

Amino acid sequence of *wt* cytochrome P-450:NADPH-P-450 reductase precursor [*Bacillus megaterium*]. Mature protein has first methionine trimmed off, therefore numbering shifts by one in Smolke paper (Michener and Smolke. 2012. High-throughput enzyme evolution in *Saccharomyces cerevisiae* using a synthetic RNA switch. Metabolic Engineering. Vol. 14: 306-316.)

```
1      mtikempqpk tfgelknlpl lntdkpvqal mkiadelgei fkfeapgrvt
51     rylssrlik eacdesrfdk nlsqalkfvr dfagdgflfts wtheknwkka
101    hnillpsfsq gamkgyhamm vdiavqlvqk werlnadehi evpedmtrlt
151    ldtiglcgfn yrfnsfyrdq phpfitsmvr aldeamnkqlq ranpddpayd
201    enkrqfgedi kvmndlvdkl iadrkasgeg sddllthmln gkdpetgepl
251    ddeniryqii tfliaghatt sgllsfalyf lvknphvlqk aaeearvrvl
301    dpvpsykqvq qlkyvgmvlv ealrlwptap afslyakedt vlggeyplek
351    gdelmvliqg lhrdktiugd dveefrperf enpsaipqha fkpfgngqra
401    cigqqfalhe atlvlgmmlk hdfdhdhtny eldiketltl kpegfvvkak
451    skkiplggip spsteqsakk vrkkaenahn tpllvlygsn mgtaegtard
501    ladiamskgf apqvatlsh agnlpregav livtasyngh ppdnakqfvd
551    wldqasadev kgvrysvfgc gdknwattyq kvpafidatl aakgaeniad
601    rgeadasddf egtyeewreh mwsdvaayfn ldiensednk stlslqfvds
651    aadmplakmh gafstnvvas kelqqpgsar strhleieip keasyqegdh
701    lgviprnyeg ivnrvtarfg ldasqqirle aeeeklahlp laktvsveel
751    lqyvelqdpv trtqlramaa ktvcpphkve leallekqay keqvlakrlt
801    mlellekypa cemkfsefia llpsirpryy sissprvde kqasitvsvv
851    sgeawsgyge ykgiasnyla elqegdtitc fistpqseft lpkdpetpli
901    mvgpgtgvap frgfvqarkq lkeggqslge ahlyfgcrsp hedylyqeel
951    enaqsegiit lhtafsrmpn qpkyvqhvm eqdgkkliel ldqgahfyic
1001   gdgsqmapav eatlmksyad vhwvseadar lwlqqleekg ryakdvwag
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Amino acid sequence of *yCDM1* cytochrome P-450:NADPH-P-450 reductase precursor [*Bacillus megaterium*]. Mature protein has first methionine trimmed off, therefore numbering shifts by one in Smolke paper. Mutations (below sequence) from directed evolution to make an enzyme that converts caffeine into theophylline. Numbers refer to the named generation of the evolved protein.

```

1      mtikempgpk tfgelknlpl lntdkpvqal mkiadelgei fkfeapgrvt
51     rylssqrlik eacdesrfdk nlsqalkfvr dfagdgflfts wtheknwkka
           W   I   L   A
           1   1   1   1
101    hnullpsfsq qamkgyhamm vdiavqlvqk werlnadehi evpedmtrlt
151    ldtiglcgfn yrfnsfyrdq phpfitsmvr aldeamnklq ranpddpayd
           V   W
           1   1
201    enkrqfgedi kvmndlvdki iadrkasgeq sddllthmln gkdpetgepl
251    ddeniryqii tfliaghatt sgllsfalyf lvknphvlqk aaeearlvv
301    dpvpsykqvk qlkyvgmvln ealrlwptap afslyakedt vlggeyplek
           F   W
           1   1
351    gdelmvlipq lhrdktiwgd dveefrperf enpsaipqha fkpfgngqra
401    cigqqfalhe atlvlgmmlk hdfdfehtny eldiketltl kpegfvvak
451    skkiplggip spstegsakk vrkkaenahn tpllvygsn mgtaegtard
501    ladiamskgf apqvatldsh agnlpregav livtasyngh ppdnakqfvd
551    wldgasadev kgvrysvfgc gdknwattyq kvpafidctl aakgaeniad
601    rgeadasddf egtyeewreh mwsdvaayfn ldiensednk stlslqfvds
651    aadmplakmh gafstnvvas kelqqgsar strhleieip keasyqegdh
701    lgviprnyeg ivnrvtarfg ldasqqirle aeeeklahlp laktvsveel
751    lqyvelqdpv trtqlramaa ktvcpphkve leallekqay keqvlakrlt
801    mllelekypa cemkfsefia llpsirpryy sissprvde kqasitvsvv
851    sgeawsgyge ykgiasnyla elqegdtitc fistpqseft lpkdpetpli
901    mvgpgtgvap frgfvqarkq lkeggqslge ahlyfgcrsp hedylyqeel
951    enaqsegiit lhtafsrmpn qpkyvqhvm eqdgkkliel ldqgahfyic
1001   gdgsqmapav eatlmksyad vhwvseadar lwlqqleekg ryakdvwag

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Amino acid sequence of *yCDM3* cytochrome P-450:NADPH-P-450 reductase precursor [Bacillus megaterium]. Mature protein has first methionine trimmed off, therefore numbering shifts by one in Smolke paper. Mutations (below sequence) from directed evolution to make a enzyme that converts caffeine into theophylline. Numbers refer to the named generation of the evolved protein. Generation 3 contains mutations from generations 1 and 3.

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1      mtikempqpk tfgelknlpl lntdkpvqal mkiadelgei fkfeapgrvt
51     rylssqrlik eacdesrfdk nlsqalkfvr dfagdgflfts wtheknwkka
           F W I L A
           3 1 1 1 1
101    hnullpsfsq qamkgyhamm vdiavqlvqk werlnadehi evpedmtrlt
151    ldtiglgcfn yrfnsfyrdq phpfitsmvr aldeamnlq ranpddpayd
           V W
           1 1
201    enkrqfqedi kvmndlvdk iadrkasgeq sddlthmln gkdpetgepl
251    ddeniryqii tfliaghett sgllsfalyf lvknphvlqk aaeearlvv
301    dpvpsykqvk qlkyvgmvln ealrlwptap afslyakedt vlggeyplek
           F W
           1 1
351    gdelmvlipq lhrdktiwgd dveefrperf enpsaipqha fkpfgngqra
401    cigqqfalhe atlvlgmmlk hdfdhdhtny eldiketltl kpegfvvkak
451    skkiplggip spsteqsakk vrkkaenahn tp1lvlygsn mgtaegtard
501    ladiamskgf apqvatldsh agnlpregav livtasyngh ppdnakqfvd
551    wldqasadev kgvrysvfgc gdknwattyq kvpafidetl aakgaeniad
601    rgeadasddf egtyeewreh mwsdvaayfn ldiensednk stlslqfvds
           T
           3
651    aadmplakmh gafstnvas kelqqpgsar strhleielp keasyqegdh
701    lgviprnyeg ivnrvtarfg ldasqqirle aeeeklahlp laktvsveel
751    lqyvelqdpv trtqlramaa ktvcpphkve leallekqay keqvlakrlt
801    mllelekypa cemkfsefia llpsirpryy sissprvde kqasitvsyv
851    sgeawsgyge ykgiasnyla elqegdtitc fistpqseft lpkdpetpli
901    mvgpgtgvap frgfvqarkq lkeqqgslge ahlyfgcrsp hedylyqeel
951    enaqsegiit lhtafsrmprn qpkyvqhvm eqdgkkliel ldqgahfyic
1001   gdgsqmapav eatlmksyad vhwqseadar lwlqqleekg ryakdvwag

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Amino acid sequence of *yCDM5* cytochrome P-450:NADPH-P-450 reductase precursor [Bacillus megaterium]. Mature protein has first methionine trimmed off, therefore numbering shifts by one in Smolke paper. Mutations (below sequence) from directed evolution to make a enzyme that converts caffeine into theophylline. Numbers refer to the named generation of the evolved protein. Generation 5 contains mutations from generations 1, 3 and 5. Amino acid 73 was F in generation 3.

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1      mtikempqpk tfgelknlpl lntdkpvqal mkiadelgei fkfeapgrvt
          H                      S
          5                      5
51     rylssqrlik eacdesrfdk nlsqalkfvr dfagdgflfts wtheknwkka
          I W I L A
          5 1 1 1 1
101    hnullpsfsq qamkgyhamm vdiavqlvqk werlnadehi evpedmtrlt
151    ldtiglcgfn yrfnsfyrdq phpfitsmvr aldeamnklq ranpddpayd
          V W
          1 1
201    enkrqfgedi kvmndlvdk iadrkasgeq sddllthmln gkdpetgepl
251    ddeniryqii tfliaghatt sgllsfalyf lvknpvhvlqk aaeearvvlv
301    dpvpsykqvk qlkyvgmvln ealrlwptap afslyakedt vlggeyplek
          F W
          1 1
351    gdelmvlipq lhrdktiwgd dveefrperf enpsaipqha fkpfgngqra
401    cigqqfalhe atlvlgmmlk hdfdhdhtny eldiketltl kpegfvvakak
451    skkiplggip spstegsakk vrkkaenahn tpllvygsn mgtaegtard
501    ladiamskgf apqvatldsh agnlpregav livtasyng h ppdnakqfvd
551    wldgasadev kgvrysvfgc gdknwattyq kvpafideti aakgaeniad
601    rgeadasddf egtyeewreh mwsdvaayfn ldiensednk stlslqfvds
          T
          3
651    aadmplakmh gafstnvvas kelqqpgsar strhleielp keasyqegdh
701    lgviprnyeg ivnrvtarfg ldasqqirle aeeeklahlp laktvsveel
751    lqyvelqdpv trtqlramaa ktvcpphkve leallekqay keqvlakrlt
801    mllelekypa cemkfsefia llpsirpryy sissprvde kqasitvsvv
851    sgeawsgyge ykgiasnyla elqegdtitc fistpqseft lpkdpetpli
901    mvpgptgvap frgfvqarkq lkeggqslge ahlyfgcrsp hedylyqeel
951    enaqsegiit lhtafsrmpn qpkyvqhvm eqdgkkliel ldqgahfyic
1001   gdgsqmapav eatlmksyad vhwvseadar lwlqqleekg ryakdvwag

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Amino acid sequence of *yCDM6* cytochrome P-450:NADPH-P-450 reductase precursor [Bacillus megaterium]. Mature protein has first methionine trimmed off, therefore numbering shifts by one in Smolke paper. Mutations (below sequence) from directed evolution to make a enzyme that converts caffeine into theophylline. Numbers refer to the named generation of the evolved protein. Generation 6 contains mutations from generations 1, 3, 5 and 6. Amino acid 73 was F in generation 3.

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1      mtikempqpk tfgelknlpl lntdkpvqal mkiadelgei fkfeapgrvt
              H                      S
              5                      5
51     rylssqrlik eacdesrfdk nlsqalkfvr dfagdgflfts wtheknwkka
              I W I L A
              5 1 1 1 1
101    hnullpsfsq qamkgyhamm vdiavqlvqk werlnadehi evpedmtrlt
151    ldtiglgcfn yrfnsfyrdq phpfitsmvr aldeamnklq ranpddpayd
              V W
              1 1
201    enkrqfqedi kvmndlvdkl iadrkasgeq sddllthmln gkdpetgepl
251    ddeniryqii tfliaghett sgllsfalyf lvknphvlqk aaeearlvv
301    dpvpsykqvk qlkyvgmvln ealrlwptap afslyakedt vlggeyplek
              F W
              1 1
351    gdelmvlipq lhrdktiwgd dveefrperf enpsaipqha fkpfgngqra
401    cigqqfalhe atlvlgmmk hdfdhdhtny eldiketltl kpegfvvkak
              G
              6
451    skkiplggip spsteqsakk vrkkaenahn tplllygsn mgtaegtard
501    ladiamskgyf apqvatldsh agnlpregav livtasyngh ppdnakqfvd
551    wldqasadev kgvrysvfgc gdknwattyq kvpafidetl aakgaeniad
601    rgeadasddf egtyeewreh mwsdvaayfn ldiensednk stlslqfvds
      T
      3
651    aadmplakmh gafstnvas kelqqgsar strhleielp keasyqegdh
701    lgviprnyeg ivnrvtarfg ldasqqirle aeeeklahlp laktvsveel
751    lqyvelqdpv trtqlramaa ktvcpphkve leallekqay keqvlakrlt
801    mllelekypa cemkfsefia llpsirpryy sissprvde kqasitvsvv
851    sgeawsgyge ykgiasnyla elqegdtitc fistpqseft lpkdpetpli
901    mvgpgtgvap frgfvqarkq lkeqqgslge ahlyfgcrsp hedylyqeel
951    enaqsegiit lhtafsrmpn qpkyvqhvm eqdgkkliel ldqgahfyic
1001   gdgsqmapav eatlmksyad vhwvseadar lwlqqleekg ryakdvwag

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Amino acid sequence of *yCDM7* cytochrome P-450:NADPH-P-450 reductase precursor [Bacillus megaterium]. Mature protein has first methionine trimmed off, therefore numbering shifts by one in Smolke paper. Mutations (below sequence) from directed evolution to make a enzyme that converts caffeine into theophylline. Numbers refer to the named generation of the evolved protein. Generation 7 contains mutations from generations 1, 3, 5, 6 and 7. Amino acid 73 was F in generation 3.

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1      mtikempqpk tfgelknlpl lntdkpvqal mkiadelgei fkfeapgrvt
          H                      S
          5                      5
51     rylssqrlik eacdesrfdk nlsqalkfvr dfagdgflfts wtheknwkka
          I W I L A
          5 1 1 1 1
101    hnullpsfsq qamkgyhamm vdiavqlvqk werlnadehi evpedmtrlt
151    ldtiglgcfn yrfnsfyrdq phpfitsmvr aldeamnklq ranpddpayd
          V          V W
          7          1 1
201    enkrqfqedi kvmndlvdkl iadrkasgeq sddllthmln gkdpetgepl
251    ddeniryqii tfliaghett sgllsfalyf lvknphvlqk aaeearlvv
301    dpvpsykqvq qlkyvgmvln ealrlwptap afslyakedt vlggeyplek
          F W
          1 1
351    gdelmvlipq lhrdktiwgd dveefrperf enpsaipqha fkpfgngqra
401    cigqqfalhe atlvlgmmk hdfdfehtny eldiketltl kpegfvvkak
          G
          6
451    skkiplggip spsteqsakk vrkkaenahn tplllygsn mgtaegtard
501    ladiamskgyf apqvatldsh agnlpregav livtasyngh ppdnakqfvd
551    wldqasadev kgvrysvfgc gdknwattyq kvpafidetl aakgaeniad
601    rgeadasddf egtyeewreh mwsdvaayfn ldiensednk stlslqfvds
          T
          3
651    aadmplakmh gafstnvas kelqqpgsar strhleielp keasyqegdh
701    lgviprnyeg ivnrvtarfg ldasqqirle aeeeklahlp laktvsveel
751    lqyvelqdpv trtqlramaa ktvcpphkve leallekqay keqvlakrlt
801    mllelekypa cemkfsefia llpsirpryy sissprvde kqasitvsvv
851    sgeawsgyge ykgiasnyla elqegdtitc fistpqseft lpkdpetpli
901    mvgpgtgvap frgfvqarkq lkeqqqslge ahlyfgcrsp hedylyqeel
951    enaqsegiit lhtafsrmpn qpkyvqhvm eqdgkkliel ldqgahfyic
1001   gdgsqmapav eatlmksyad vhwvseadar lwlqqleekg ryakdvwag

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Amino acid sequence of *yCDM8* cytochrome P-450:NADPH-P-450 reductase precursor [*Bacillus megaterium*]. Mature protein has first **methionine** trimmed off, therefore numbering shifts by one in Smolke paper. Mutations (below sequence) from directed evolution to make a enzyme that converts caffeine into theophylline. Numbers refer to the named generation of the evolved protein. Generation 8 contains mutations from generations 1, 3, 5, 6, 7 and 8. Amino acid 73 was F in generation 3. Amino acid 88 was A in generations 1-7.

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1      mtikempgpk tfgelknlpl lntdkpvqal mkiadelgei fkfeapgrvt
          H S
          5 5
51     rylessrlik eacdesrfdk nlsqalkfvr dfagdgflfts wtheknwkka
          I W I L S
          5 1 1 1 8
101    hnullpsfsq qamkgyhamm vdiavqlvqk werlnadehi evpedmtrlt
151    ldtiglcgfn yrfnsfyrdq phpfitsmvr aldeamnlq ranpddpayd
          V W
          7 1 1
201    enkrqfgedi kvmndlvdkl iadrkasgeq sddlthmln gkdpetgepl
251    ddeniryqii tfliaghett sgllsfalyf lvknphvlqk aaeearvly
301    dpvpsykqvq qlkyvgmvl n ealrlwptap afslyakedt vlggeyplek
          F W
          1 1
351    gdelmvlipq lhrdktiugd dveefrperf enpsaipqha fkpfgngqra
401    cigqqfalhe atlvlgmmlk hdfd fedhtny eldiketltl kpegfvvak
          G
          6
451    skkiplggip spsteqsakk vrkkaenahn tpll vlygsn mgtaegtard
501    ladiamskgf apqvatldsh agnlpregav livtasyng h ppdnakqfvd
551    wldqasadev kgvrysvfgc gdknwattyq kvpafideti aakgaeniad
601    rgeadasddf egtyeewreh mwsdvaayfn ldiensednk stlslqfvds
          T
          3
651    aadmplakmh gafstnvas kelqqgsar strhleielp keasyqegdh
701    lgviprnyeg ivnrvtarfg ldasqqirle aeeeklahlp laktvsveel
751    lqyvelqdpv trtqlramaa ktvcpphkve leallekqay keqvlakrlt
801    mlellekypa cemkfsefia llpsirpryy siss sprvde kqasitvsvv
851    sgeawsgyge ykgiasnyla elqegdtitc fistpqseft lpkdpetpli
901    mvgpgtg vap frgfvqarkq lkeggqslge ahlyfgcrsp hedylyqeel
951    enaqsegiit lhtafsrmpn qpkyvqhvm eqdgkklie lldqqahfyic
1001   gdgsqmapav eatlmksyad v hqvseadar lwlqqleekg ryakdvwag

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