500ng of plasmid is spotted on to 3MM paper within the pencil circle, before putting in a sealable bag.  For plasmid recovery, cut the circle out, add 50 l of TE, vortex and let rehydrate for 5 min. Quick spin, the supernatant liquid can then be used to transform competent bacteria.

Suggest start with 5l for transformation, using cells that are at least 10\*7 in efficiency. 10\*8 would be better.

There are no particular problems in growing this plasmid. Routinely the BL21(DE3) strain is used for cloning and preparation of the plasmid. However, as is commonly known, this strain is compromised in transformation efficiency and will produce a lot fewer colonies than standard cloning strains such as XL1-blues or DH5alpha. If there is a problem in transforming BL21s then try a standard high efficiency cloning strain. Once grown up the plasmid can be retransformed into BL21s.

**EXPRESSION AND PURIFICATION OF BG4 ANTIBODY**

Inoculate in 2 ml 2xTY media+2% glucose+50 mg/ml kanamycin

Grow ON at 200 rpm at 30C

Inoculate 200 ml of cell culture in 100 ml auto-induction media

Grow at 37C at 250 rpm for 6 h

Then ON at 25C at 280 rpm

Spin down cell culture for 30’ at 4C at 4000g

Resuspend pellet in 8 ml TES, leave 10’ on ice

Add 12 ml TES diluted 1:5, leave 15’ on ice

Spin down for 10’ at 8000g at 4C

Rotate for 1 h at RT with Nickel affinity/anti-his tag beads

Purify on column washing twice with cold PDS+100 mM NaCl+10 mM imidazole, pH 8.0

Elute with PBS+250 mM imidazole, pH 8.0

Dialyze ON in PBS in cold room

Dialyze again OD in PBS in cold room

Store at 4C for few weeks.

**BUFFERS**

**TES buffer**

50 mM Tris/HCl pH 8.0

1 mM EDTA pH 8.0

20% sucrose

filter, store at 4C

**Auto-induction Medium**

Supplement basic ZY medium as follows;

2mM MgSO4

0.2 x Metals Mix (a small precipitate forms on addition)

1 x 5052

1 x M

50μg/ml kanamycin

**1000x metals mix (Stock Solution) - add in order written (per 10ml)**

3.6ml distilled MilliQ water

50mM stock FeCl3.6H2O (dissolved in ~0.1M HCL) 5ml of a 0.1M

20mM stock CaCl2 200 μl of a 1M

10mM stock MnCl2.4H2O 100 μl of a 1M

10mM stock ZnSO4.7H2O 100 μl of a 1M

2mM stock CoCl2.6H2O 100μl of a 0.2M

2mM stock CuCl2.2H2O 200μl of a 0.1M

2mM stock NiCl2.6H2O 100μl of a 0.2M

2mM stock Na2MoO4.2H2O 200μl of a 0.1M

2mM stock Na2SeO3.5H2O (ppts, then dissolves rapidly) 200μl of a 0.1M

2mM stock 0.1M H3BO3 200μl of a 0.1M

Autoclave the stock solutions of the individual metals (except the FeCl3 in HCL)

Mix aseptically and store at room temperature

**ZY medium (base medium) (per litre)**

N-Z amine AS (Sigma Aldrich: N4517) 10g

Yeast Extract 5g

Dissolve in H2O, autoclave & store at room temperature

**1M MgSO4 (Stock Solution)**

Dissolve in H2O and autoclave.

Store at room temperature.

**50x 5052 (Stock Solution)**

25% (w/v) glycerol (weigh in beaker)

2.5% (w/v) glucose

10% (w/v) α-lactose

Dissolve in H2O, autoclave & store at room temperature

**50xM (Stock Solution)**

1.25M KH2PO4

2.5M NH4Cl

0.25M Na2SO4

Dissolve in H2O, autoclave and store at room temperature.

**VECTOR MAP**

For details of pSANG10 please see: Martin, C.D., *et al*. (2006). A simple vector system to improve performance and utilisation of recombinant antibodies. BMC Biotechnology *6*, 46.



