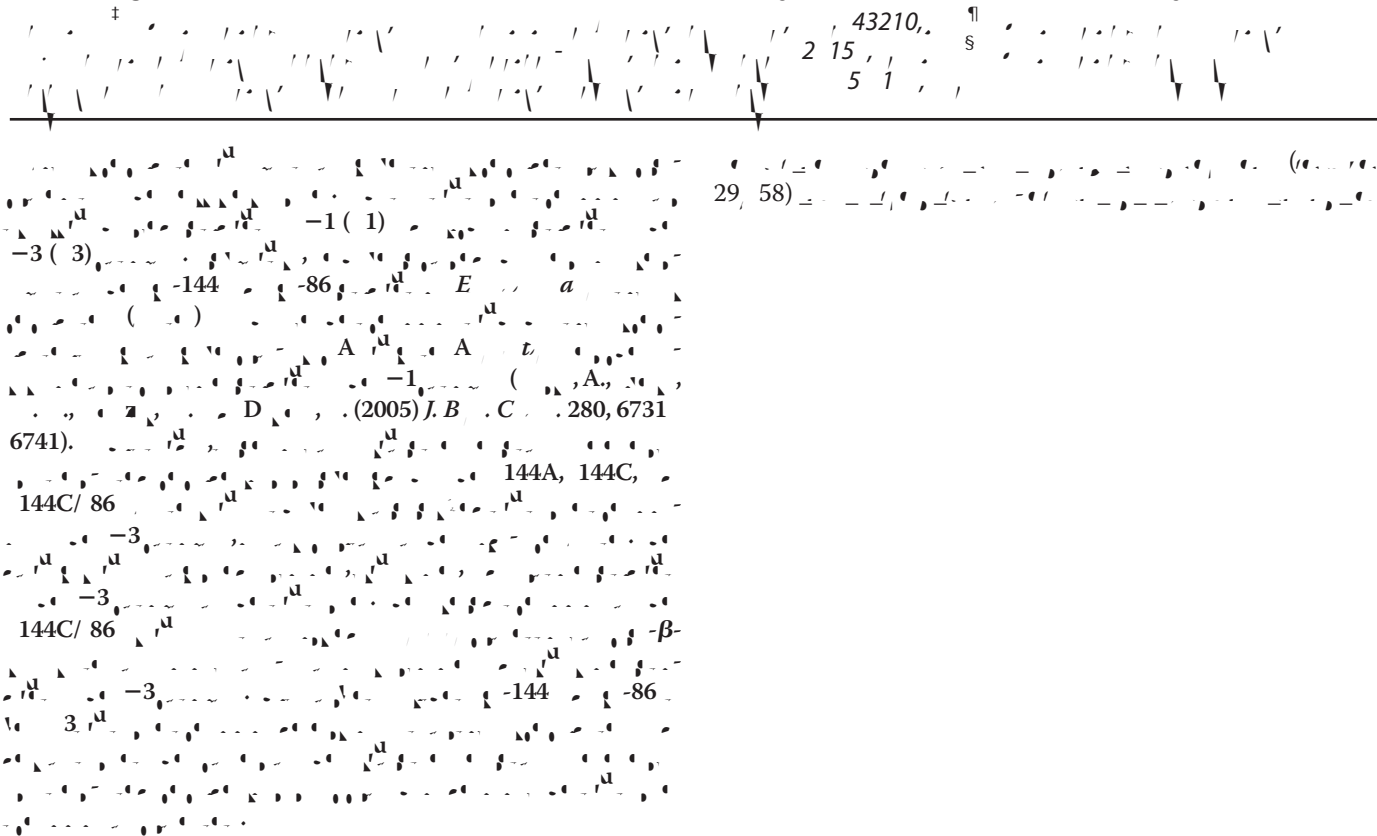# Altered −3 Substrate Specificity of *Escherichia coli* Signal Peptidase 1 Mutants as Revealed by Screening a Combinatorial Peptide Library*

Özlem Doğan Ekici, Andrew Karla, Mark Paetzel, Mark O. Lively, Dehua Pei, and Ross E. Dalbey[1]

... 43210, ...
2 15 ...
5 1 ...

... ...
... −1 (1)
... −3 (3) ...
... -144 ... -86 ... *E* ... *a*
... ( ) ...
... A ... A ...
... −1 ... (, A., ...
... D ... (2005) *J. B. C.* 280, 6731
6741). ...
... 144A, 144C,
144C/86 ...
... −3 ...
... −3
144C/86 ... -β-
... −3 ... -144 ... -86
3 ...
... ...

... (29, 58) ...

Pr... ... ...
... S ... (1). ... T...
(2). ... D... ...
... ...

I *Escherichia coli*, ... (SP ... I)[2] ...
... 37 D (3). T ...

R [...] SP [...] A‑*X*‑A [...] I [...] SP [...] S1 [...] S3 [...] SP [...] (10, 20).

I [...] I‑144 [...] I‑144/I‑86 [...] (FRET) [...] T [...] −3 [...] P3 [...] I [...] SP [...] I144C/I86T [...] ‑β‑ [...] −3 [...] T [...] I‑144 [...] I‑86 [...] −3 [...]

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids — T [...] *E. coli* [...] DH5α [...] *E. coli* [...] SP [...] I [...], IT41, [...] D [...] N [...] (21). T [...] RD8, [...] SP [...] I [...] ING [...] UC19 [...] T [...] G 119HE [...] D [...] A [...] K [...].

Construction of Plasmids — T [...] SP [...] β‑ [...] T [...] F [...] SP [...] ET23 [...] (19) [...]

⊠(D [...] T [...] ‑326(262 [...] (192 [...] T [...])‑186([...])‑186([...])‑189 26(156⊠6205⊠T ⊠7(T23)‑262([...])‑1‑18 [...] 127.2945 [...])‑1‑18 [...]

α- -4- (10

500 μ 0.1% 500 μ CH$_3$CN).

T MALDI-TOF 1 μ

Peptide Sequencing

(F . 2*B*). T

MALDI-MS

I PED/MS . T NH$_2$

(F . 1*B*). A N-

SP . T (B)
(*a*, F . 1*B*). T F
E
(F . 1*B*,).T
N-
TOF NH$_2$-

(... = 28,908 D_). T... ...(232.4 D_) ... H. -P/. (234.3 D_), ... NH$_2$- ... H.-P/. ... β- ... A ... −3 ... −1 ... P/. ... −1 ... M13 ... (39).

The SPase I144C/I86T Mutant Can Cleave Pre-β-lactamase Mutants with Non-canonical −3 Residues B ... +2 ... β- ... P/. -2 ... A ... F . 3A, ... SP_ ... −3 A/ P2F β- ... I ... I144C/I86T SP_ ... −3 A/ P2F β- ... S ... I144C/I86T SP_ ... −3 G. β- ... (F . 3B), ... −3 T/ β- ... (F . 3C). T ... SP_ ... −3 G. β- ... (F . 3B). I _

Ile-101 -132. T −3 T

DISCUSSION

I Ile-86 Ile-144. *E. coli* SP −3 U FRET Ile-144

PED/MS

20 30 1 $1/

C

( )