use the PCR machine program)
5. Add 12.5 μl of 1 μg / μl M13mp18 (Affymetrix) and then I adjust the total volume to 70 μl by additing 37.5 μl milli Q water.
6. Add 3.68 20x SSC buffer[4] and incubate for 2 min at 90-95 ^{o}C
7. Switch off the heat block and leave overnight
8. The next day use a microspin column (pack for 1 min at 3,000g then spin mix through for 2 min at 3,000g)
9. Add 126.32 μl of mQH2O to make vol up to 200 μl giving us a 10 nM stock

[4] For **20x SSC** dissolve 75.3g NaCl and 88.2g of Na_3 Citrate in 800ml H_2O. Adjust to pH 7.0 using HCl then adjust the volume to 1L and autoclave. This makes 20x SSC which is 3M NaCl, 300mM Na_3 Citrate (150mM, 15mM final respectively)

Cell Methods

Making Electrocompetant E.coli

1. Thaw a sample of XL1 Blue/BL21 and streak 10μl onto a plate with relevant resistance. Leave overnight
2. Set up a 10ml starter culture from a single colony. Leave overnight
3. Inoculate 500ml of LB in a baffles glass growth flask, grow at 37^{o}C until the optical density at λ595 reaches 0.7
4. Leave the flask in a cold room (4^{o}C) for 30 min to stop growth (PRE-CHILL ALL BOTTLES H2O ETC)
5. Divide the cells into two pre-chilled sterile centrifuge bottles and harvest (3,500 g x 17min at 4^{o}C)
6. Wash in 200mL dH2O and harvest (6,000 g x 12min at 4^{o}C)[5]
7. Repeat step 6
8. Resuspend in 100mL dH2O and harvest (6,000 g x 12min at 4^{o}C)
9. Resuspend in 20mL dH2O and divide into two sterile pre-chilled centrifuge tubes. Harvest (6,000 g x 12min at 4^{o}C)
10. Resuspend in autoclaved, pre-chilled dH2O (4ml or 6ml for XL1-Blues or BL21 respectively)
11. Snap freeze 80μl aliquots and store at -80^{o}C

[5] Washing steps are performed gently but without delay in a 4^{o}C cold room with pre-chilled autoclaved dH2O. Resuspension is done by swirling

Electroporation

Electroporation allows transformation of cells by temporarily creating holes in the cell membrane which allow the passage of charges molecules (in this case plasmid DNA). The physical mechanism involves multiple phases. Phase 1 is a <1 ms 300-400 mV pulse which creates a voltage across the membrane by inducing an ion migration from the surrounding solution. When the critical field is reached a pre-pore forms (a small (∼3ÊÅ) conductive hydrophobic defect). Phase 2 involves rearrangements at the pore edge which lead to a hydrophillic interface.

STERILE TECHNIQUE THROUGHOUT

1. Get the competent cells (XL1 blues/BL21s) out of the fridge (A20 -80) and defrost on ice
2. Get the plasmid sample and defrost on ice (NB the plasmid sample should be around 100 ng/μl not much above, lower conc. Will be ok just reduce the transformation efficiency)
3. Chill electro cuvetts on ice
4. Set the electroporation machine to Ec2 (this setting is always used unless ligation is being performed)
5. When defrosted put 1 μl of the plasmid into ∼50-85 μl of competent cells (although one aliquot is usually ok aka 80 μl). Gently pipette mix up and down with the p100.
6. Leave on ice for 2 min
7. Transfer the mixture into a pre-chilled electrocuvette (Ensure the liquid is touching both plates)
8. Insert the cuvette into the machine making sure its is the correct way round
9. Using the p1000 collect 1 ml of LB
10. Pulse the cells and quickly add the 1ml of LB instantly mixing by pipetting up and down. After a quick mix transfer the sample to labelled eppendorfs
11. Incubate for 1 hour at 37^{o}C
12. Inoculate an agar plate with ∼250 μl of the broth(containing relevant plasmid resistance to select for transformants)

Glycerol Stocks

Glycerol stocks are the ultimate long term storage. Plasmids are stored in vivo, the cells are protected by the anti freeze agent glycerol and snap frozen in liquid nitrogen (N2) then stored in a -80 freezer.

1. Grow a 5ml overnight culture containing all selective antibiotic conditions. [6]
2. Spin down 10ml to get a pellet (4000rpm 15 min in a fixed angle centrifuge)
3. Discard the supernatant.
4. Resuspend the pellet with LB plus 25-50% glycerol
5. Transfer the suspension into a sterile eppendorf tube. **Special tubes are available for stocks to be added to the lab strain collection.**
6. Snap freeze the sample in liquid N_2 **Undergraduates and masters students must do this under supervision!**
7. Store in the -80 freezer

[6] e.g. pET22b is amp resistant and XL1 blues are tet resistance. The working conc. of tet is 5μl/ml (therefore 50μl in 10ml) and amp is 1 μl/ml (therefore 10μl in 10 ml) NB amp breaks down after incubated growth so if possible adding half again every $\sim$4 hours is a good idea. Carbenicillin has the same activity as ampicillin but does not break down.

Purification Methods

TEV Protease

General information

The TEV protease on this plasmid (pRK793) is a double mutant (L56V/S135G) which reportedly improves solubility and avoids autocatalytic inactivation.

It is expressed in the cell as an MBP-fusion but undergoes self-cleavage to cut off the MBP, creating His-TEV:

GHHHHHHHGESLFKGPRDYNPISSTICHLTNESDGHTTSLYG IGFGPFIITNKHLFRRNNGTLVVQSLHGVFKVKNTTTLQQHLID GRDMIIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQTKSM SSMVSDTSCTFPSGDGIFWKHWIQTKDGQCGSPLVSTRDGF IVGIH-SASNFTNTNNYFTSVPKNFMELLTNQEAQQWVSGWRL NADSVL-WGGHKVFMVKPEEPFQPVKEATQLMNRRRRR

Macrolab purified TEV will cleave 100% of a control substrate in 2 hr at RT at a molar ratio of 1:50 TEV to substrate (equivalent to 1:125 by mass).

In general, we recommend using TEV at 1:20 by mass, unless you have already tested your substrate and found that you can use less. Some substrates may require significantly more TEV. We do not recommend repeated freeze-thaw cycles. TEV retains full activity for several years when stored at -80^{0}C.

TEV is not inhibited by PMSF (1 mM), pepstatin A (1 mM), or complete protease inhibitor cocktail (Roche).

Transformation and Expression

1. Transform into Rosetta2(DE3)pLysS (other expression strains e.g. BL21 should work but cannot be guaranteed). The plasmid is AmpR, but we use Carbenicillin for selection.

2. Inoculate 2YT (1L) + 100 μg/ml Carbenicillin with 5 ml of an overnight starter culture.

3. Grow at 37oC to OD of approximately 0.6, induce with 0.5 mM IPTG, and harvest after 2.5 hours of growth at 37^{0}C.

4. Pellet cells 4000 rpm 15 mins 4^{o}C.

5. Resuspend in 20 ml Nickel A buffer per L cells (25 mM HEPES pH 7.5, 400 mM NaCl, 80 mM imidazole, 10% glycerol) with 1 μg/ml leupeptin, 1 μg/ml pepstatin, 0.5 mM PMSF, 5 mM BME.

6. Freeze cells at -80^{o}C.

Purification(column sizes are for 2L cells)

1. Thaw cells and homogenize with 3 passes through Avestin C3 at 10,000-15,000 psi (or sonicate with your own protocol).

2. Clarify lysate 15000 rpm, 30 mins, 4^{o}C in SS34.

3. Load lysate onto 5 ml HisTrap FF Crude equilibrated in Nickel A buffer + 5 mM BME.

4. Elute with Nickel B buffer (as for Nickel A but 400 mM imidazole) + 5 mM BME.

5. Desalt into IEX A buffer (25 mM HEPES pH 7.5, 100 mM NaCl, 10% glycerol) + 5 mM BME. If there is precipitate at this stage, filter the sample (0.2 μm).

6. Load onto 5 ml HiTrap CaptoS (GE Healthcare) equilibrated in IEX A + 5 mM BME.

7. Elute with step to 100% IEX B buffer (25 mM HEPES pH 7.5, 400 mM NaCl, 10% glycerol) + 1 mM DTT.

8. Adjust concentration to 2 mg/ml and freeze in aliquots. Yield is typically 25-30 mg per L.

TEV protease purified by this method is >99% pure by SDS-PAGE

Appendix

Door Codes

- Lab A20: 2480x
- Computer Room: 4568y
- A Floor Locker Room: 1345y
- Radioactive Lab:
- Radioactive Lab Freezer:
- B floor autoclave: 1723x
- NMR

Useful Websites

- Bioinformatic Tools- http://www.ebi.ac.uk/Tools/emboss/
- Bioinformatic Tool Repository- http://www.expasy.org
- *E. coli* Genome- http://genolist.pasteur.fr/Colibri/
- *B. subtilis* Genome- http://genolist.pasteur.fr/SubtiList/
- Largest Genome Repository- http://www.uniprot.org
- Human Gene Compendium- http://www.genecards.org
- Radioactive Ordering- https://workspace.nottingham.ac.uk/display/CBS/Radioisotope+Order+Form
- HMM Protein Database- http://pfam.xfam.org
- Database Alignment Tool- http://blast.ncbi.nlm.nih.gov/Blast.cgi
- Model Threader- http://swissmodel.expasy.org
- PyMol PDB Viewer- http://pymol.org/educational/
- US Protein Data Bank- http://www.rcsb.org/pdb/home/home.do

- EU Protein Data Bank- http://www.ebi.ac.uk/pdbe/
- SAS Tutorials and Scatter Download- http://www.bioisis.net
- SAS Discussion Forum- http://www.saxier.org
- Agresso Ordering- https://agresso.nottingham.ac.uk/
- MW, pI and Ext Calculator- http://web.expasy.org/protparam/
- pI Range Calculator- http://protcalc.sourceforge.net

Project Codes

- Geoff Briggs- RB05EB
- Matthew Green- RA05KA [7]
- Chemistry General Waste Disposal- A10550

[7] Currently in use.

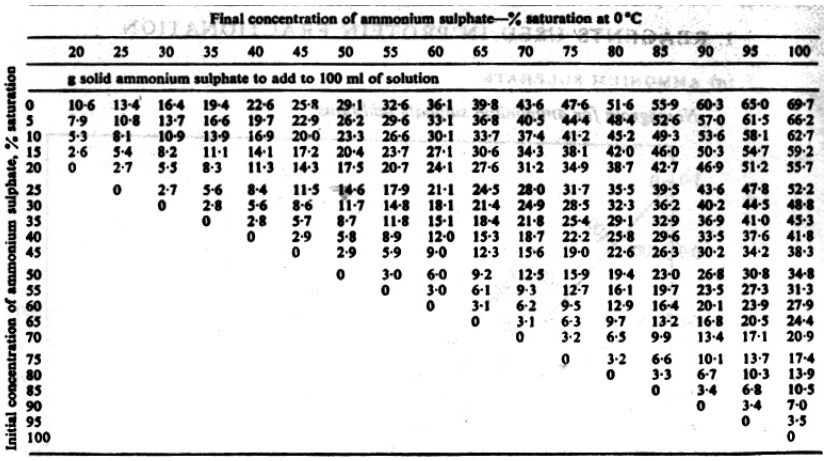
Extinction Coefficients

The Extinction coefficient normally quoted is cm^{-1} M^{-1}, i.e. a 1 molar solution in a 1 cm cell will give an absorbance of 10930. However, a more useful value is cm^{-1} mg/m^{-1}, i.e. a 1 mg/ml solution in a 1 cm cell gives an absorbance of 0.57.

You will notice that if you take the value of 10930 and divide it by the protein MW of ∼18000, then you get something close to 0.57.

Protein	Ext	MW
B. subtilis SSB	0.576769	18742.3
B. subtilis G23C C51V SSB	0.575477	18784.4
E. coli SSB	1.4693	18975
B. subtilis DnaG	0.662	68800.4
B. subtilis DnaE	0.616114	125350
B. subtilis his SSB $^{\Delta 107-171}$	0.583499	14138.8
B. subtilis DnaA	0.724353	50859.2
B. subtilis DnaC	0.466578	50602.5
B. subtilis DnaD	1.24029	27638.8
B. subtilis DnaI	0.653756	36114.4
B. subtilis Fully Deuterated SSB		19992.7
B. subtilis PolC	0.799	162663.3
B. subtilis DnaE	0.690	125349.9

Table 7: Extinction coefficients and MW of the proteins currently being used. If your protein is not here but you would like it to be included for easy reference please email matthew.green@nottingham.ac.uk to make a request.



Initial concentration of ammonium sulphate, % saturation	Final concentration of ammonium sulphate—% saturation at 0 °C															
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
	g solid ammonium sulphate to add to 100 ml of solution																
0	10·6	13·4	16·4	19·4	22·6	25·8	29·1	32·6	36·1	39·8	43·6	47·6	51·6	55·9	60·3	65·0	69·7
5	7·9	10·8	13·7	16·6	19·7	22·9	26·2	29·6	33·1	36·8	40·5	44·4	48·4	52·6	57·0	61·5	66·2
10	5·3	8·1	10·9	13·9	16·9	20·0	23·3	26·6	30·1	33·7	37·4	41·2	45·2	49·3	53·6	58·1	62·7
15	2·6	5·4	8·2	11·1	14·1	17·2	20·4	23·7	27·1	30·6	34·3	38·1	42·0	46·0	50·3	54·7	59·2
20	0	2·7	5·5	8·3	11·3	14·3	17·5	20·7	24·1	27·6	31·2	34·9	38·7	42·7	46·9	51·2	55·7
25		0	2·7	5·6	8·4	11·5	14·6	17·9	21·1	24·5	28·0	31·7	35·5	39·5	43·6	47·8	52·2
30			0	2·8	5·6	8·6	11·7	14·8	18·1	21·4	24·9	28·5	32·3	36·2	40·2	44·5	48·8
35				0	2·8	5·7	8·7	11·8	15·1	18·4	21·8	25·4	29·1	32·9	36·9	41·0	45·3
40					0	2·9	5·8	8·9	12·0	15·3	18·7	22·2	25·8	29·6	33·5	37·6	41·8
45						0	2·9	5·9	9·0	12·3	15·6	19·0	22·6	26·3	30·2	34·2	38·3
50							0	3·0	6·0	9·2	12·5	15·9	19·4	23·0	26·8	30·8	34·8
55								0	3·0	6·1	9·3	12·7	16·1	19·7	23·5	27·3	31·3
60									0	3·1	6·2	9·5	12·9	16·4	20·1	23·9	27·9
65										0	3·1	6·3	9·7	13·2	16·8	20·5	24·4
70											0	3·2	6·5	9·9	13·4	17·1	20·9
75												0	3·2	6·6	10·1	13·7	17·4
80													0	3·3	6·7	10·3	13·9
85														0	3·4	6·8	10·5
90															0	3·4	7·0
95																0	3·5
100																	0

Figure 1: This table is useful for quickly calculating the amount of ammonium sulphate to add to 'cut'. It is especially useful when you already have a solution containing some ammonium sulphate, say 20%, and you are taking it higher.

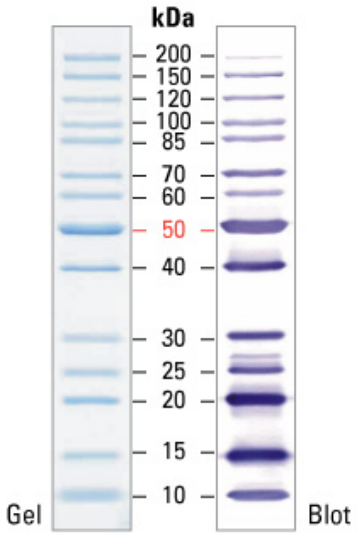


Figure 2: SDS-PAGE band profile of the Thermo Scientific PageRuler Unstained Protein Ladder. Images represent 8-16% Tris-glycine gels (SDS-PAGE). Gel was stained with coomassie blue dye; blot was detected with a Strep-Tactin-AP Conjugate and NBT/BCIP substrate.

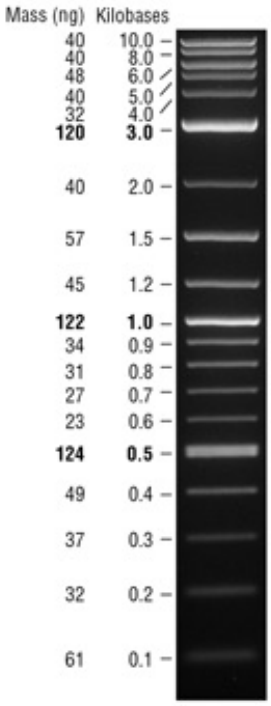


Figure 3: A number of proprietary plasmids are digested to completion with appropriate restriction enzymes to yield 17 bands suitable for use as molecular weight standards for agarose and polyacrylamide gel electrophoresis. The digested DNA includes fragments ranging from 50-1,350 base pairs. The 200 and 500 base pair bands have increased intensity to serve as reference points.

Comes supplied with 1 vial of Gel Loading Dye, Purple (6X), no SDS. 2-Log DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel. Mass values are for 1 μg/lane.

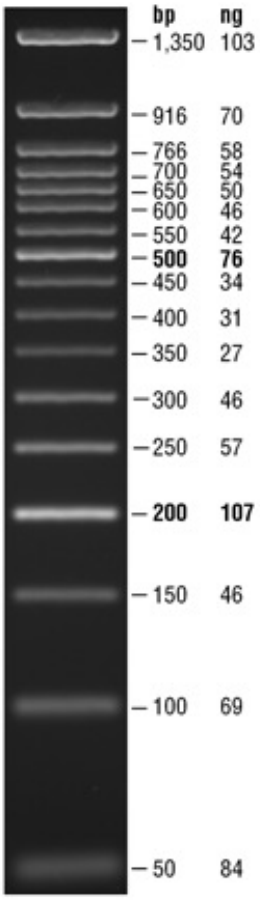


Figure 4: 50 bp DNA Ladder visualized by ethidium bromide staining on a 1.8% TBE agarose gel. Mass values are for 1 μg/lane.

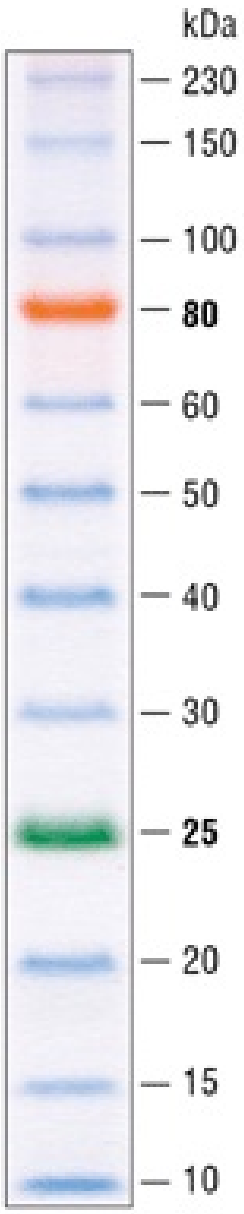


Figure 5: ColorPlus Prestained Protein Ladder, Broad Range (10-230 kDa) is a mixture of highly purified recombinant proteins covalently coupled to a blue dye that resolves into 12 sharp bands when electrophoresed. The protein concentrations are carefully balanced for even intensity. Both the orange 80 kDa and the green 25 kDa bands serve as the reference indicators. The covalent coupling of the dye to the proteins affects their electrophoretic behavior on SDS-PAGE gels relative to unstained proteins (1). The apparent molecular weights of the ColorPlus Prestained Protein Ladder bands were determined on Invitrogen Novex 10Ð20% Tris-glycine SDS-PAGE gels (1,2) by comparison to the Protein Ladder. The ColorPlus Prestained Protein Ladder is ideally suited for use in SDS-PAGE and western blotting applications. It allows continuous monitoring of protein separations during electrophoresis and also provides a quick and easy way to assess blotting efficiency (3).

The ColorPlus Prestained Protein Ladder is designed for use with Tris-glycine gels.