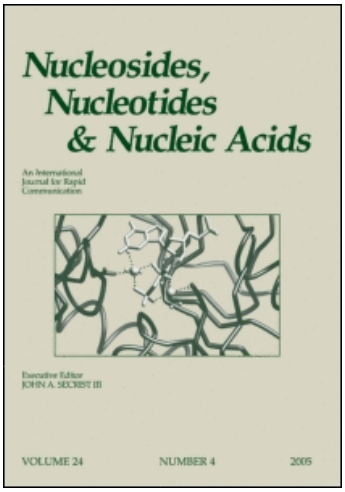

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Chemical Synthesis and Cloning of a Gene Fragment Designed to Aid Polypeptide Purification

L. D. Bell[a]; J. C. Smith[a]; R. B. Derbyshire[a]; E. Cook[a]; L. Dunthorne[a]; J. Viney[a]; S. Brewer[a]; H. Sassenfeld[a]
[a] Searle Research and Development, Buckinghamshire, U.K.

To cite this Article Bell, L. D. , Smith, J. C. , Derbyshire, R. B. , Cook, E. , Dunthorne, L. , Viney, J. , Brewer, S. and Sassenfeld, H.(1985) 'Chemical Synthesis and Cloning of a Gene Fragment Designed to Aid Polypeptide Purification', Nucleosides, Nucleotides and Nucleic Acids, 4: 1, 307
To link to this Article: DOI: 10.1080/07328318508077902
URL: http://dx.doi.org/10.1080/07328318508077902

NUCLEOSIDES & NUCLEOTIDES, 4(1&2), 307 (1985)

CHEMICAL SYNTHESIS AND CLONING OF A GENE FRAGMENT DESIGNED TO AID POLYPEPTIDE PURIFICATION

L.D.Bell*, J.C.Smith, R.B.Derbyshire, E.Cook, L.Dunthorne, J.Viney, S.Brewer, H.Sassenfeld.
Searle Research and Development, PO Box 53, Lane End Road, High Wycombe, Buckinghamshire, U.K.

Summary: The design, synthesis and cloning of a 43 bp DNA duplex coding for polyarginine is described. It has been used to modify the isoelectric point of human urogastrone and thereby facilitate purification by ion-exchange chromatography.

Purification of genetically engineered proteins from a bacterial lysate can be difficult on a large scale. Ion exchange chromatography is particularly suited to large scale purification but is unlikely to resolve polypeptides of similar overall charge. We decided to investigate a general method by which the isoelectric point of any protein could be modified. As a model system we chose urogastrone for which we have previously synthesised a gene (1).

A polyarginine C-terminal tail of 6 residues was chosen since this was shown to have minimal effect on biological activity, it could readily be removed with carboxypeptidase B and significantly increases the pI of urogastrone. The gene sequence was carefully designed to avoid potential problems related to tRNA limitation and secondary structure in the mRNA.

We have shown that cells containing polyarginine fused urogastrone grow at a similar rate to those containing only urogastrone. In addition the polyarginine tail confers an increased resistance to endogenous proteases.

Ion exchange HPLC data have shown the significant advantage of this general method for polypeptide purification.

REFERENCES

(1) Smith et al., Nucleic Acids Research. (1982), 10, 4467-4481.

0732-8311/85/0401-0307$3.50/0