Primer Design for ~~synthetic~~ Biosynth. C-terminal segment of PAX_1 HUMAN. (resides 38-557)

Friday, 2/14/03

PAX_HUMAN (see p. 3-54 for amino acid sequence)

NCBI
a isoform
res. 38 → cac

5' atggacgacctcgacgccctgctggcggacttggagtctaccacctcccacatctccaaacggcctgtgttcttgtcgga
ggagacccctactcatacccactggaaacacacatacaggagattgccgtgccacccccgtcccccccacccccgt
ccagcgaggccctcaatggcacaatccttgacccttagaccagtggcagcccagcggctcccgattcatccaccagcag
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tgagcacgtccctgggcagcaacctttctgaactcgaccgcctgctgctggaactgaacgctgtacagcataacccgcca
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c
ggcccagtgtggagagtctcttggatgaactggagagctccgtgcccagccccgtccctgccatcactgtgaaccagggc
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cgaagcccgggagccagctggacagcatgctggggagcctgcagtctgacctgaacaagctgggggtcgccacagtcgcc
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ggcctactgtcgcaaggactacttcgacatgttcgcacccaagtgtggcggctgcgcccgggccatcctggagaactata
tctcagccctcaacacgctgtggcatcctgagtgctttgtgtgccgggaatgcttcacgccattcgtgaacggcagcttc
ttcgagcacgacgggcagccctactgtgaggtgcactaccacgagcggcgcggctcgctgtgttctggctgccagaagcc
catcaccggccgctgcatcaccgccatggccaagaagttccaccccgagcacttcgtctgtgccttctgcctcaagcagc
tcaacaagggcaccttcaaggagcagaacgacaagccttactgtcagaactgcttcctcaagctcttctgctag 3'

- Residues 1-36 (Met → Gly, ATG → GGA) synthesized as the thioester.
- Want an N → C mutation at residue 37
- IEGR sequence (cleaved after the R by Factor Xa) added directly ~~following the~~ preceding the Cys.

- PCR product will be cloned into pCR T7 TOPO TA Expression Vector (to be expressed in E. coli)

for IEGRC ATA GAA GGA AGA TGC (38) HTYQ... (550) NCFLKLFC

Xa cuts (after Arg & Cys)

Cys

aa PAX

(From DNA snider

note: there are some rare codons here - should have chosen more common codons for e. coli

PCR of pieces for ~~Z~~...His...IEGR-Cys (PAX 38→end) Monday 2/17/03

FORWARD PRIMER 10 uM

BVPAXFOR (029473-1) **02/13/2003**

36 mer
5'-ATA GAA GGA AGA TGC CAC ACA TAC CAG
GAG ATT GCC

1 OD = 29.9 µg = 2701 pmol
MW = 11084.33 E260 = 370.2 L/mmol-cm
Tm = 70.1 °C ([Oligo]=250 pM; [Salt]=50 mM)

Yield = 3.6 OD; Purified (RPC) 9723.6 pmol

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BACKWARD PRIMER 20 uM

BVPAXBAC (029473-2) **02/13/2003**

24 mer
5'-CTA GCA GAA GAG CTT GAG GAA GCA

1 OD = 29.9 µg = 4024 pmol
MW = 7435.95 E260 = 248.5 L/mmol-cm
Tm = 56.3 °C ([Oligo]=250 pM; [Salt]=50 mM)

Yield = 4.5 OD; Purified (RPC) 1810.8 pmol

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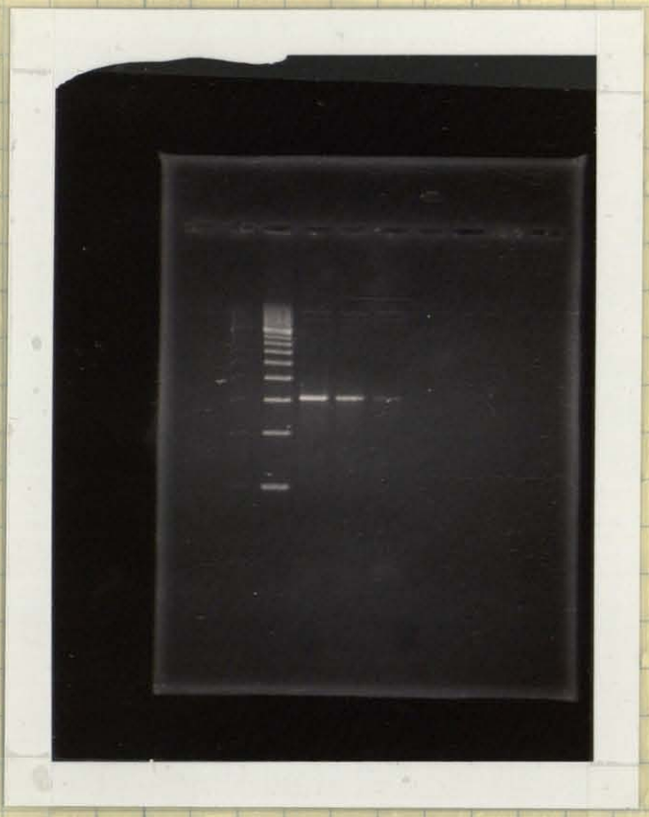
		# 1	# 2	# 3	
1	10× PCR buffer	5 uL	5 uL	5 uL	
2	5 mM dNTPs	5 uL	2 uL	2 uL	
3	50 mM MgCl2	1.5 uL	1.5 uL	1.5 uL	~~(2 uM)~~
4	Forward primer (1 uM)	5 uL	5 uL	10 uL	(2 uM)
5	Back primer (1 uM)	2.5 uL	2.5 uL	5 uL	(2 uM)
6	DNA Template (127.5 ng)	0.5 uL	0.5 uL	0.5 uL	
7	Taq Polymerase * (1 unit/uL)	1 uL	1 uL	1 uL	
8	sterile water (uL)	29.5	32.5	25	
		50 uL	50 uL	50 uL	

* Note: Do not use the platinum Taq - it has proofreaders that will get rid of the A overhangs needed for insertion into the vector. (perhaps just Hi Fi that has proofing)

PCR products



lane 1: 500 bp molecular rular
lane 2: PCR conditions 1
lane 3: PCR conditions 2
lane 4: PCR conditions 3

Yea molecular biology!

1 2 3 4
gel: 1.5% agarose in TAE buffer
500 bp molecular ruler (500, 1000, 1500, 2000, etc.)

TOPO Cloning and Transformation of PAX (Cys. 38 → end) Tuesday 2/18/03

Cloning Reaction (w/ Chemically Competent E. coli)

Fresh PCR Product	2 μL (from PCR #2 p. 3-73)
salt soln. (1.2 M NaC, 0.06 M $MgCl_2$) (from TOPO Kit)	1 μL
sterile water	2 μL (to 5 μL total so far)
TOPO® vector	1 μL

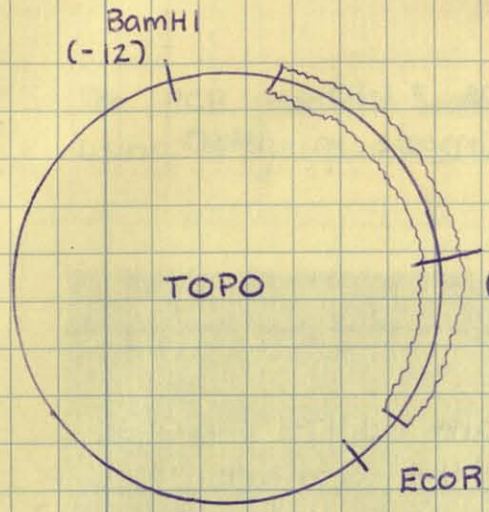
- mixed gently and incubated 5 min. at room temp. (Flicked w/ finger for mixing, then tapped back down.)
- Reaction was placed on ice untill the Transformation.

Transformation Reaction (Following directions for the One shot® TOP10F' Transformation Rxn. p. 12 TOPO instructions)
Following the transformation 2 LB plates were spread, one with 10 μL and one with 50 μL from the transformation. To spead, a speader sterilized by burning EtOH was used on an antibiotic containing LB plate. 50 μL of SOC were added to each plate before spreading. The plates were incubated overnight at 37° C.

Wednesday 2/19/03

- 5 colonies from each plate were selected for analysis. With a small (pink size) pipet tip, the colony was touched. The tip was then released into a clear snap like loose top tube containing 3mL of LB media w/ an antibiotic. The tubes were numbered #1 - #10 and incubated overnight shaking at 37°C.

Mini Prep and Restriction Digest — Thursday, 2/21/03

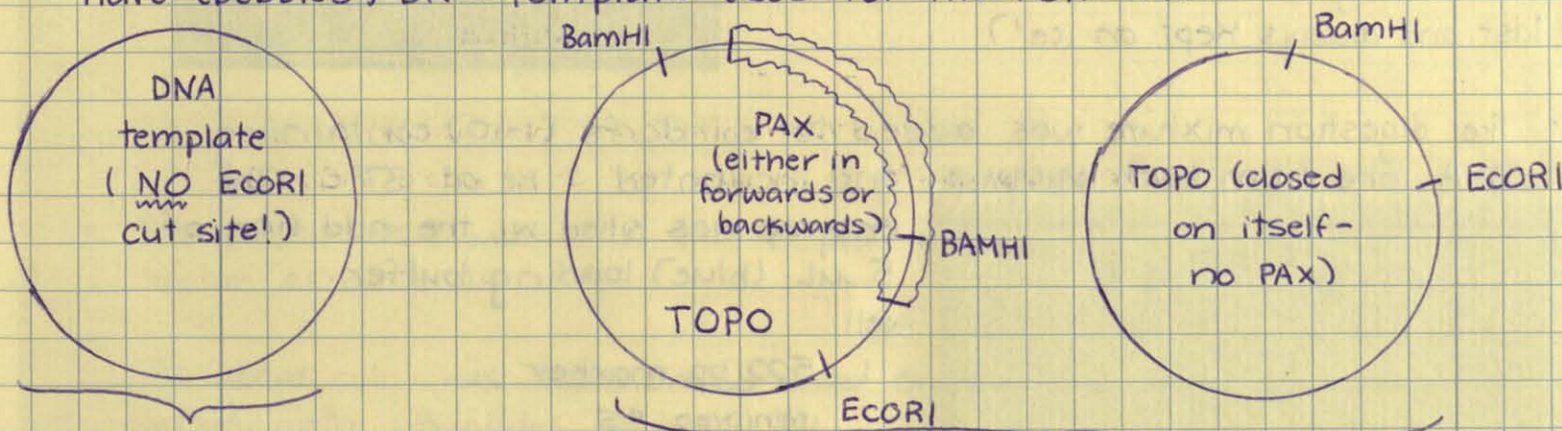


insert: IEGRC-PAX (38-557)
set 0 at ↑ first residue, I.

vector	2,872 bp
PAX insert	1,578 bp
	4,450 bp

- Minipreps on the 10 colonies (#1-10 grown up on the previous page) were performed following the miniprep kit conditions.

- The products (#1-10) of the minipreps were digested with EcoRI. This is an initial screen to determine which colonies have the TOPO vector (either with or wlout the PAX insert) and which colonies have (Debbie's) DNA template used for the PCR rxn.



will NOT linearize in EcoRI digest — WILL linearize in EcoRI digest

- The first digest (EcoRI) is simply to distinguish whether of not the colonies contain the DNA template or the TOPO vector in some form. Once this is determined, the colonies containing the TOPO vector will be digested with BamHI. This will determine whether the vector contains the PAX insert and if that insert was ligated in forwards or backwards.