

***Cryptococcus neoformans* Prp8 Intein: An In Vivo Target-Based Drug Screening System in *Saccharomyces cerevisiae* to Identify Protein Splicing Inhibitors and Explore Its Dynamics**

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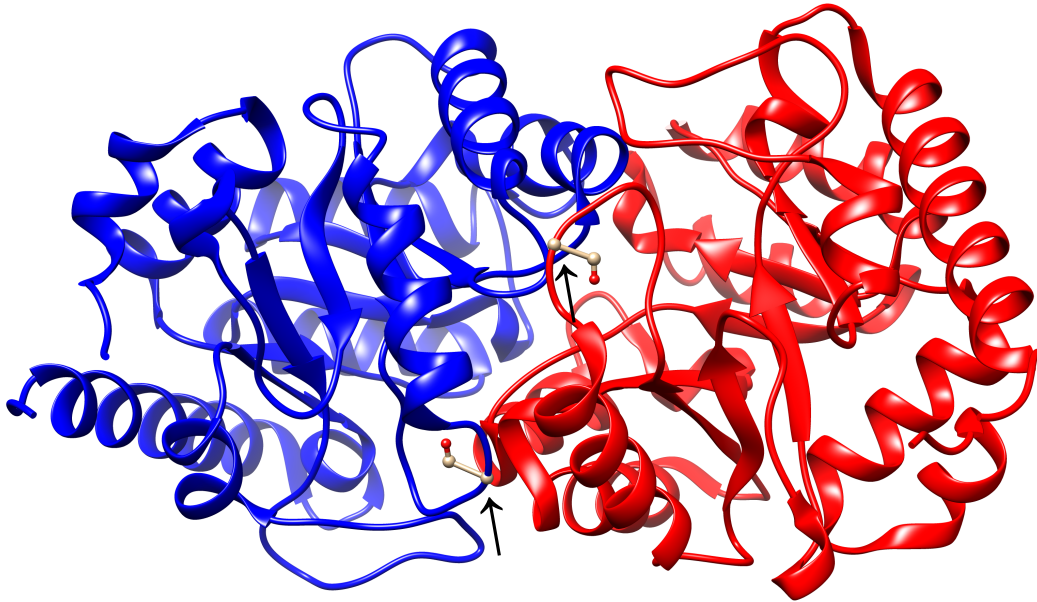
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Figure S1 – 3D structure of the *S. cerevisiae* Ura3 dimer and CnePrp8i insertion site.



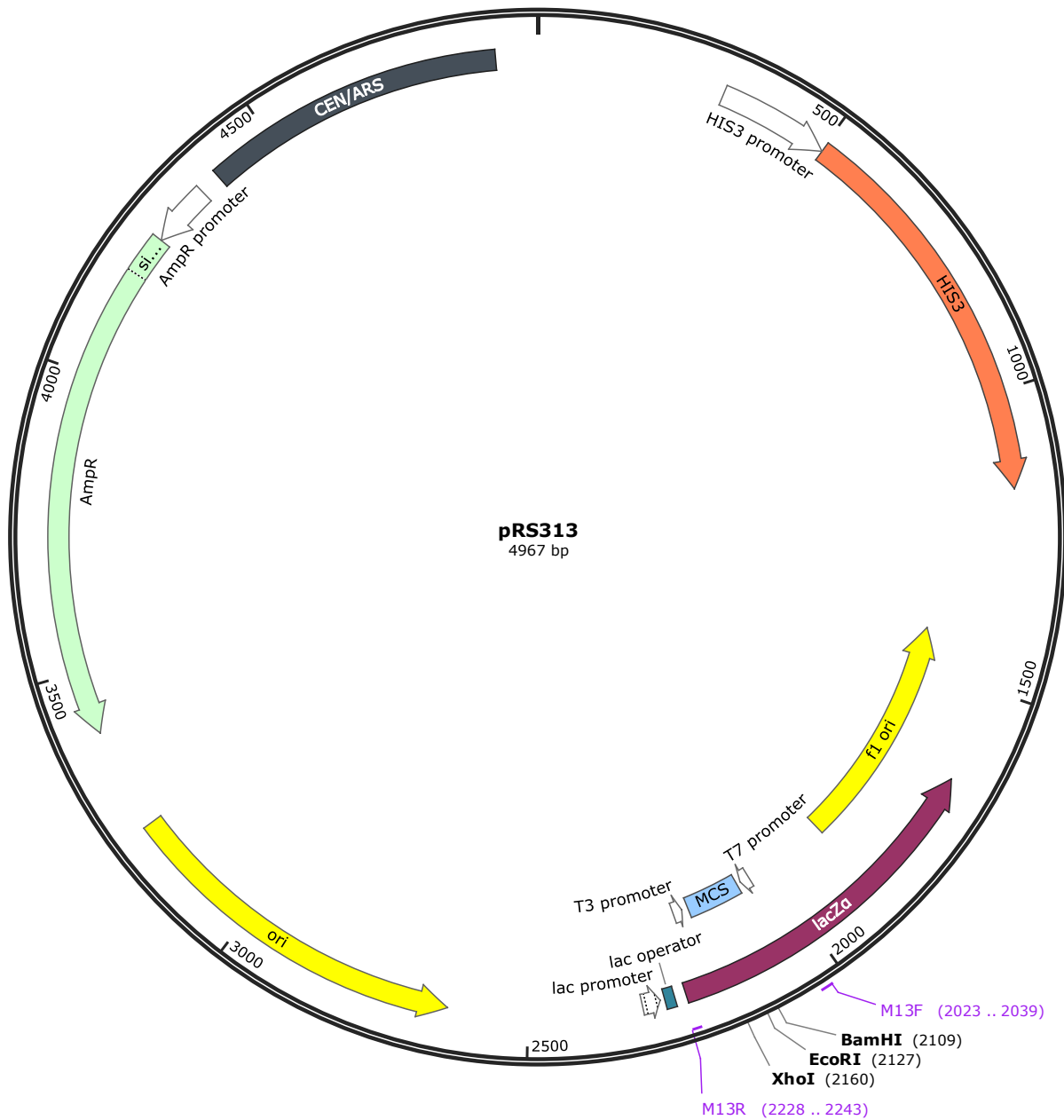
CnePrp8i insertion site (black arrows) and Serine 158 have been highlighted. Based on X-ray diffraction crystal structure from PDB 1DQX – Orotidine 5'-phosphate Decarboxylase complexed to 6-hydroxyuridine 5'-phosphate (BMP) at 2.40 Å resolution. For the purpose of this figure, BMP molecules have been removed.

Table S1 – Primers used in this work

#	Primer Name	Sequence 5' → 3'	Modification	T _a (°C) *	Use
Set 1	M13F	GTAAACGACGGCCAGT	-	55	Insert cloning amplification using the plasmid backbone
	M13R	AACAGCTATGACCATG	-		
Set 2	pCnePrp8-Intein_F	TGTCTGCAGAATGGTACTCG	-	55	CnePRP8i amplification, used for RT-PCR
	pCnePrp8-Intein_R	CAATACCAAATAGTCATGACG	-		
Set 3	P35	CGCGGATCCGCTTTTCAATTCAATTCATC	-	59	Insert amplification using the URA3 promoter and URA3 terminal site
	P102	CGCAAGCTTTTAGTTTTGCTGGCCGCATCTTC	-		
Set 4	p3s	ATGTCGAAAGCTACATATAAGG	-	62	Insert amplification using the URA3 initial and terminal sites, used for RT-PCR.
	p102s	TTAGTTTTGCTGGCCGCATCTTC	-		
Set 5	ppRCT2(C→A)_1F	GCTCTGCAGAATGGTACTCG	5' phosphorylated	65	pRInt whole plasmid amplification, used for site-directed mutagenesis
	ppRCT2(C→A)_1R	GGCCTTGCATGACAATTCTG	5' phosphorylated		
Set 6	ppRCT2(N→A)_2F	TCCGGCCTATCTACTGGAGA	5' phosphorylated	65	pRInt whole plasmid amplification, used for site-directed mutagenesis
	ppRCT2(N→A)_2R	AGCGTGCAATACCAAATAGTCA	5' phosphorylated		
Set 7	Mut1F	AGAATTGTCATGCAAGGCCGC	-	57	Identification of site-directed mutagenesis, used the screen ligated plasmids
	Mut2R	CCAGTAGATAGGCCGGAAGC	-		

* Annealing Temperature.

Figure S2 – pRS313 Plasmid Map and Sequence



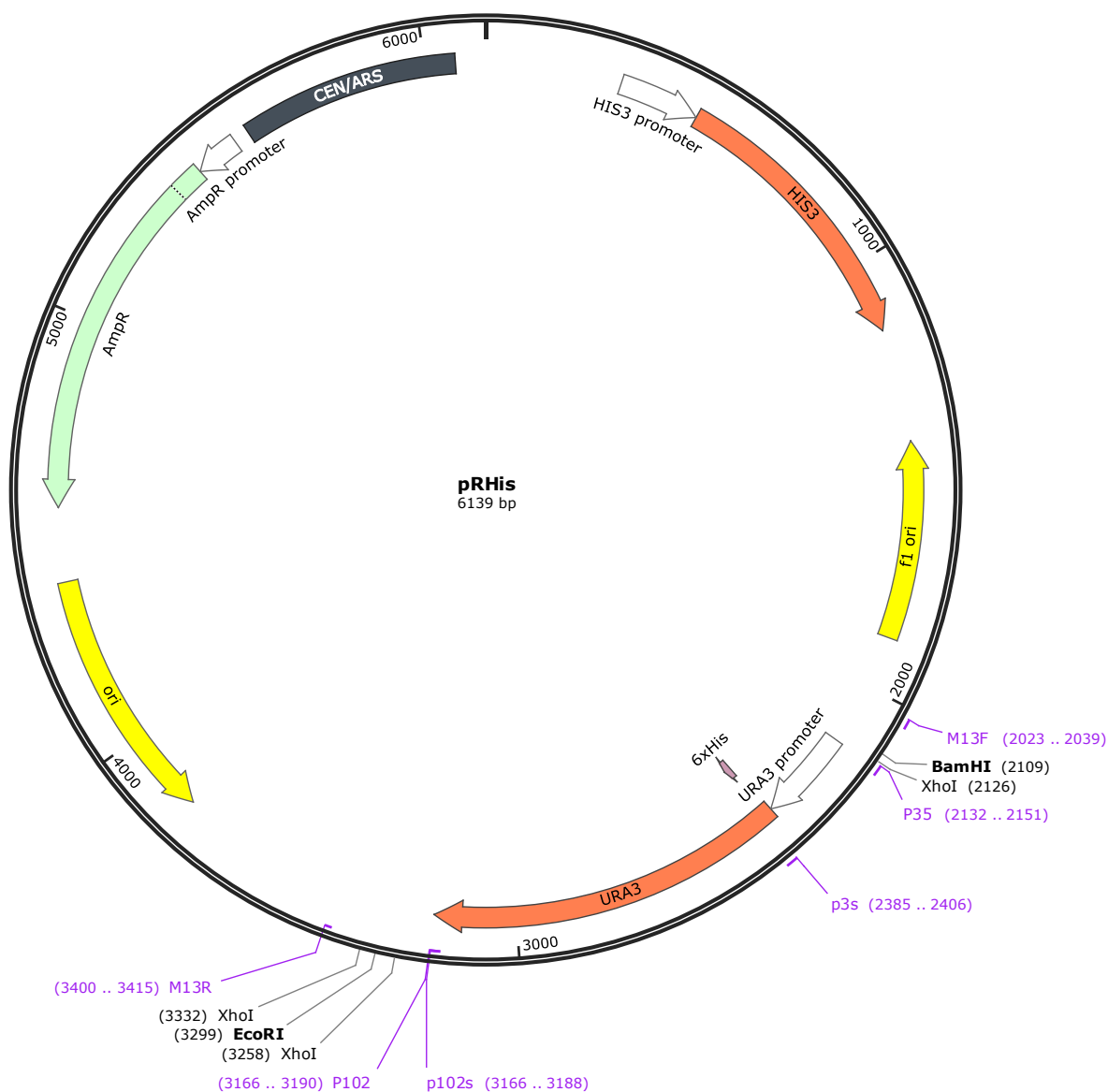
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Figure S3 – pRHis Plasmid Map and Sequence



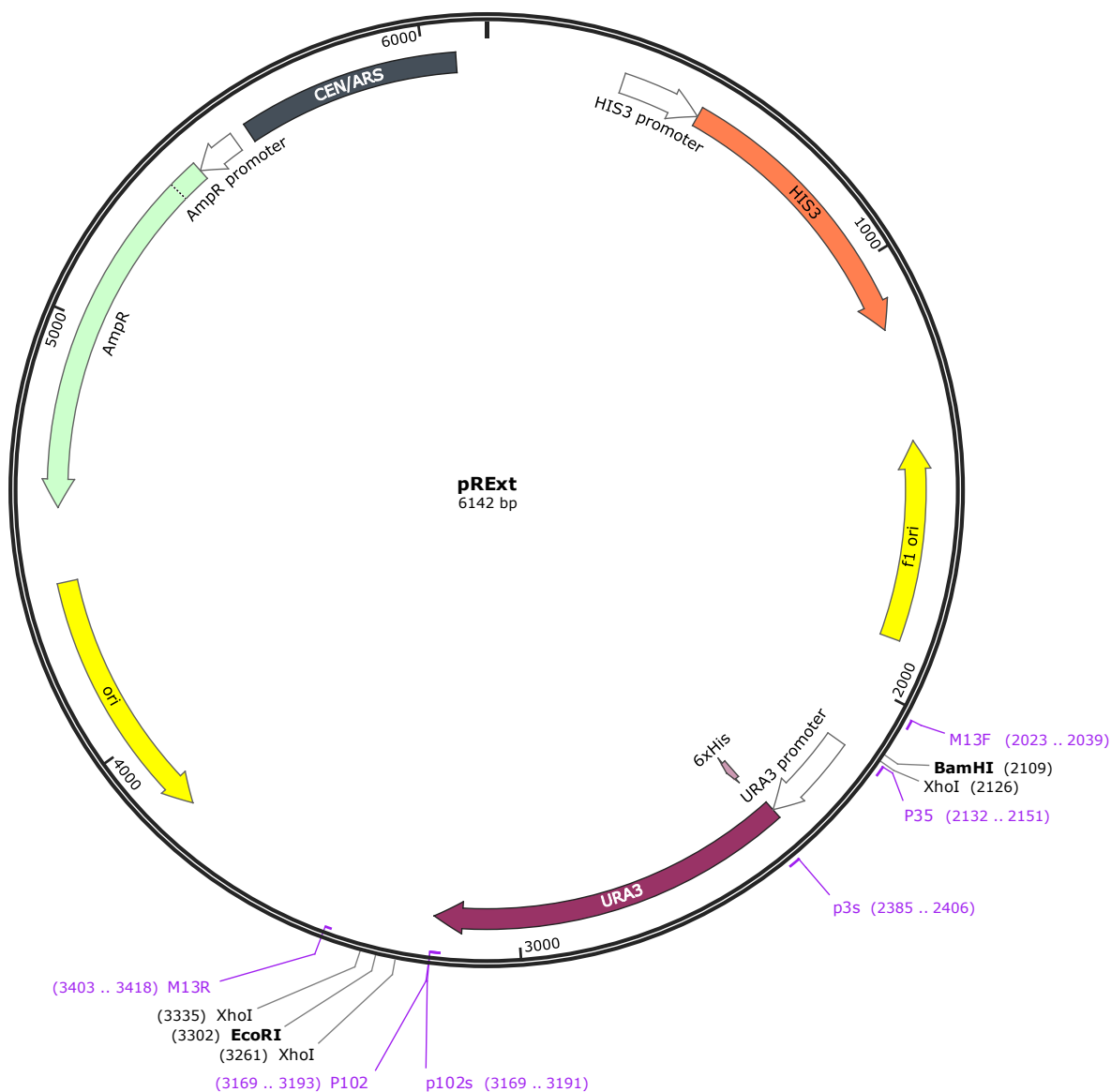
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Figure S4 – pRExt Plasmid Map and Sequence



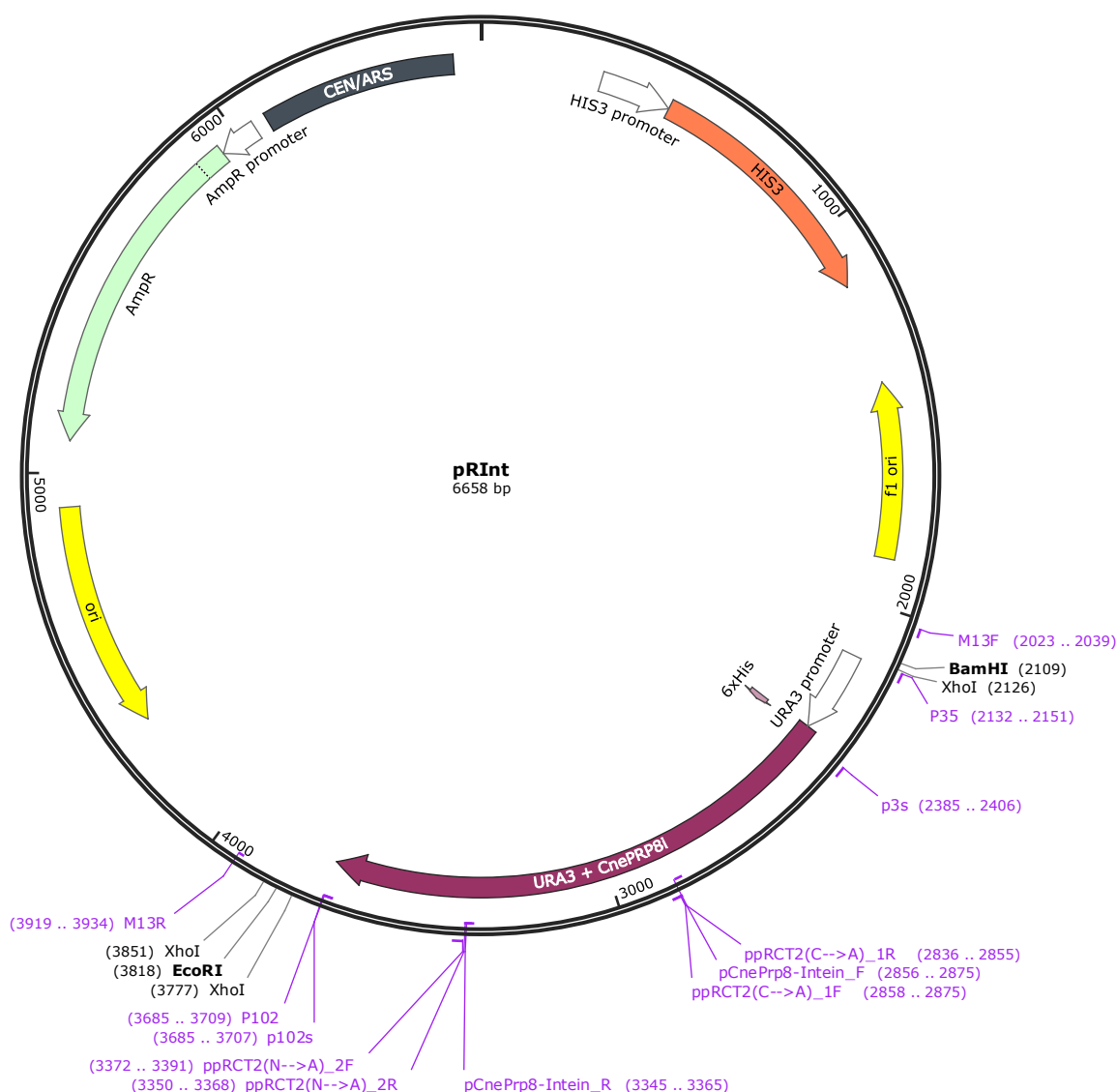
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Figure S5 – pRInt Plasmid Map and Sequence



>pRInt (6658 bp)

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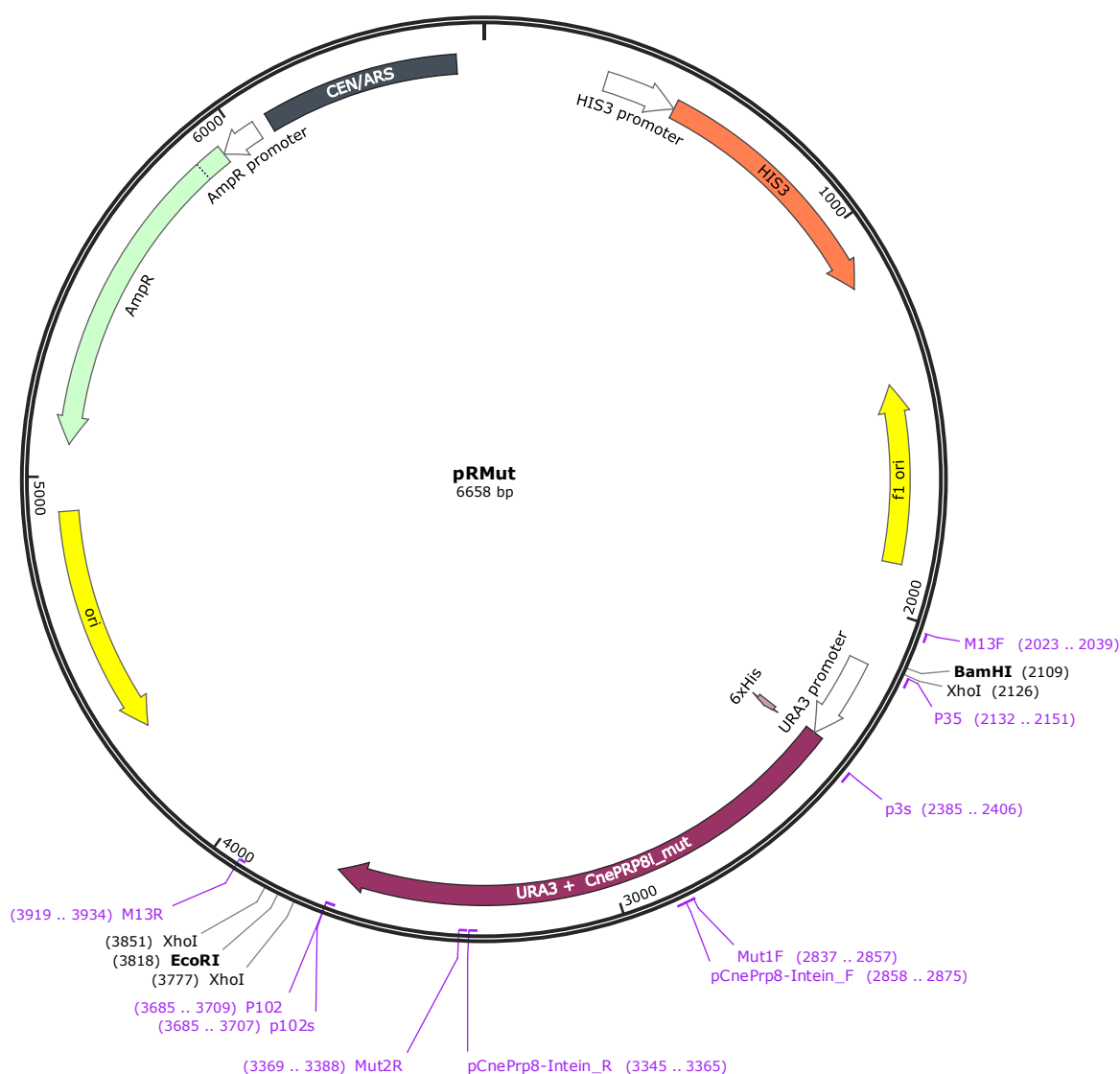
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Figure S6 – pRMut Plasmid Map and Sequence



>pRMut (6658 bp)

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TACGCATCAATTCTATCGAGCTTGAGGACGAGCCTACGAAGTGGTCCGTTTTTGTGGTTGACAA
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TCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGAAGGGATGCTAAGGTAGAGGGTG
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tc

Figure S7 – Alignment of the Proteins generated by this work, highlighting its modifications

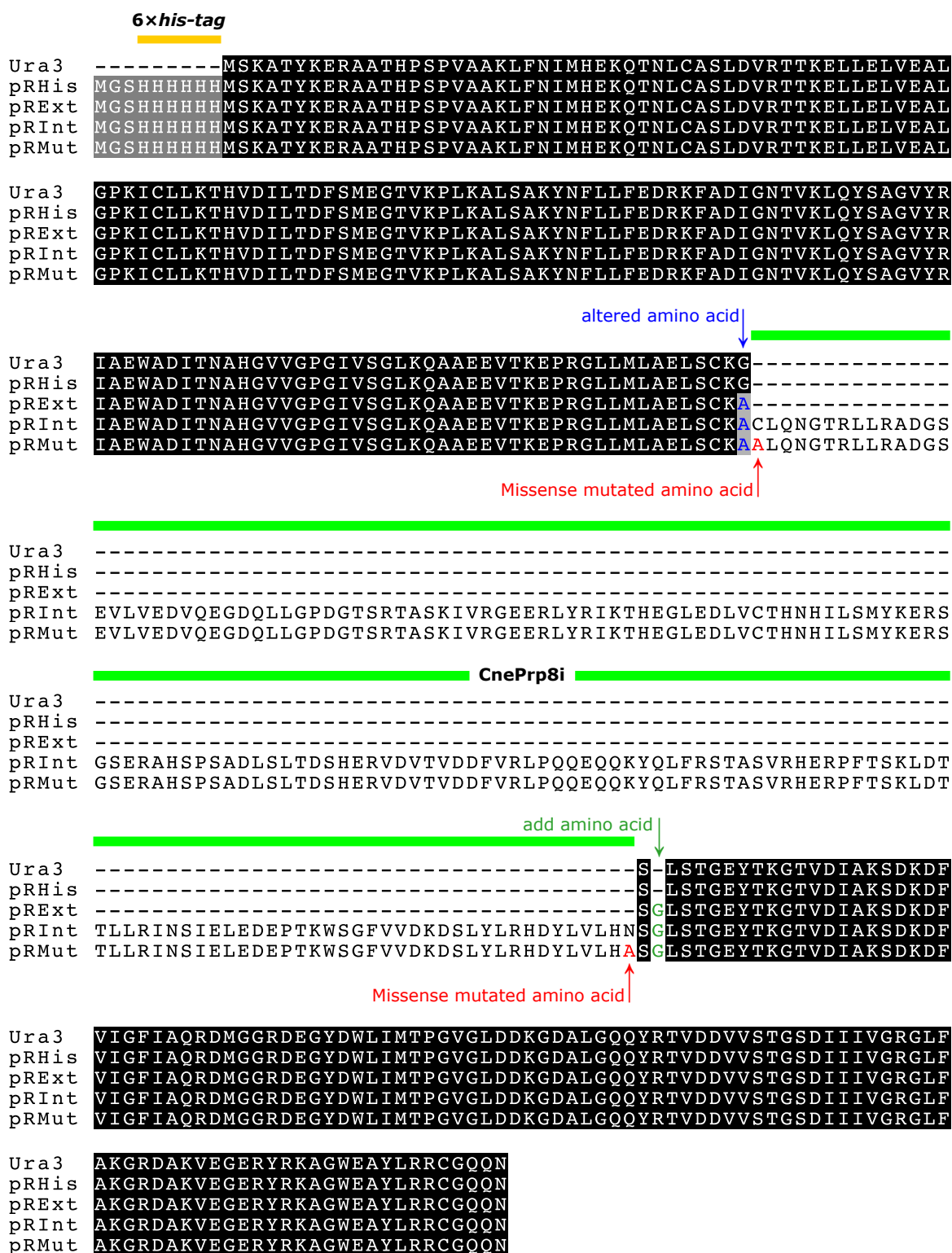


Figure S8 – Protein sequences generated by this work, highlighting its modifications

>Ura3

MSKATYKERAATHPSPVAAKLFNIMHEKQTNLCASLDVRTTKELLELVEALGPKICLLKTHVDI
LTDFSMEGTVKPLKALS AKYNFLLFEDRK FADIGNTVKLQYSAGVYRIA EWADITNAHGVVGP
IVSGLKQAAEEVTKEPRG LLM LAELSCKGSLSTGEYTKGTVDIAKSDKDFVIGFIAQRDMGGRD
EGYDWLIMTPGVGLDDKGDALGQQYRTVDDVVSTGSDIIIVGRGLFAKGRDAKVEGERYRKAGW
EAYLRRCGQQN*

>pRHis

MGS **HHHHHH**MSKATYKERAATHPSPVAAKLFNIMHEKQTNLCASLDVRTTKELLELVEALGPKI
CLLKTHVDILTDFSMEGTVKPLKALS AKYNFLLFEDRK FADIGNTVKLQYSAGVYRIA EWADIT
NAHGVVGP GIVSGLKQAAEEVTKEPRG LLM LAELSCKGSLSTGEYTKGTVDIAKSDKDFVIGFI
AQRDMGGRDEGYDWLIMTPGVGLDDKGDALGQQYRTVDDVVSTGSDIIIVGRGLFAKGRDAKVE
GERYRKAGWEAYLRRCGQQN*

>pRExt

MGS **HHHHHH**MSKATYKERAATHPSPVAAKLFNIMHEKQTNLCASLDVRTTKELLELVEALGPKI
CLLKTHVDILTDFSMEGTVKPLKALS AKYNFLLFEDRK FADIGNTVKLQYSAGVYRIA EWADIT
NAHGVVGP GIVSGLKQAAEEVTKEPRG LLM LAELSCK **A** **Sc** LSTGEYTKGTVDIAKSDKDFVIGF
IAQRDMGGRDEGYDWLIMTPGVGLDDKGDALGQQYRTVDDVVSTGSDIIIVGRGLFAKGRDAKV
EGERYRKAGWEAYLRRCGQQN*

>pRInt

MGS **HHHHHH**MSKATYKERAATHPSPVAAKLFNIMHEKQTNLCASLDVRTTKELLELVEALGPKI
CLLKTHVDILTDFSMEGTVKPLKALS AKYNFLLFEDRK FADIGNTVKLQYSAGVYRIA EWADIT
NAHGVVGP GIVSGLKQAAEEVTKEPRG LLM LAELSCK **A** **CLQNGTRLLRADGSEVLVEDVQEGDQ**
LLGPDGTSRTASKIVRGEERLYRIKTHEGLEDLVCTH NHILSMYKERSG SERAHSPSADLSLTD
SHERVDVTVD D FVRLPQQEQQKYQLFRSTASVRHERPFTSKLDTTLLRINSIELEDEPTKWSGF
VVDKDSL YLRHDYLV LHN **Sc** LSTGEYTKGTVDIAKSDKDFVIGFIAQRDMGGRDEGYDWLIMTP
GVGLDDKGDALGQQYRTVDDVVSTGSDIIIVGRGLFAKGRDAKVEGERYRKAGWEAYLRRCGQQ
N*

>pRMut

MGS **HHHHHH**MSKATYKERAATHPSPVAAKLFNIMHEKQTNLCASLDVRTTKELLELVEALGPKI
CLLKTHVDILTDFSMEGTVKPLKALS AKYNFLLFEDRK FADIGNTVKLQYSAGVYRIA EWADIT
NAHGVVGP GIVSGLKQAAEEVTKEPRG LLM LAELSCK **A** **CLQNGTRLLRADGSEVLVEDVQEGDQ**
LLGPDGTSRTASKIVRGEERLYRIKTHEGLEDLVCTH NHILSMYKERSG SERAHSPSADLSLTD
SHERVDVTVD D FVRLPQQEQQKYQLFRSTASVRHERPFTSKLDTTLLRINSIELEDEPTKWSGF
VVDKDSL YLRHDYLV LHN **Sc** LSTGEYTKGTVDIAKSDKDFVIGFIAQRDMGGRDEGYDWLIMTP
GVGLDDKGDALGQQYRTVDDVVSTGSDIIIVGRGLFAKGRDAKVEGERYRKAGWEAYLRRCGQQ
N*