Construction of the expression vector RosettaBlue(DE3)pLysS/pUAB055 and purification of PspA/Rx1 AA1..302

The protein is called UAB055 (or for publication … it could be called PspA/Rx1 AA1..302-CLADE 2). This contains AA#1 - ~#302 of PspA protein from strain Rx1 plus a 6x His tag on the C-terminus. By serotype and sequence type this recombinant protein is clade 2 and Family 1 (see papers 2-4 for definitions of what this means).

Construction of vector. An internal gene fragment of the pspA Rx1 gene, encoding PspA/Rx1 AA1..302, was amplified by polymerase chain reaction from the Streptococcus pneumoniae strain Rx1 using the oligonucleotides 5’GCCATGGAAGAATCTCCCGTAGCC3’ (pspA1-F) and 5’CTCGAGTTCTGGGGCTGGAGTTTC3’(pspA6-R). Reactions were carried out for 30 cycles in a total volume of 50 ml in a cocktail containing 3.0 mM MgCl2, 125 mM dNTPs, 50 picomole of each primer, and 2.5 units of Taq DNA Polymerase. The cycle was 94°C, 1 min.; 55°C, 1 min; 72°C, 5 minutes. This amplified gene fragment was initially cloned into pCRII (Invitrogen, Inc.) by a T-tailed method forming plasmid pUAB026.

After digestion of pUAB026 with Ncol and XhoI, the 918 bp pspA gene fragment was then incorporated between Ncol and XhoI sites of a vector (pET20b, Novagen, Inc.) with a strong T7 promoter and translation signals. DNA sequence confirmed that the recombinant plasmid pUAB055 contained the expected 0.9 kb pspA gene fragment inserted between the pelB leader sequence and the His-tag site in vector pET20b. pUAB055 was transformed into the E. coli strain RosettaBlue (DE3)pLysS for protein production. This strain contains a chromosomal copy of the T7 promoter under control of the inducible UV5 promoter. Upon induction with IPTG, a truncated protein containing amino acids #1 to #302 of the wild type mature PspA protein is expressed. The six histidine residues present on the C-terminus of the recombinant protein simplify its purification by nickel chromatography.

Protein Purification To purify the protein overexpressed by construct pUAB055, we are currently using the Novagen system of binding buffers for the affinity nickel chromatography step. Thus, the protein is released in the following buffer: 20 mM Tris-HCl pH 7.9, 500 mM NaCl, 200 mM imidazole. It is being sent to you in that buffer. Screening by Coomassie Blue staining only shows a single band; we estimate it to be above 90% purity.

A picture of the protein as it appears on a Coomassie blue-stained gel is below:
Sequences of the Insert

The protein sequence of the insert is

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EESPVASQSKAEKDYDAAKDKANNAKAVEDAQKLDDAKAAQKPYEDDKKTEEEKAALAEKAAASEEMDKAVA
AVQQAYLAYQQATDKAADDKMLSDEAKKEEAMKREEEAKTKNFTVRAMVPQPLAEKKKSEEAKQKAPLET
KLEEAHKAKEEKATEAQKVDAAEVAPQAIAEALENQVHRLEQELKIDESEDYAKEGFRAPLQSKL
DAAKAKLSKLEELSDKIDELDAEIAKLEDQLKAAEENNNVYDFFKEGLEKTIAKKAELEKTEADLKAKAVN
PEKPAAPETPAPE
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The DNA sequence of the insert is

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GAAGAATCTCCCGTAGCCAGAGCTAAAAAAGGCATAAAATGCTGCAAAAGTGCTGCTAGAAATATGACGAG
GATAGAAATCTCCCGTAGCCAGAGCTAAAAAAGGCATAAAATGCTGCAAAAGTGCTGCTAGAAATATGACGAG
GATAGAAATCTCCCGTAGCCAGAGCTAAAAAAGGCATAAAATGCTGCAAAAGTGCTGCTAGAAATATGACGAG
GATAGAAATCTCCCGTAGCCAGAGCTAAAAAAGGCATAAAATGCTGCAAAAGTGCTGCTAGAAATATGACGAG
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These fall between the *NcoI* sites and *XhoI* sites on the primers. (RE sites cut off in above seqs).

The signal peptide and His tag are coming from the pET20b vector so a few more amino acids at the end should come and I think a Met gets added on the beginning when we ligated into the Nco - if you look at the cleavage site for signal peptide. So the actual recombinant protein will be something like 309 amino acids after processing. 302 above are the native PspA amino acids.

Coating for ELISA method to determine antibody titers to PspA. We have performed an initial test of this preparation of PspA for coating to ELISA plates. Based upon total protein concentrations determined by the Biorad protein assay, we coated Nunc MaxiSorp plates (Nalge Nunc International, Denmark) with 1, 2, 4, and 6 microgram per milliliter of this protein in phosphate buffered saline overnight. Antibody titers were tested with previous ELISA results using a human antibody pool for which human antibody titers to PspA in each of the PspA clades are known. UAB099 from this batch performed comparably with the initial PspA-clade3 protein used to determine titers. The optimum concentration for coating plates in our assay was judged to be 2 ug/mL.
References


On PspA clades and families:

