

Primers/ Sequencing of FAK (857-1053) and GIT (622-761) constructs (p. 86)

Invitrogen Custom Primers			
Certificate of Analysis			
Primer: 1 (Order Line No.: 2)		Primer Number:	89419G02
Primer Name:	BACGIT121804	Primer Length:	48
Researcher:	Elizabeth Vogel	Scale of Synthesis:	10 N
Sequence (5' to 3'):	(DNA) - GCC CCC TTT TGC GGC CGC TCA CTG CTT CTT CTC TCG GGT GAT GGT		
Molecular Weight ($\mu\text{g}/\mu\text{mole}$):	14,668.6	μg per OD:	31.68
Millimolar Extinction Coeff.: (OD/ μmole):	463.0	nmoles per OD:	2.16
Purity	Desalt	OD's	3.43
Tm (1M Na ⁺)	113	μg 's	108.80
Tm (50 mM Na ⁺)	91	nmoles	7.4
% GC Content:	63	Notes: 108.8 μg in 217 μL = 0.5 $\mu\text{g}/\mu\text{L}$	
Primer: 2 (Order Line No.: 4)		Primer Number:	89419G03
Primer Name:	BACFAK121804	Primer Length:	48
Researcher:	Elizabeth Vogel	Scale of Synthesis:	10 N
Sequence (5' to 3'):	(DNA) - GCC CCC TTT TGC GGC CGC TTA GTG GGG CCT GGA CTG GCT GAT CAT TTT		
Molecular Weight ($\mu\text{g}/\mu\text{mole}$):	14,741.6	μg per OD:	30.63
Millimolar Extinction Coeff.: (OD/ μmole):	481.3	nmoles per OD:	2.08
Purity	Desalt	OD's	3.22
Tm (1M Na ⁺)	112	μg 's	98.56 in 197 μL
Tm (50 mM Na ⁺)	91	nmoles	6.7
% GC Content:	60	Notes:	

Invitrogen Custom Primers			
Certificate of Analysis			
Primer: 1 (Order Line No.: 1)		Primer Number:	89385E02
Primer Name:	FORGIT121804	Primer Length:	39
Researcher:	Elizabeth Vogel	Scale of Synthesis:	10 N
Sequence (5' to 3'):	(DNA) - GCC GGA ATT CGT GAA GAC TTC CAC CCA GAG CTG GAA AGC		
Molecular Weight ($\mu\text{g}/\mu\text{mole}$):	12,011.8	μg per OD:	27.59
Millimolar Extinction Coeff.: (OD/ μmole):	435.3	nmoles per OD:	2.30
Purity	Desalt	OD's	2.96
Tm (1M Na ⁺)	105	μg 's	81.61
Tm (50 mM Na ⁺)	83	nmoles	6.8
% GC Content:	56	Notes: 163.36 μL	
Primer: 2 (Order Line No.: 3)		Primer Number:	89385E03
Primer Name:	FORFAK121804	Primer Length:	39
Researcher:	Elizabeth Vogel	Scale of Synthesis:	10 N
Sequence (5' to 3'):	(DNA) - GCC GGA ATT CGT GCT GGT AAC CAG CAC ATA TAT CAG CCT		
Molecular Weight ($\mu\text{g}/\mu\text{mole}$):	11,952.8	μg per OD:	28.17
Millimolar Extinction Coeff.: (OD/ μmole):	424.3	nmoles per OD:	2.36
Purity	Desalt	OD's	2.47
Tm (1M Na ⁺)	102	μg 's	69.69
Tm (50 mM Na ⁺)	80	nmoles	5.8
% GC Content:	51	Notes: 141.69 in 139.4 μL	

2 minipreps for each protein (from p. 5-86) were sequenced w/ both 5' and 3' pGEX vector primers (although the constructs were short enough that the 5' could sequence the entire protein). For each submission, the following was added to a 600 μL eppendorf:

- 0.64 μL (3.2 pmol) of 5 pmol/ μL 5' or 3' pGEX primer
- 6 μL (200-500 ng, I hope) of the miniprep
- 5.36 μL of sterile water

(The sequencing results can be opened in Fetch (Mac) or FileZilla (PC) and analyzed w/ DNA assist. host: biopolymers.mit.edu user: Imperiali, password: GLYCO ~ then go to Pub folder \rightarrow Imperiali)

GIT- miniprep A

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N50439_05_VOG_IMP_C01.seq
GAAGACTTCCACCCAGAGCTGGAAAGCCTGGATGGAGACCT
AGATCCTGGGCTTCCCAGCACAGAGGATGTCACTTGAAGACAGAGCAGGTCCACCAAGAACATTGAGGAAGTGTGCGGG
CAGCCCAGGAGTTCAAGCATGACAGCTTCGTGCCCTGCTCAGAGAAGATCCATTGGCTGTGACCGAGATGGCTCCCTC
TTCCCAAAGAGGCCAGCCCTGGAGCCAGTGCAGGCTCACTGCGGCTGCTCAACGCCAGCGCTACCGGCTGCAGAGTGA
GTCCCGAAGACAGTGCCTCCAGAGCCGGCCGCCAGTGGACTTCCAGCTGCTGACTCAGCAGGTGATCCAGTGCCT
ATGACATGCCAAGGCTGCCAAGCAGCTGGTACCATCACCACCCGAGAGAAGAAGCAGTGA

EDFHPELESLDGLDPGLPSTEDVILKTEQVTKNIQELLRAAQEFKHDSFVPCSEKIHLAVTEMASLFPKRPALPEVRRS
LRLLNASAYRLQSECRKTPPEPGAPVDFQLLTQQVIQCAVDIAKAAKQLVTITREKKQ

EDFHPELESLDGLDPGLPSTEDVILKTEQVTKNIQELLRAAQEFKHDSFVPCSEKIHLAVTEMASLFPKRPALPEVRRS
LRLLNASAYRLQSECRKTPPEPGAPVDFQLLTQQVIQCAVDIAKAAKQLVTITREKKQ
    
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622-761
 GIT (857-1053)
 \leftarrow DNA (converted below to prot.)
 \leftarrow desired seq.
 \leftarrow DNA \rightarrow prot- "actual"

Rare codons in GIT:	AGG (Arg)	1	CTA (Leu)	1
	AGA (Arg)	0	CCC (Pro)	4
	ATA (Ile)	0		

The GIT1 construct from miniprep A was transformed into BL-21 (DE3) CodonPlus-RP competent cells as described in ntbk 4-7, but scaled down for 50 μ L of cells. 50 μ L from the transformation were spread onto LB plates w/ 50 μ L SOC (plates w/ carb. and chlor.) and the plates were incubated overnight at 37°C. Tons of colonies grew and 1 was selected and grown up in 8 mL LB/antibiotics overnight.

Sequencing results from miniprep A of FAK (857-1053)

starts at pro-val-gly
rest on DNA: GCT GGT AAC CAG CAC ATA TAT CAG

N504339_01_VOG_IMP_F12.seq

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CCTGTGGGTAACCCAGATCATGCCGCTCCACCAAAGAAGCCCCCTCGCCCTGGAGCCCCCACTTGGGCAGCCTCGCGAG
CCTGAACAGCCCCGTGGACAGCTACAACGAAGGCGTGAAGATCAAGCCACAGGAAATCAGCCCTCCTCCTACGGCCAACC
TGGACCGCTCCAATGACAAAAGTCTATGAGAAATGTAACCGGGCTGGTAAAGCTGTATAGAGATGTCCAGTAAAAATACAG
CCAGCTCCGCCAGAGGAGTACGTGCCCATGGTAAAGGAGGTTGGCTTGGCGCTGAGAACCCTTGCTAGCAACAGTGGATGA
GTCGCTGCCAGTGCTTCTGCAAGCACCCACAGAGAGATTTGAGATGGCCAGAAACTGCTGAACTCTGACCTGGCTGAGC
TCATTAACAAGATGAAGCTGGCCAGCAGTACGTATGACCAGCCTGCAGCAGGAGTACAAGAAGCAAATGCTGACGGCT
GCTCAGCTCTGGCTGTGGATGCCAAGAAGCTGCTGGATGTCATCGATCAAGCCAGACTGAAAAATGATCAGCCAGTCCAG
GCCCACTAAGCGGCCGCTGACTGACTGACGATCTGCTCGCGCTTTCGGTGATGACGGTGAAAAACCTCTGACAC
ATGACGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCCGTGAGGG
    
```

sequence

- ✓ AGNQHIYQ
- ✓ PVGKPDHAAPPKPPRPGAPHLGSLASLNSPVDSY
- ✓ NEGVKIKPQEISPPPTANLDRSNDKVYENVTGLVKAVIEMSSKIQ
- ✓ PAPPEEYVPMVKEVGLALRTLATVDESLPVL PASTHREIE
- ✓ MAQKLLNSDLAELINKMKLAQQYVMTSLQQEYKQMLTA
- ✓ AHALAVDAKNLLDVIDQARLKMISQSRPH

in silico sequencing

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AGNQHIYQ
PVGKPDHAAPPKPPRPGAPHLGSLASLNSPVDSY
NEGVKIKPQEISPPPTANLDRSNDKVYENVTGLVKAVIEMSSKIQ
PAPPEEYVPMVKEVGLALRTLATVDESLPVL PASTHREIE
MAQKLLNSDLAELINKMKLAQQYVMTSLQQEYKQMLTA
AHALAVDAKNLLDVIDQARLKMISQSRPH
    
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Yea! Perfect!

← DNA from sequencing

← reported protein sequence

← converted from DNA sequencing

rare codons in FAK:	AGG (Arg)	1	CTA (Leu)	1
	AGA (Arg)	3	CCC (Pro)	5
	ATA (Ile)	3		

Since it is unclear which set of rare codons will be more problematic for E. coli translation, the FAK construct was transformed into both Codon Plus RP and RIL cells (RIL were not (DE3), RP(DE3) ~ only bic all we had were (DE3) versions, not bic (DE3) was needed.) Plenty of colonies grew up on each plate.

1 colony each from the RP and RIL cells was grown up in 8 mL of LB w/ carbenecillin and chloramphenicol overnight.