Selective Amplification of DNA Fragments Coding for the G Domain of Factors IF2 and EF-Tu, Two G Proteins from the Myxobacterium *Stigmatella aurantiaca*

L. Bremaud, B. Derijard,¹ and Y. Cenatiempo

Institut de Biologie Moléculaire et d'Ingénierie Génétique, Centre National de la Recherche Scientifique (CNRS) URA1172, Université de Poitiers, 86022 Poitiers Cedex, France

Two DNA fragments of the genome of the myxobacterium *Stigmatella aurantiaca* were selectively amplified by PCR. These fragments encode a segment of the G domain of translational initiation factor IF2 and elongation factor EF-Tu, two GTP-binding proteins. This was made possible by carefully designing the primers for this reaction to avoid the amplification of every G-domain-encoding region of the genome. The sequence of two pairs of primers was deduced from highly conserved regions, namely G₁ and G₃ and/or their vicinal amino acids, within each subfamily (Initiation and elongation factors, respectively) of GTP-binding proteins. On the basis of the expected size, one band was selected in each experiment, cloned into a vector, and sequenced. This showed unambiguously after comparison analysis that they belong to the IF2 and EF-Tu genes, respectively. This strategy seems suitable for the amplification of a segment of any gene coding for a G protein from any origin.

Selective amplification of DNA fragments is desired as the first step in gene cloning experiments, especially when the use of homologous probe is required. This situation occurs when dealing with a family of proteins with members that display strong similarities in their primary structure. In this respect, an interesting example is provided by the widely spread GTP-binding proteins (G proteins).

The members of the G protein superfamily⁽¹⁾ are involved in diverse biological processes such as transmembrane signaling, control of proliferation and differentiation, and initiation and elongation of protein synthesis in prokaryotes and eukaryotes.⁽²⁻⁵⁾ In spite of these different roles, similar molecular mechanisms occur for GTP binding (active form) followed by its hydrolysis into GDP (inactive form). Conserved primary and presumably tertiary protein structures were identified, especially within the G domain bearing the most conserved motifs.^(1,6)

It is now accepted that at least four conserved regions, termed G₁ to G₄, are found throughout all the superfamily, but with an even higher homology within subgroups such as translational initiation factors, elongation factors, small eukaryotic GTPases like Ras proteins, and subunits of signal-transducing G proteins. Consensus sequence motifs have been proposed: GXXXXGKS/T, D-Xn-T, DXXG, and NKXD within G₁, G₂, G₃, and G₄, respectively.⁽¹⁾

We began studying GTP-binding proteins in *Stigmatella aurantiaca*, a myxo-

bacterial species, because under starvation these prokaryotic cells undergo a developmental cycle leading to the formation of particular structures termed fruiting bodies.^(7,8) Multicellular development of myxobacteria is sustained by signal exchanges presumably transduced by G proteins, as in most eukaryotes.^(9,10) Two soluble G proteins, translational initiation IF2 and elongation factor EF-Tu, may also play a role, directly or indirectly, in this developmental process, via the selection and translation of particular mRNAs.

IF2 and EF-Tu are present in different forms in bacteria, and they arise from different processes. In *Escherichia coli*, one gene, *infB*, directs the synthesis of the various forms of IF2, caused by the presence of several translational initiation sites on *infB* mRNA,⁽¹¹⁾ whereas EF-Tu appears as two polypeptides bearing a different amino acid at the carboxyl terminus. The latter are coded by two genes, *tufA* and *tufB*, differing only in 13 positions out of a total of 1185 nucleotides; 8 of the 13 positions are located within the region encoding the G domain.^(12,13)

In this report we show that it is possible to amplify specifically a segment of the genes for IF2 and for EF-Tu. This was performed by designing different pairs of primers, taking into account the most conserved sequences, but also the main differences among subfamilies, within and at the border of the consensus regions G₁ and G₃.

The amplified DNA fragments, which were identified unambiguously by se-

¹Present address: Howard Hughes Medical Institute Research Laboratories, University of Massachusetts Medical Center, II Biotech Park, Worcester, Massachusetts 01605 USA.

quencing, will now be used as homologous probes to clone the corresponding genes of *S. aurantiaca*.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

S. aurantiaca DW4 was obtained from D. White (Indiana University, Bloomington). This strain was grown at 30°C in 1% Bactocastone (Difco), with 8 mM MgSO₄, and harvested at ~4.10⁸ cells/ml. *E. coli* TG1 was grown at 37°C in 2× YT medium.⁽¹⁴⁾

DNA Preparation

Genomic DNA of *S. aurantiaca* DW4 was extracted by the procedure described by Starich and Zissler.⁽¹⁵⁾

Primer Synthesis

Degenerate oligonucleotides used for PCR were produced by the phosphoramidite method as described previously,⁽¹⁶⁾ using a Gene Assembler Synthesizer (Pharmacia LKB). Upstream and downstream primers, respectively, were

appended with *Eco*RI and *Bam*HI restriction sites at their 5' terminus.

PCR Amplification

PCR was performed with a Gene ATAQ Pharmacia Thermocycler. Amplification of the desired segments was performed in a 50-μl reaction mixture containing 1 μg of *S. aurantiaca* genomic DNA; 50 pmoles of each primer; 200 μM each of dATP, dTTP, dCTP, and dGTP; *Taq* DNA polymerase buffer [10 mM Tris-HCl (pH 9), 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100; and 1.25 units of *Taq* DNA polymerase.

Phenol-extracted *S. aurantiaca* genomic DNA was denatured for 5 min at 100°C in the presence of both primers, overlaid with 50 μl of mineral oil (Sigma), and then cooled rapidly on ice. Next, dNTPs, *Taq* DNA polymerase buffer, water, and *Taq* DNA polymerase were added to the mineral oil and dropped by a brief centrifugation.

Amplification with IF2 primers (*infB* G₁ and G_{3a}) was carried out as follows: Each cycle consisted of denaturation at 94°C for 30 sec, annealing at 65°C for 30 sec, and extension at 72°C for 1 min. This cycle was repeated 30 times. Ampli-

fication with Tu primers (*tuf* G₁ and G'_{3a}) was almost identical, except that the annealing temperature was dropped to 56°C.

Twenty microliters of each reaction mixture was loaded onto a 2% agarose gel. After electrophoresis, amplification products were visualized by ethidium bromide staining. PCR products were purified using the freeze-squeeze method.⁽¹⁷⁾ After extraction and ethanol precipitation, amplification products were digested with *Bam*HI (BRL) and *Eco*RI (BRL). DNA fragments were subcloned into M13mp19, digested previously with the same restriction enzymes, and transformed into *E. coli* TG1. The single-stranded template was sequenced by the dideoxy chain termination method,⁽¹⁸⁾ using a fluorescent universal M13 primer and T7 DNA polymerase. Sequencing was performed with an automated sequencer (A.L.F., Pharmacia).

Computer Analysis of DNA Sequences

Computer analysis of PCR fragments was performed using DNAsis software (Hitachi).

RESULTS

Primer Design

The first stage of this work was to design two sets of primers to amplify specifically a segment of the genes *infB* and *tuf* of *S. aurantiaca* coding for two GTP-binding proteins, respectively, IF2 and EF-Tu. As shown in Figure 1A, to obtain an amplification of a G-protein-encoding gene segment, we took advantage of (1) conserved amino acid sequences G₁ and G₃ to design the primers *infB* G₁, *infB* G_{3a}, and *tuf* G₁, which overlap those consensus sequences and upstream amino acids; and (2) a conserved stretch of 8 amino acids, located downstream from G₃, in the Tu subgroup of the G-protein family to design *tuf* G'_{3a}.

We had to take into account the GC content (~70%) of the myxobacterial genome, especially at the third position of codons where it can be as high as 90%.⁽¹⁹⁾ Moreover, the high GC content of the primers could theoretically lead to secondary structures, because of self-annealing, and to primer-dimer formation. This was estimated by using the OLIGOTEST version 2.0 software,⁽²⁰⁾

FIGURE 1 Conserved amino acid sequences in the G domain of IF2 and EF-Tu (A) and nucleotide sequence of primers used in PCR (B). (A) G₁ and G₃ represent two consensus motifs found throughout G proteins; broken lines correspond to IF2 and EF-Tu, respectively. Only those amino acids taken into account to design the primers (*infB* G₁, *infB* G_{3a}, *tuf* G₁, and *tuf* G'_{3a}) are displayed. The size (in bp) of the expected PCR fragments is also indicated. (B) The nucleotide sequence of degenerate primers is described. At degenerated positions, in the absence of any other indication, each base represents 50% of the mixture, whereas underlined or doubly underlined bases represent 66.6% and 83.3%, respectively, of the mixture.

FIGURE 2 Agarose gel analysis of the PCR-amplified products. (Left) The size of relevant markers separated in lane 1 (pBluescript restricted with *Hpa*II); (lanes 2,3) amplification with *infB* G₁ and *infB* G_{3a} from *E. coli* and *S. aurantiaca* genomic DNA, respectively; (lanes 4–6) control experiments with *infB* G₁ alone, *infB* G_{3a} alone, and without DNA, respectively; (lanes 7,8) amplification similar to lanes 2 and 3 but with *tuf* G₁ and *tuf* G_{3a}; (lanes 9,10) controls with *tuf* G₁ alone and *tuf* G_{3a}, respectively. (Right) The positions of the expected bands are indicated by arrows and their theoretical sizes are shown.

which indicated that self complementarity within the primers did not exceed 5 bp but that contiguous and noncontiguous complementarity could occur between mainly *tuf* G₁ and *tuf* G_{3a}, thus leading to primer-dimer formation in PCR. Finally, directional cloning in a sequencing vector was done by adding bases at the 5' terminus of the primers (restriction sites).

Figure 1B shows the sequence of the degenerate primers that were synthesized following the designed specifications. As indicated, only a few positions were degenerated on the basis of codon bias determined from the analysis of 18 sequenced genes of another myxobacterium, *Myxococcus xanthus* (B. Dérjard, unpubl.). This bacterial species was chosen because insufficient data were available to define codon preferences in the genome of *S. aurantiaca*.

Amplification

PCR experiments were performed with the two pairs of primers, *infB* G₁/*infB* G_{3a}, and *tuf* G₁/*tuf* G_{3a}, and included negative and positive controls as described in Figure 2. Positive controls were carried out by using *E. coli* genomic DNA, the IF2 and EF-TU G domains of which were already described.^(2,21,22) The first set of primers, *infB* G₁ and G_{3a}, was used to amplify a segment of *infB*,

the IF2 gene. A major amplified product of the same size was obtained with *E. coli* and *S. aurantiaca* DNA (lanes 2 and 3). Two weaker bands of a smaller size were observed with the latter. The lower of these two bands may correspond to an amplification product arising from *infB* G_{3a} alone (lane 5), whereas the origin of the other one remains unexplained. The expected size of the major band was 178 bp (162 bp plus 16 bp attributable to the appended *Eco*RI and *Bam*HI sites). This length is compatible with its observed size (180–200 bp).

In the case of the second set of primers, a unique band seems to correspond to an amplified segment of the *E. coli* EF-Tu gene (*tufA* or *tufB*, lane 7), whereas the corresponding band amplified from the genome of *S. aurantiaca* appears weaker than a shorter one, presumably a primer-dimer or a nonspecific amplified product (lane 8). However, we did not try to optimize further the PCR, as the upper band migrates at the expected level (~270 bp) and the estimated amount of DNA was sufficient for subsequent cloning.

Identification of PCR Fragments

The putative *infB* and *tuf* segments of *S. aurantiaca* were inserted into M13mp19 before sequencing. A comparison of the DNA sequence of these PCR fragments (excluding the above described appended restriction sites) with their *E. coli* counterpart is presented in Figure 3. The size of the corresponding fragments is absolutely identical in both bacterial species. A 71.5% identity was calculated between *E. coli* and *S. aurantiaca* 162-bp fragments (Fig. 3, bottom), leading to a 81.5% amino acid identity (Fig. 4, bottom). Therefore, one can conclude that the *S. aurantiaca* fragment has been amplified from the *infB* gene. The situation may be more complex with the 255-bp fragment, as two genes for EF-Tu may be present within the genome of *S. aurantiaca*. However, there is little doubt about the nature of this fragment, which seems to belong to a *tuf* gene. When compared with the *E. coli* *tufB* sequence (Fig. 3, top), the percentage of identity reaches 69.4% (76.5% for the deduced protein sequence; Fig. 4, top). Whether the se-

<i>S. aurantiaca</i>	AACATCGGCACGATCGGCCACGTTGACCACGGGAAGACGTCGCTGACGGCCGCCATCACC
<i>E. coli</i>	AACGTCGGTACTATCGGCCACGTTGACCATGGTAAACCAACGCTGACCGCTGCAATCACT
	770 780 790 800 810
<i>S. aurantiaca</i>	AAGGTGCTGGCCAAGACGGGCGGCCACGTTCTCGGCTATGACAGAATCGACAAGGCC
<i>E. coli</i>	ACCGTACTGGCTAAACCTACGGCGGTGCTGCTGCGCATTGACAGATCGATAACGCG
	830 840 850 860 870
<i>S. aurantiaca</i>	CCCGAGGAGCGTGAGCGCGGCATCACCATCTCCACGGCGCAGCTGGAGTACCAGACGAAG
<i>E. coli</i>	CCGGAAGAAAAAGCTGCTGGTATCACCATCAACACTTCTCAGTTGAATACGACACCCCG
	890 900 910 920 930
<i>S. aurantiaca</i>	AACCGCCACTACGCGCACGTTGGACAGTCTCGGCCACGCCGACTACGTGAAGAACATGATT
<i>E. coli</i>	ACCGTCACTACGACACGTTAGACTGCCCGGGGACGCCGACTATGTTAAACATGATC
	950 960 970 980 990
<i>S. aurantiaca</i>	ACCGGCGCGGCGCAG
<i>E. coli</i>	ACCGTGTCTGCGCAG
	1010
<i>S. aurantiaca</i>	GTGGTGACGATCATGGGCCACGTCGACCACGGCAAGACGACCTCTGGACGCCATCCGC
<i>E. coli</i>	GTTGTGACCATCATGGGTACGTTGACCATGGTAAACCTCTGCTGCTGACACTATTCGT
	1330 1340 1350 1360 1370
<i>S. aurantiaca</i>	GCGGCCAAGCTGGGCTCGGGTGAGGCGGGCGGCATCACCAGCACATCGGCGCTACAGC
<i>E. coli</i>	TCAACGAAAGTGGCTCTGGCGAAGCGGGCGGCATTACCCAGCACATTGGTGATACCCAC
	1390 1400 1410 1420 1430
<i>S. aurantiaca</i>	GTCGCCACGGCCCGGGGAGACATCAAGTTCTCGACACCCCC
<i>E. coli</i>	GTTGAAACTGAAACGGCATGATCACCTTCTGGACACCCCC
	1450 1460 1470

FIGURE 3 Nucleotide sequence of the PCR fragments from *S. aurantiaca* and their alignment with *E. coli* *tufB* (top) and *infB* (bottom) regions coding for part of the G domain of EF-Tu and IF2, respectively. The nucleotide sequence of the restriction sites added to the 5' end of the primers is not displayed (see text and Fig. 1). Identical bases are indicated by colons.

FIGURE 4 Amino acid sequence of *S. aurantiaca* gene products deduced from Fig. 3 aligned with a segment of EF-TU (top) and IF2 (bottom). Conserved sequences are boxed.

quenced fragment represents the equivalent of *tufA* or *tufB* gene cannot be assessed from this simple alignment (see Discussion).

DISCUSSION

The goal of this work was to define a simple strategy to obtain a homologous probe to increase the probability of cloning some, but not all, genes coding for G proteins in a myxobacterial species, *S. aurantiaca*. We thought that the choice of primers based on consensus sequences within the G domain but limited to subfamily homologies was critical. Furthermore, we expected the main problem to be the nature of the myxobacterial genome, namely its high GC content. According to the nucleotide sequence of the amplified products, the strategy appears to be successful in the two chosen cases (*infB* and *tuf* segments), in spite of the fact that the amplification was obscured in the second example, probably by primer-dimer formation.

Although these segments represent relatively short portions of the genes, interesting information can already be deduced from their nucleotide sequences. The calculated GC percent in the *infB* sequence, including the primers, reaches 75%, a value higher than that observed in the *tuf* sequence, ~65%. More impressive is this percentage at the third codon

position, 96% (*infB*) and 92% (*tuf*). This establishes that we are dealing with a DNA amplified from myxobacteria and rules out any possibility of amplification from contaminating DNA, especially *E. coli*.

Finally, we compared these new sequences with homologous regions in the genome of *E. coli* and other microorganisms. Four *infB* sequences have already been published, out of which only one is from Gram-negative bacteria (*E. coli*)⁽²¹⁾ and three from gram-positive ones (*Bacillus subtilis*, *Streptococcus thermophilus*, and *Streptococcus faecium*).^(23–25) It is interesting, knowing that myxobacteria are Gram-negative bacteria, that the homology found with the corresponding sequences is the highest with the *E. coli* genome (71.5%). Lower percentages are obtained with the above mentioned Gram-positive bacteria (66.5%, 68%, and 60%, respectively). Such values extended to the overall gene will be important in terms of evolutionary studies.

The presence of two *tuf* genes in *E. coli*^(12,13), or even three in *Streptomyces ramocissimus* (L.P. Woudt, K. Rietveld, M. Verdurmen, J. Van Haarlem, G.P. Van Wezel, E. Vijgenboom, and L. Basch, unpubl.), raises the question of whether such a situation occurs in *S. aurantiaca* and, consequently, which fragment has been amplified in our experiments. We examined the sequence, especially at those positions where differences be-

tween *tuf* genes have been described in *E. coli*, and were not able to come to a conclusion because three types of situations were encountered: At some positions a nucleotide is identical to that present in *tufA* (such as a C at nucleotide 789), or in *tufB* (such as a C at nucleotide 800), or even absent in both genes (such as a G at nucleotide 798). Therefore, the sequence of the entire gene as well as the surrounding region of the *S. aurantiaca* genome is required to solve this problem, because *tufA* is part of a complex operon including a gene (*fusA*) coding for the EF-G, another G protein, whereas *tufB* is preceded by tRNA genes.^(12,13)

Finally, an analysis of the deduced protein sequences shows a very high degree of conservation within their G domains and confirms that a choice of primers designed only from consensus sequences such as G₁ or G₃ could have led to the simultaneous amplification of at least two G-domain-encoding regions. For instance, a long stretch of identical amino acids is found within and after G₁ of IF2 and EF-Tu of *S. aurantiaca*.

Either amplified fragments or oligonucleotides generated from the most diverged regions within the sequenced segments could be used to clone individual genes. We chose the amplified fragments as homologous probes to screen a λgt11 library of *S. aurantiaca* DW4/3.1 genomic DNA (kindly provided by H.U. Schairer, Zentrum für Molekular Biologie, University of Heidelberg, Germany). On the basis of preliminary sequence data, we have identified clones bearing *infB* and *tufB*, respectively (L. Bermaud, B. Dérjard, and Y. Cenatiempo, in prep.).

The strategy proposed here may be extended to other sets of primers and eventually different prokaryotic or eukaryotic organisms. This would allow for the cloning of any gene coding for a member of the very important superfamily of G proteins.

ACKNOWLEDGMENTS

We are grateful to Didier Delourme for his help in the preparation of this manuscript.

NOTE ADDED IN PROOF

The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank, and DDBJ nucleotide sequence data bases under the accession

numbers X75300 and X75301 for *tufB* and *infB*, respectively.

REFERENCES

1. Bourne, H.R., D.A. Sanders, and F. McCormick. 1991. The GTPase superfamily: Conserved structure and molecular mechanism. *Nature* **349**: 117–127.
2. Cenatiempo, Y., F. Deville, J. Dondon, M. Grunberg-Manago, C. Sacerdot, J.W.B. Hershey, H.F. Hansen, H.U. Petersen, B.F.C. Clark, M. Kjeldgaard, T.F.M. La Cour, K.K. Mortensen, and J. Nyborg. 1987. The protein synthesis initiation factor 2 G-domain. Study of a functionally active C-terminal 65-kilodalton fragment of IF2 from *Escherichia coli*. *Biochemistry* **26**: 5070–5076.
3. Pfeuffer, T. and E.J.M. Helmreich. 1988. Structural and functional relationships of guanosine triphosphate binding proteins. *Curr. Topics Cell Regul.* **29**: 129–216.
4. Bourne, H.R., D.A. Sanders, and F. McCormick. 1990. The GTPase superfamily: A conserved switch for diverse cell functions. *Nature* **348**: 125–132.
5. Gibbs, J.B. and M.S. Marshall. 1989. The ras oncogene—An important regulatory element in lower eucaryotic organisms. *Microbiol. Rev.* **53**: 171–185.
6. Dever, T.E., M.J. Glynias, and W.C. Merrick. 1987. GTP binding domain: Three consensus elements with distinct spacing. *Proc. Natl. Acad. Sci.* **84**: 1814–1818.
7. Wireman, J.W. and M. Dworkin. 1975. Morphogenesis and developmental interactions in myxobacteria. *Science* **189**: 516–522.
8. Shimkets, L.J. 1990. Social and developmental biology of the myxobacteria. *Microbiol. Rev.* **54**: 473–501.
9. Dérillard, B., M. Ben Aïssa, B. Lubochinsky, and Y. Cenatiempo. 1989. Evidence for a membrane-associated GTP-binding protein in *Stigmatella aurantiaca*, a prokaryotic cell. *Biochem. Biophys. Res. Commun.* **158**: 562–568.
10. March, P.E. 1992. Membrane-associated GTPases in bacteria. *Mol. Microbiol.* **6**: 1253–1257.
11. Sacerdot, C., G. Vachon, S. Laalami, F. Morel-Deville, Y. Cenatiempo, and M. Grunberg-Manago. 1992. Both forms of translational initiation factor IF2 (α and β) are required for maximal growth of *Escherichia coli*. Evidence for two translational initiation codons for IF2 β . *J. Mol. Biol.* **225**: 67–80.
12. Yokota, T., H. Sugisaki, M. Takanami, and Y. Kaziro. 1980. The nucleotide sequence of the cloned *tufA* gene of *Escherichia coli*. *Gene* **12**: 25–31.
13. An, G. and J.D. Friesen. 1980. The nucleotide sequence of *tufB* and four nearby tRNA structural genes of *Escherichia coli*. *Gene* **12**: 33–39.
14. Davis, L.G., M.D. Dibner, and J.F. Battey, eds. 1986. *Basic methods in molecular biology*. Elsevier, Amsterdam, The Netherlands.
15. Starich, T. and J. Zissler. 1989. Movement of multiple DNA units between *Myxococcus xanthus* cells. *J. Bacteriol.* **171**: 2323–2336.
16. Hartmann, R.K., N. Ulbrich, and V.A. Erdmann. 1987. An unusual rRNA operon constellation: In *Thermus thermophilus* HB8 the 23S/5S rRNA operon is a separate entity from the 16S rRNA operon. *Biochimie* **69**: 1097–1104.
17. Tautz, D. and M. Renz. 1983. An optimized freeze-squeeze method for the recovery of DNA fragments from agarose gels. *Anal. Biochem.* **132**: 14–19.
18. Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain termination inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
19. Mesbah, M., U. Premachandran, and W.B. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39**: 159–167.
20. Beroud, C., C. Antignac, C. Jeanpierre, and C. Junien. 1990. Un programme informatique pour la recherche d'amorces pour l'amplification par PCR. *Médecine/Sciences* **9**: 901–903.
21. Sacerdot, C., P. Dessen, J.W.B. Hershey, J. Plumbridge, and M. Grunberg-Manago. 1984. Sequence of initiation factor IF2 gene: Unusual protein features and homologies with elongation factors. *Proc. Natl. Acad. Sci.* **81**: 7787–7791.
22. Laalami, S., C. Sacerdot, G. Vachon, K. Mortensen, H.U. Sperling-Petersen, Y. Cenatiempo, and M. Grunberg-Manago. 1991. Structural and functional domains of *E. coli* initiation factor IF2. *Biochimie* **73**: 1557–1566.
23. Brombach, M., C.O. Gualerzi, Y. Nakamura, and C.L. Pon. 1986. Molecular cloning and sequence of the *Bacillus stearothermophilus* translational initiation factor IF2 gene. *Mol. Gen. Genet.* **205**: 97–102.
24. Friedrich, K., M. Brombach, and C.L. Pon. 1988. Identification, cloning and sequence of the *Streptococcus faecium infB* (translational initiation factor IF2) gene. *Mol. Gen. Genet.* **214**: 595–600.
25. Shazand, K., J. Tucker, R. Chiang, K. Stansmore, H.U. Sperling-Petersen, M. Grunberg-Manago, J.C. Rabinowitz, and T. Leighton. 1990. Isolation and molecular genetic characterization of the *Bacillus subtilis* gene (*infB*) encoding protein synthesis initiation factor 2. *J. Bacteriol.* **172**: 2675–2687.

Received August 23, 1993; accepted in revised form October 14, 1993.

Selective amplification of DNA fragments coding for the G domain of factors IF2 and EF-Tu, two G proteins from the myxobacterium *Stigmatella aurantiaca*.

L Bremaud, B Derijard and Y Cenatiempo

Genome Res. 1993 3: 195-199

References

This article cites 24 articles, 8 of which can be accessed free at:
<http://genome.cshlp.org/content/3/3/195.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

Focused on your science.

USC
SCIENTIFIC
SIDE BY SCIENCE

To subscribe to *Genome Research* go to:
<https://genome.cshlp.org/subscriptions>