



























![](_page_4_Figure_1.jpeg)

![](_page_4_Figure_2.jpeg)

![](_page_5_Figure_0.jpeg)

![](_page_5_Figure_1.jpeg)

![](_page_5_Figure_2.jpeg)

![](_page_5_Figure_3.jpeg)

![](_page_6_Figure_0.jpeg)

![](_page_6_Figure_1.jpeg)

![](_page_6_Figure_2.jpeg)

![](_page_6_Figure_3.jpeg)

![](_page_7_Figure_0.jpeg)

![](_page_7_Figure_1.jpeg)

![](_page_7_Figure_2.jpeg)

![](_page_8_Figure_0.jpeg)

![](_page_8_Figure_1.jpeg)

Spotting assay	Kinetic parameters for E1->E2 ubiquitin transfer													
Carbenicillin (µg/mL)	-	50												
c9 ChE1 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	El	E2	K <sub>cat</sub> (S <sup>-1</sup> )	<i>К</i> <sub>м</sub> (µМ)	$K_{cat}/K_{M}$ (M <sup>-1</sup> S <sup>-1</sup> )	Rel. K <sub>cat</sub> /K <sub>M</sub>								
3A   v0	ChE1 v4.5	Ubc9	$0.15 \pm 0.02$	$0.42 \pm 0.12$	$3.6 \pm 0.8 \times 10^{5}$	410								
V0	ChE1 v0.2	Ubc9	$2.0 \pm 0.1 \times 10^{-3}$	$2.4 \pm 0.5$	$8.8 \pm 2.2 \times 10^{2}$	1								
v1.2 v2.8 00000000	Uba1	Ube2G1	4.7 ± 1.2	$3.9 \pm 0.6$	$1.2 \pm 0.2 \times 10^{6}$	1400								
v3.3 v4.5	Uba1	E2-25K	1.4 ± 0.2	9.6 ± 4.9	$1.6 \pm 0.5 \times 10^{5}$	180								
In vitro ubiquitylation														
NH <sub>2</sub>	Ubc9	O Ub	(%) 407-	$100 - t_{12} = 50 - t_{12}$	+ Cr + Cr + Cr + Cr 15 min + Ub	nE1 v4.5 nE1 v0.2 pa1								
Ubiguitin GFP	Ubc9 30 uM FP , 37 °C		5	0	2 4 Time (h)									

		566	579	610	623	628	634	636	637	640	647	661	672	680	681	688	689	699	700	726	742	748	754	755	759	769	781	784	786	805	813	815 8	823 8	24 84	6 855	856	8
	<u>v0</u>	N	s	D	V	Т	Е	Т	L	Α	V	Q	H	N	Ν	N	F	Α	Р	М	Q	Α	۷	Q	F	۷	V	S	L	Y	D	s	V.	<u> </u>	<u>A</u>	T	+
Round 1	v1.1	_										L	L	S		Y	1							_					_	_	_	_	_	V	V	S	Ļ
epPCR	v1.2	-					V	Α																_	V	A			_	_	_	- 1	E	_	_	-	J
	v1.3	-	N	_		_	_	_		_	_		_			<u>Y</u>			_		_			-	_	_	_	_	_	_	_	-	_	_	_		+
Round 2	v2.3	-	N		Α					T						Y								L	-				_	_	-	-	-		-	-	ł
еррск	V2.9	•	N	_		-			Ρ							Y	÷.							-	-	A	•			-	E	- 1	E		-	-	ł
	V2.10	S	N	_												Y								-	-		A		н	-	-	-	-			-	ł
Davia d 0	V2.11	-	N		-	_		_		Ŧ						- <u>¥</u>	÷	_							-	A	_	~	-	-	-		E				-
nound 3	v3.1	-	N		A								-			v	÷	G	-	v			A	-	-	۸		C	-	-	-	- 1			,	-	ł
epron	v3.2	-	N	E												v	÷				-	c		-	-	~			-	-	-	- 1				-	f
	v3.3	-	N	-												v	÷					3		-	-	۸			-	-	E	- 1	E		-	-	h
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Shuffling	v4.2	-		-						Ť			1			v	÷			-		S		-	-	~			-	- 1		- 1	F	V	1		f
+NNK	v4.3	-	Ν	Е						T			L			Ŷ	i.					S				Α				С		- 1	-	v	1		1
	v4.4	-	Α							т						w	L					S				Α				-	Е		E		-	-	f
	v4.5									Т						Y	1					S			1					1	Е	1	Е				1
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![](_page_9_Figure_0.jpeg)

![](_page_9_Figure_1.jpeg)

![](_page_9_Figure_2.jpeg)

![](_page_9_Figure_3.jpeg)

![](_page_10_Figure_0.jpeg)

![](_page_10_Picture_1.jpeg)