

TITLE PrM mutants: strategy for cloning

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Desired mutants:

*note that in M. Schumacher notation, D22 = residue 2
b/c she removed the signal sequenced & added M22.
↳ Add 20 to her numbering system

>PrIM
MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYNNVETDADTNTNSPIYAFKSIADASNQANGEKLGAVLTNNNGAVKIPNSIKPKNMYMK
DQATAAQGLEKYRAERDEINNNIKKLESQKLGWRIKVVAEQAKLSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNGPKAYFNDIAKPVLS
HLNAAWDSATSLNYVDYRSNIDMFAGARVVLWNGQYNINSKDAATAIEFNDITNYLTITQIKSLQGSDDTTKAVSLYADANTLTYYTITIGDLSVQK
KIASPRTALSICEASLISIRTNKVTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLKLDGNWALKAAATNAIRSTIGSDSILYVKGHTLML
ISTSSMVDAFIKAIAENEIYQVSDADRVLFQGNACNFTAAKNSNNPIVKAMIAASKQIAGQLPKGQVIDTVFEEKVYLALQSTMFENLSNVLPEKE

>PrIM_mpk1: Mutations in the pocket region: arginine, two tryptophans and one tyrosine to alanine
MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYNNVETDADTNTNSPIYAFKSIADASNQANGEKLGAVLTNNNGAVKIPNSIKPKNMYMK
DQATAAQGLEKYRAERDEINNNIKKLESQKLGWRIKVVAEQAKLSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNGPKAYFNDIAKPVLS
HLNAAWDSATSLNYVDYRSNIDMFAGARVVLWNGQYNINSKDAATAIEFNDITNYLTITQIKSLQGSDDTTKAVSLYADANTLTYYTITIGDLSVQK
KIASPRTALSICEASLISIRTNKVTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLKLDGNWALKAAATNAIRSTIGSDSILYVKGHTLML
ISTSSMVDAFIKAIAENEIYQVSDADRVLFQGNACNFTAAKNSNNPIVKAMIAASKQIAGQLPKGQVIDTVFEEKVYLALQSTMFENLSNVLPEKE

>PrIM_mtip1: Mutations in the tip region: two lysine doublets to glutamic acid doublets
MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYNNVETDADTNTNSPIYAFKSIADASNQANGEKLGAVLTNNNGAVKIPNSIKPKNMYMK
DQATAAQGLEKYRAERDEINNNIKKLESQKLGWRIKVVAEQAKLSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNGPKAYFNDIAKPVLS
HLNAAWDSATSLNYVDYRSNIDMFAGARVVLWNGQYNINSKDAATAIEFNDITNYLTITQIKSLQGSDDTTKAVSLYADANTLTYYTITIGDLSVQK
KIASPRTALSICEASLISIRTNKVTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLKLDGNWALKAAATNAIRSTIGSDSILYVKGHTLML
ISTSSMVDAFIKAIAENEIYQVSDADRVLFQGNACNFTAAKNSNNPIVKAMIAASKQIAGQLPKGQVIDTVFEEKVYLALQSTMFENLSNVLPEKE

>PrIM_mtip2: Mutations in the tip region: entire tip region to polyglycine
MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYNNVETDADTNTNSPIYAFKSIADASNQANGEKLGAVLTNNNGAVKIPNSIKPKNMYMK
DQATAAQGLEKYRAERDEINNNIKKLESQKLGWRIKVVAEQAKLSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNGPKAYFNDIAKPVLS
HLNAAWDSATSLNYVDYRSNIDMFAGARVVLWNGQYNINSKDAATAIEFNDITNYLTITQIKSLQGSDDTTKAVSLYADANTLTYYTITIGDLSVQK
KIASPRTALSICEASLISIRTNKVTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLKLDGNWALKAAATNAIRSTIGSDSILYVKGHTLML
ISTSSMVDAFIKAIAENEIYQVSDADRVLFQGNACNFTAAKNSNNPIVKAMIAASKQIAGQLPKGQVIDTVFEEKVYLALQSTMFENLSNVLPEKE

PrIM vs PrIM_mpk1

PrIM	1	MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYNNVETDADTNTNSPIYAFKSIAD	60
PrIM_mpk1	1	MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYNNVETDADTNTNSPIYAFKSIAD	60
PrIM	61	ASNIQANGEKLGAVLTNNNGAVKIPNSIKPKNMYMKDQATAAQGLEKYRAERDEINNNI	120
PrIM_mpk1	61	ASNIQANGEKLGAVLTNNNGAVKIPNSIKPKNMYMKDQATAAQGLEKYRAERDEINNNI	120
PrIM	121	KKLESQKLGWRIKVVAEQAKLSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNG	180
PrIM_mpk1	121	KKLESQKLGWRIKVVAEQAKLSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNG	180
PrIM	181	PKAYFNDIAKPVLSHLNAAWDSATSLNYVDYRSNIDMFAGARVVLWNGQYNINSKDAATA	240
PrIM_mpk1	181	PKAYFNDIAKPVLSHLNAAWDSATSLNYVDYRSNIDMFAGARVVLWNGQYNINSKDAATA	240
PrIM	241	IEFNDITNYLTITQIKSLQGSDDTTKAVSLYADANTLTYYTITIGDLSVQKIASPRTAL	300
PrIM_mpk1	241	IEFNDITNYLTITQIKSLQGSDDTTKAVSLYADANTLTYYTITIGDLSVQKIASPRTAL	300
PrIM	301	SICEASLISIRTNKVTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLKLDGNW	360
PrIM_mpk1	301	SICEASLISIRTNKVTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLKLDGNW	360
PrIM	361	ALKAATNAIRSTIGSDSILYVKGHTLMLISTSSMVDAFIKAIAENEIYQVSDADRVLFQ	420
PrIM_mpk1	361	ALKAATNAIRSTIGSDSILYVKGHTLMLISTSSMVDAFIKAIAENEIYQVSDADRVLFQ	420
PrIM	421	KNACNFTAAKNSNNPIVKAMIAASKQIAGQLPKGQVIDTVFEEKVYLALQSTMFENLSN	480
PrIM_mpk1	421	KNACNFTAAKNSNNPIVKAMIAASKQIAGQLPKGQVIDTVFEEKVYLALQSTMFENLSN	480
PrIM	481	LPKEE	485
PrIM_mpk1	481	LPKEE	485

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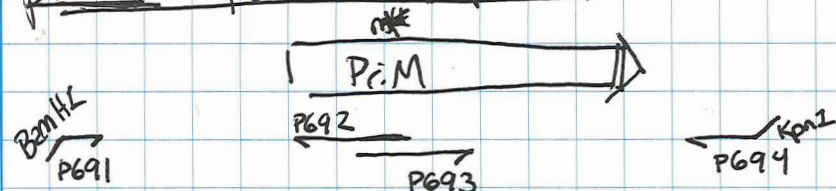
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To make "pocket" mutations (PrM-mpk1), first make a plasmid with the first set of alanine mutations using overlap extension PCR, then make the last codon change for alanine using ~~the~~ overlap extension PCR from the first plasmid to the second.

pKL114 pEX-PrM-mpk1A



amplify from gDNA:

P691 & P692 = 1089

P693 & P694 = 1062

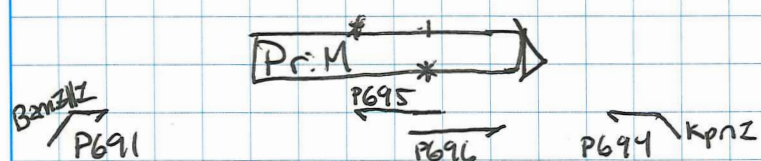
P691 & P694 = 2127

Digest pKL80 w/ BamHI/KpnI & insert fragment

Sequence plasmid using P21, P367, P368, P22

pKL115 pEX-PrM-mpk1B → Made from pKL114

amplify from pKL114 :



P691 & P695 = 1234

P694 & P696 = 922

P691 & P694 = 2127

Digest pKL80 w/ BamHI & KpnI & insert fragment

Sequence plasmid using P21, P367, P368, P22

Check for modification:

*** This modification adds a PvuII site into the PrM gene! ***

Use P697 & P698 = 499bp

Cut with PvuII : WT = 499bp

mpk1 = 280bp & 219bp

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Prim vs Prim_mt1p1

Prim	1	MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYSNVETDADTNTNSPIYAFKSIAD	60
Prim_mtip1	1	MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYSNVETDADTNTNSPIYAFKSIAD	60
Prim	61	ASNIQANGEKLARGFVLTNNGAVKIPNSIKPKNMYMKDQATAAQGLEKYRAERDEINNNI	120
Prim_mtip1	61	ASNIQANGEKLARGFVLTNNGAVKIPNSIKPKNMYMKDQATAAQGLEKYRAERDEINNNI	120
Prim	121	KKLESQKKLGWRIKVVAEQAKLKSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNG	180
Prim_mtip1	121	EELESQEELGWRIKVVAEQAKLKSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNG	180
Prim	181	PKAYFNIDIAKPVLSHLNAAWDSATSLNYVDYRSNIDMFWGARVVLWNGQYNINSKDAATA	240
Prim_mtip1	181	PKAYFNIDIAKPVLSHLNAAWDSATSLNYVDYRSNIDMFWGARVVLWNGQYNINSKDAATA	240
Prim	241	IEFNDITNYLTIQTIKSLQGSDDTTKAVSLYADANTLTYYTVTITIGDLSVQKKIASPRTAL	300
Prim_mtip1	241	IEFNDITNYLTIQTIKSLQGSDDTTKAVSLYADANTLTYYTVTITIGDLSVQKKIASPRTAL	300
Prim	301	SICEASLISIRTNTKVTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLDKLGDNW	360
Prim_mtip1	301	SICEASLISIRTNTKVTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLDKLGDNW	360
Prim	361	ALKAATNAIRSTIGSDSILYVKGHTLMLISTSSMVDAFIKAI AENEIYKQVSDADRVLFG	420
Prim_mtip1	361	ALKAATNAIRSTIGSDSILYVKGHTLMLISTSSMVDAFIKAI AENEIYKQVSDADRVLFG	420
Prim	421	KNACNFTAANKSNNPV KAMIAASKQIAGQLPKGQVIDTVFEEKVYVALQSTMFENLSNV	480
Prim_mtip1	421	KNACNFTAANKSNNPV KAMIAASKQIAGQLPKGQVIDTVFEEKVYVALQSTMFENLSNV	480
Prim	481	LPKEE 485	
Prim_mtip1	481	LPKEE 485	

Prim vs Prim_mt看2

Prim	1	MKKTTLTIALLGTIATTSVYADDLNAKIVNESVTKYSNNVETDADTNTNSPIYAFKSI	60	
Prim	mtip2	1	MKKTTLTIALLGTIATTSVYADDLNAKIVNESVTKYSNNVETDADTNTNSPIYAFKSI	60
Prim	61	ASNIQANGEKLARGFVLTTNNGAVKIPNSIKPKNNMYMKDQATAAQGLEKYRAERDEINNNI	120	
Prim	mtip2	61	ASNIQANGEKLARGFVLTTNNGAVKIPNSIKPKNNMYMKDQATAAQGLEKYRAERDEINNNI	120
Prim	121	KKLESQKKG WRIKVVAEQAKLKSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNG	180	
Prim	mtip2	121	GGGGGGGGGGGGG KVVAEQAKLKSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNG	180
Prim	181	PKAYFNIDIAKPVLSHLNAAWDSATSLNYVDYRSNIDMFWGARVVLWNGQYNINSKDAATA	240	
Prim	mtip2	181	PKAYFNIDIAKPVLSHLNAAWDSATSLNYVDYRSNIDMFWGARVVLWNGQYNINSKDAATA	240
Prim	241	IEFNDITNYLTIQTIKSLQGSDDTTKAVSLYADANTLTYYTVTITGDLSSVQKKIASPTAL	300	
Prim	mtip2	241	IEFNDITNYLTIQTIKSLQGSDDTTKAVSLYADANTLTYYTVTITGDLSSVQKKIASPTAL	300
Prim	301	SICEASLISIRTNKVTARTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLDKLGDNW	360	
Prim	mtip2	301	SICEASLISIRTNKVTARTARNIINRLSNKKAVKPIRLHLLNQTSIDDIILYDLDKLGDNW	360
Prim	361	ALKAATNAIRSTIGSDSILYVKGHTLMLISTSSMVDAFIKAIKAENEIYKQVSDADRVLF	420	
Prim	mtip2	361	ALKAATNAIRSTIGSDSILYVKGHTLMLISTSSMVDAFIKAIKAENEIYKQVSDADRVLF	420
Prim	421	KNACNFTAANKSNPNIVKAMIAASKQIAGQLPKGQVIDTVFEEKVYALQSTMFENLSNV	480	
Prim	mtip2	421	KNACNFTAANKSNPNIVKAMIAASKQIAGQLPKGQVIDTVFEEKVYALQSTMFENLSNV	480
Prim	481	LPKEE 485		
Prim	mtip2	481	LPKEE 485	

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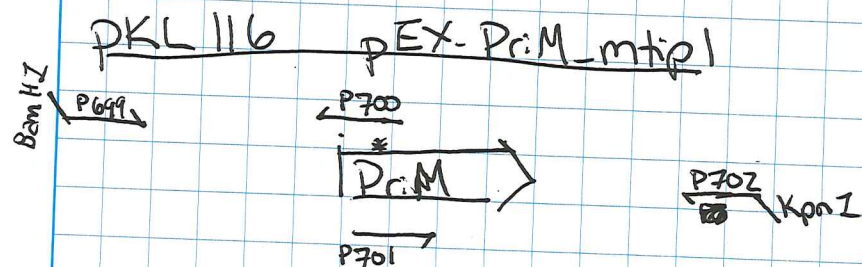
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amplify from gDNA:

P699 & P700 : ~~1010~~ 1010P701 & P702 : ~~1038~~ 1038

P699 & P702 : 2018

- Digest pKL80 w/ BamHI & KpnI, insert fragment

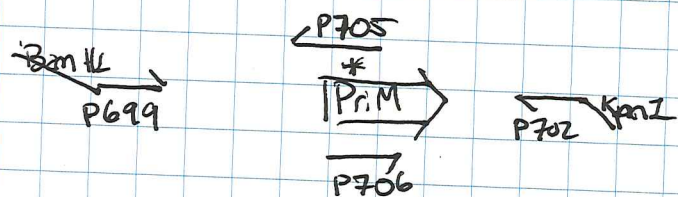
- Sequence plasmid using P21, PRP12, P368, P22

Check for modification:

PCR P703 & P704 = 500bp

Digest with SapI : WT = 500bp
mtp1 = 292 & 208 bp

PKL117 pEX-PrM-mtp2



amplify from gDNA:

P699 & P705 : 1035

P706 & P702 : 1022

P699 & P702 : 2018

- Digest pKL80 w/ BamHI & KpnI, insert fragment

- Sequence plasmid using P21, PRP12, P368, P22

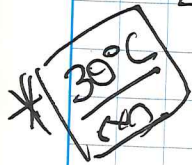
Check for modification:

PCR with P703 & P704 = 500bp

* Digest with GsuI (Fast Digest from ThermoFisher) → isoschizomer of BpmI

WT: 347 & 153 bp

mtp2: 500bp



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FastDigest GsuI information

Thermo
SCIENTIFIC

PRODUCT INFORMATION
Thermo Scientific
FastDigest

GsuI*

#FD0464

20 µL (for 20 rxns)

Lot: _____ Expiry Date: _____

* FastDigest GsuI is a proprietary formulation of GsuI, an isoschizomer of BpmI having the same recognition and cleavage specificity.

5'... C T G G A G (N)₁₆ ↓ ... 3'

3'... G A C C T C (N)₁₄ ↑ ... 5'

New Name
Same Enzyme
Same Performance

Supplied with: 1 mL of 10X FastDigest Buffer
1 mL of 10X FastDigest Green Buffer

Store at -20°C



BSA included

www.thermoscientific.com/onebio

- Use instead of BpmI b/c
- BpmI prefers substrates w/ multiple sites
- Star activity can occur w/ BpmI & extended digestion (less relevant, but noted)

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Also attempt to make a Prim mutant that is not properly folded but may still be produced & secreted. M. Schumacher suggested: Trp199, Phe400, Phe455
 Attempt Trp199 (my numbering Trp 219) first:

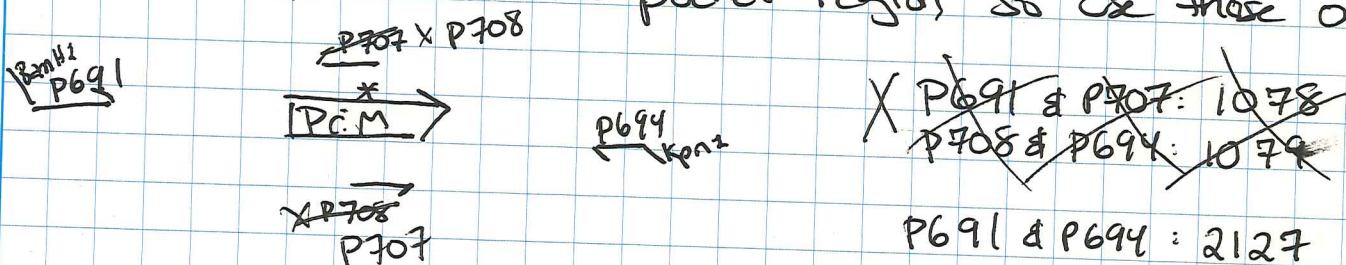
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>Prim
MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYSNNVETDADTNTNSPIYAFKSAADASNIQANGEKLARGFVLTNNGAVKIPNSIKPKNMYMK
DQATAAQGLEKYRAERDEINNNIKKLESQKKGWRIKVVAEQAKLSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNGPKAYFNDIAKPVLS
HLNAAWSATSLNYVDYRSNIDMFAGARVVLWNGQYNINSKDAATAIEFNDITNYLTITQIKSLQGSDDTKAVSLYADANTLTYTTIGDLSSVQK
KIASPRTALSICEASLISIRTNKVTARNIINRLSNKKAVKPIRLHLLNQTSDIILYDLKLDGNWALKAAATNAIRSTIGSDSILYVKGHTLML
ISTSSMVDAFIKAIAENEIYQVSDADRVLFKGNACNFTAAKNSNNPIVKAMIAASKQIAGQLPKGQVIDTVFEKVVYALQSTMFENLSNVLPKEE

>Prim_unst1
MKKTLTIALLGTIATTSVYADDLNAKIVNESVTKYSNNVETDADTNTNSPIYAFKSAADASNIQANGEKLARGFVLTNNGAVKIPNSIKPKNMYMK
DQATAAQGLEKYRAERDEINNNIKKLESQKKGWRIKVVAEQAKLSINTKIDILKGIENGDKAYAEKIAQFNIVKNITVNGPKAYFNDIAKPVLS
HLNAAWSATSLNYVDYRSNIDMFAGARVVLWNGQYNINSKDAATAIEFNDITNYLTITQIKSLQGSDDTKAVSLYADANTLTYTTIGDLSSVQK
KIASPRTALSICEASLISIRTNKVTARNIINRLSNKKAVKPIRLHLLNQTSDIILYDLKLDGNWALKAAATNAIRSTIGSDSILYVKGHTLML
ISTSSMVDAFIKAIAENEIYQVSDADRVLFKGNACNFTAAKNSNNPIVKAMIAASKQIAGQLPKGQVIDTVFEKVVYALQSTMFENLSNVLPKEE
```

PKL118 ^{PEX} Pr.M_unst1

Change TGG → GCT & also D216 GAT → GAC to make PciI site.

This mutation is near the pocket region so use those oligos.



- Digest PKL80, insert fragment BamHI/KpnI
 - Sequence using P21, P367, P368, P22

- Check for modification: PCR w/ P367 & P698 = 784bp

Digest with PciI: WT = 784 bp
 unst1 = 440 bp, 344 bp

Corrected * P691 & P708 - 1082
 P707 & P694 - 1087

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