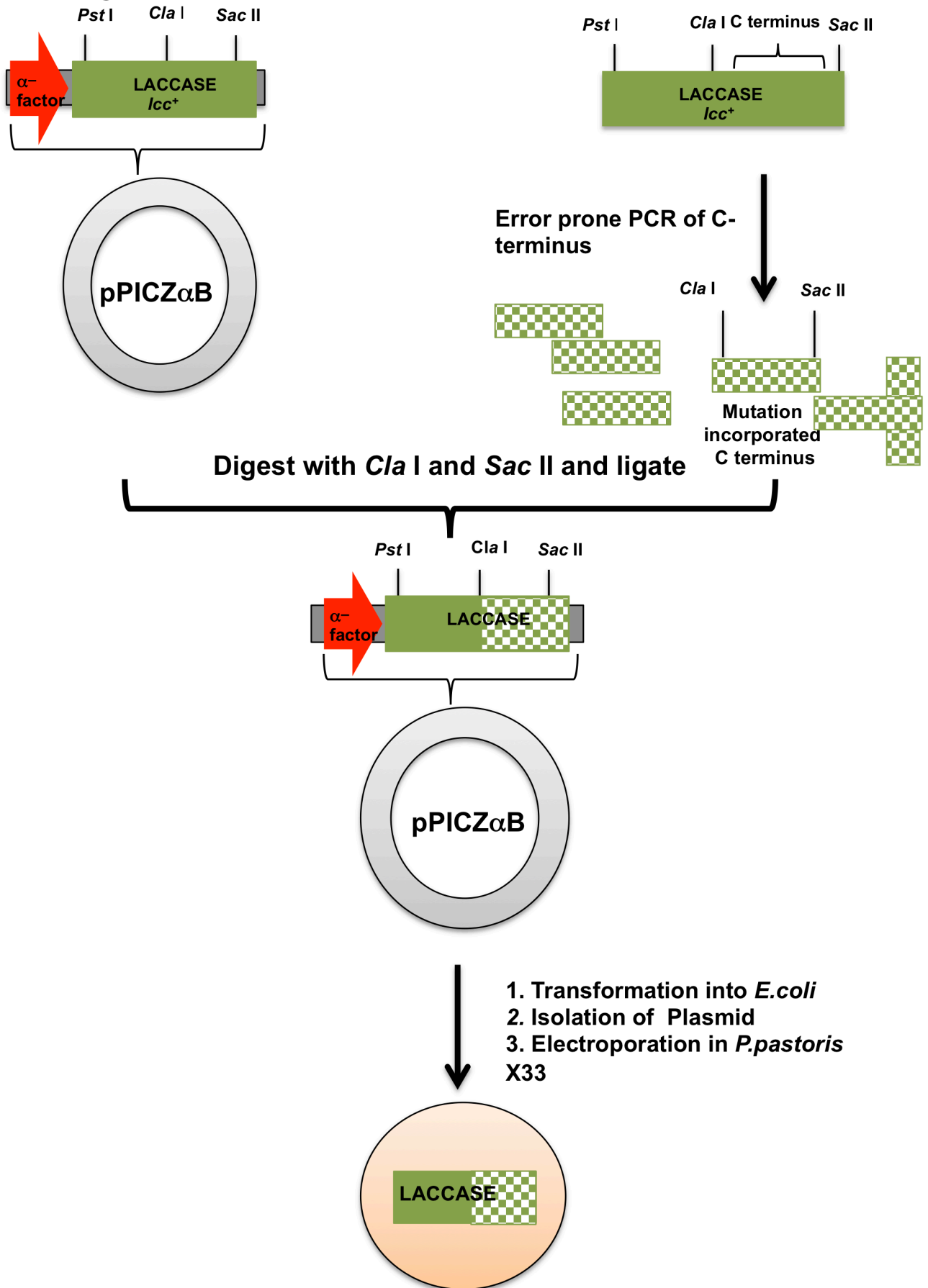
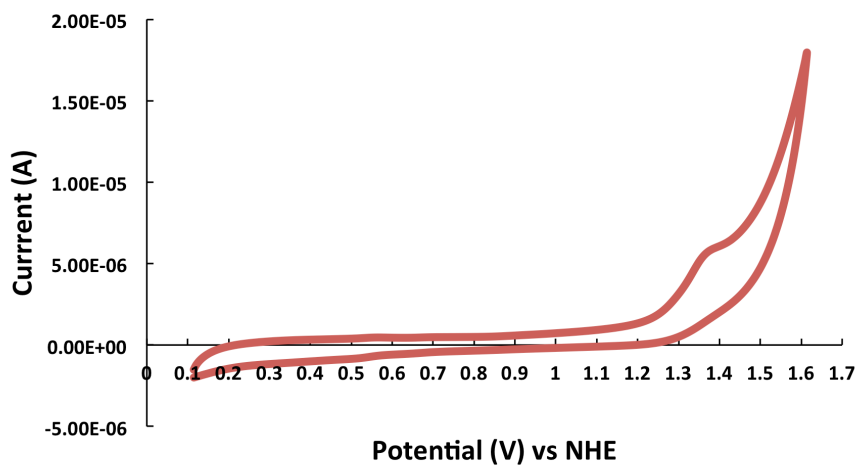


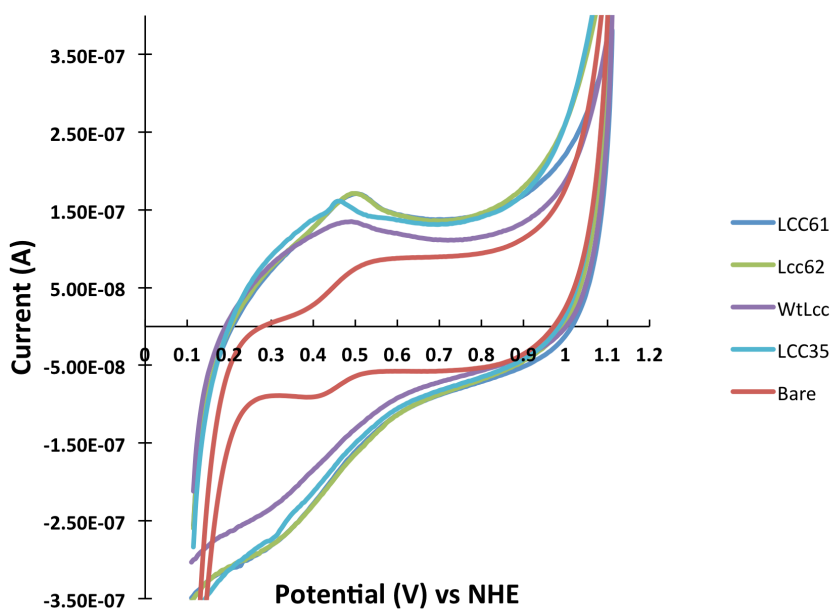
Supplementary Fig. 1. Strategy of mutagenesis of the 816-nt segment of *C. bulleri lcc⁺* gene



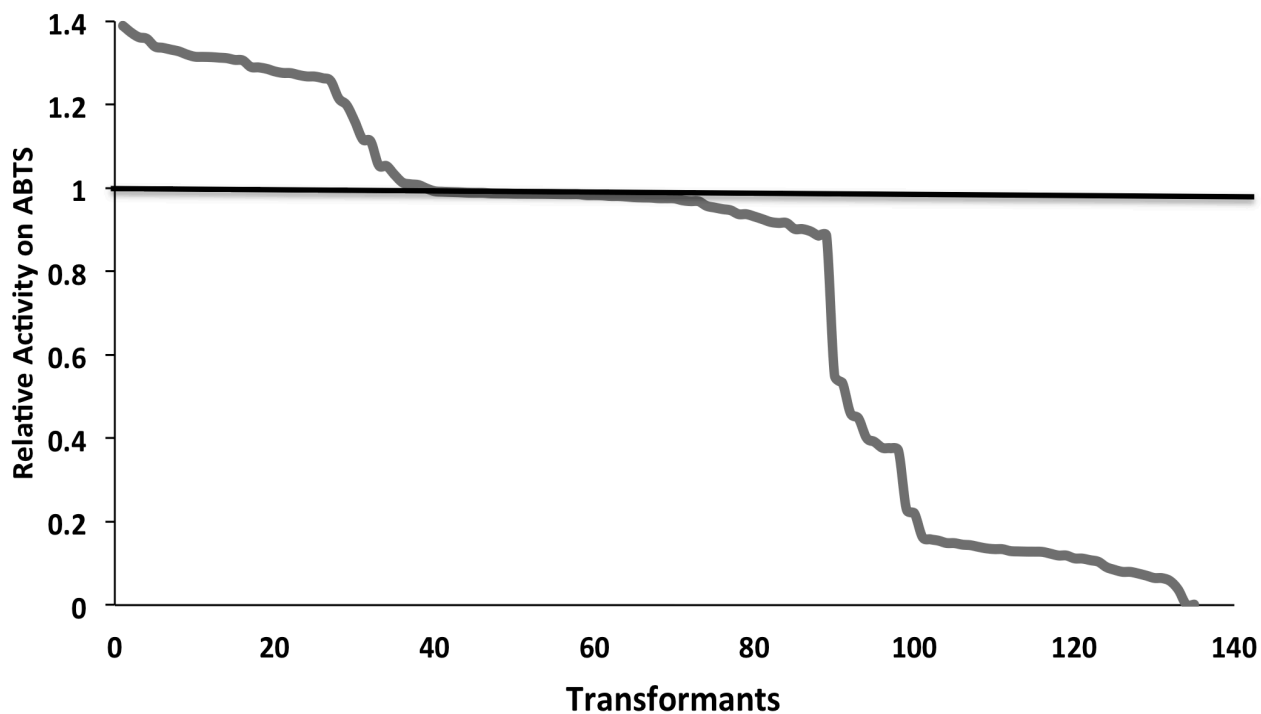
Supplementary Fig 2a. Oxidation potential of RB21



Supplementary Fig 2b Comparison of redox potential of the WtLcc and the mutant variants Lcc35, Lcc61 and Lcc62



Supplementary Fig. 3 Relative ABTS oxidizing activity of C-terminus laccase mutants. Activity on ABTS is plotted in descending order. X-axis shows the range in which different transformants display the indicated relative ABTS activity. The horizontal line shows the activity of the WtLcc.



Supplementary Fig. 4 Sequence alignment of the 816 nt segment (towards C-terminus) of the wild type *Icc⁺* gene of *C. bulleri* (CyB) with bacterial, fungal and plant laccases using Clustal Omega. The amino acids boxed by black or grey color represent the 'inactive' or the 'more active' variants respectively. CoC: *Coprinus cinereus* (Q9Y780); TrVr: *Trametes versicolor* (Q96UT7); TrT: *Trametes trogii* (Q9HDQ0); MeA: *Melanocarpus albomyces* (Q70KY3); PyC: *Pycnoporus cinnabarinus* (O59896); PeO: *Pleurotus ostreatus* (Q12739); TrV: *Trametes villosa* (Q99055); TrV2: *Trametes villosa* 2 (Q99049); CoH: *Coriolus hirsuta* (Q02497); CotA: *Bacillus subtilis* (B9W2C5); ArT: *Arabidopsis thaliana* (Q9LMS3). Numbers in brackets are the UNIPROT ID of these enzymes.

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CyB  IDGHTFT--IIEVDGQVNVTPVEVDSIQIFAGQRYSEFLNANQPVNDYWIR-AKPNIAGK---VTFDGGGINS-ILRYAGAPDTPDTPTSQTPNSA--PMVET-----D-----L 326
CoC  IDGHELT--IIEVDGQLTEPHTVDRLQIFPTGQRYSEFLDANQPVNDYWIR-AQPNKGRNLAGTFANGVNSA-ILRYAGAAADPTTSANPNPA--QLNEA-----D-----L 326
TrVr IDGHNMT--IIEETDSINTAPLVVDSIQIFAAQRYSEFLEANQAVNDYWIR-ANPNFG-N---VGFTEGGINS-ILRYDGAAPVEPTTQTTSIA--PLNEV-----N-----L 326
TrT  IDGHSLT--VIEADSVNLKPQTVVDSIQIFAAQRYSEFLNADQDVGNYWIR-ALPNSG-T---TGFVGGVNSA-ILRYDGAAPVEPTTQTPSIN--PLVES-----A-----L 325
MeA  LVNHTMT--VIAADMVFNAMTVDSLFLAVGQRYSDVIDASRAPDNYWFN-VTF--GGQACGGSLNHPHAA-IFHYAGAPGLPTDEGTPPVVHQCCLDTL-----D-----V 388
PyC  IDGHTMT--VIEADSVNTOPLVDSIQIFAAQRYSEFLDASQPVNDYWIR-ANPAFG-N---VGFAGGINS-ILRYDGAPEVEPTTQTTSIA--PLNEA-----D-----L 326
PeO  IDGHSLL--VIEADAVNIPIITVDSIQIFAGQRYSEFLTANQAVNDYWIR-ANPNLG-S---TGFVGGVNSA-ILRYAGATEDDPTTSST--ST--PLEET-----N-----L 337
TrV  IDGHNMT--IIEADAVNHEPLTVDSIQIYAGQRYSEFLTADQDIDNYFIR-ALPSAG-T---TSPDGGINS-ILRYSGASEVDPTTETTSVL--PLDEA-----N-----L 327
TrV2 IDGHTMT--IIEADGQNTOPHQVGLQIFAAQRYSEFLNANQAVNNYWIR-ANPNRANT---TGFANGINS-ILRYKGAPIKEPTTQNTIRN--FLWET-----D-----L 322
CoH  IDGHDLT--IIEVDSINSQPLVDSIQIFAAQRYSEFLNADQDVGNYWIR-ANPNFG-N---VGFAGGINS-ILRYDGAADPVEPTTQTTPK--PLNEV-----D-----L 326
CotA LDNGGEFIQVGSDDGLLPRSVKLNSEFLAPAERYDIIIDFTAYEGESIIL-----ANSAGCGDVPNPETDANIMQFRVTKPLAQKDESRL-----D-----351
ArT  VANHTLT--VVEVDVAVYTKPVHTKAMIIAPGQTTLLLRADQLSGGEFLIAATPYVTS---VTFDGGGINS-ILRYAGAPDTPDTPTSQTPNSA--PMVET-----D-----L 326

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CyB  HPLENPGAPGGSNADVPLNLA--IA-----FGS-NLKFYVNGA---TFA-PPNPVVLQIILSGAQTA-Q-----DLLPTGSVYTLPAVKV-----D-----IEI 402
CoC  HALIDPAAPGIPTGAADVNLN--FQ-----LGFSGGRFTINGT---AYE-SPSVPTLQIMSGAQSA-N-----DLLPAGSVYELPRNQV-----D-----VEL 403
TrVr HPLVATAVPGSPVAGGVDLAIN--MA-----FNFNGTNFFINGA---SFT-PPTVPVLLQIISGAQNA-Q-----DLLPSGSVYSLPSNAD-----D-----IEI 403
TrT  TTLEGTAAAPGSPAPGGVDLAIN--MA-----PGFAGGKFTINGA---SFT-PPTVPVLLQIILSGAQSA-Q-----DLLPSGSVYSLPANAD-----D-----IEI 402
MeA  RPVVPRSVVNSFVKRPDNTLP--VA-----LDLTGTPLFVWKVNGSDINVDWPKIIDIYLLTNGTS-----YPVSDNIVQV-----D-----DAVDQWTYW 467
PyC  HPLTMPVPGRPEAGGVDKPLN--MV-----FNFNGTNFFINNH---SFV-PPSVVLLQIILSGAQAA-Q-----DLVPDGSVYVLPSSNS-----D-----IEI 403
PeO  VPLENPGAPPPPVGADININLAMA-----PDTTFELTINGV---PFL-PPTAPVLLQIILSGASTA-A-----SLLPSGSYIYELEANKV-----D-----VEI 416
TrV  VPLDSPAAPGDPNIGGVYALN--LD-----FNFDGTNFFINDV---SFV-SPTVPVLLQIILSGTSTA-A-----DLLPSGSLFAVPSNST-----D-----IEI 404
TrV2 HPLTDPRAPGLPFKGGVDHALN--LN-----LTFNGSEFFINDA---PFV-PPTVPVLLQIILNGTLDA-N-----DLLPPGSVYNLPPDST-----D-----IEL 399
CoH  HPLATMAVPGSPVAGGVDTAIN--MA-----FNFNGTNFFINGA---SFV-PPTVPVLLQIISGAQNA-Q-----DLLPSGSVYSLPSNAD-----D-----IEI 403
CotA -PEYLASYPVQH-ERIQNIRT-----LKLAGTQDEYGRPVLLLNK-----RWHDPVT---GAP-----D-----KVGTEIWI 409
ArT  KSLGSAKYPC-KVPTKIDKRVIITISLNLQDCPLNQTCGYAGKRFFAS--MNNISFVRPP--ISILESYKKQSKGVVSLDFPEKPPNRFDTGVDPVSNMTEFGTKLFEVEFGSRLEI 469

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CyB  SIPGG---TTGFPHPFHLHGHTFDVRSAGS-----SVYNYDNP-----VRRDAVN-TGGA--GDNVTIR-FLTDNAPFWLHCHIDWHLEGLAVFAEDVPTIAAS 493
CoC  VVPAG---VLGGPFPFHLHGHAFAVRSAGS-----STYNYVNP-----VKRDVVS-LGVT--GDNVTIR-FVTDNPPWFHCHIEFHLMNLAVFAEDMAMTVDA 494
TrVr SFPATA-AAPGAPHPFHLHGHAFAVRSAGS-----TVYNYDNP-----IFRDVVS-TGTPAAGDVTIR-FRTDNPSPWFLHCHIDFHLEAGFAVFAEDIPDVASA 498
TrT  SLPATA-AAPGFPHPFHLHGHTFAVRSAGS-----STYNYDNP-----VYRDVVS-TGSP--GDNVTIR-FRTDNPSPWFLHCHIDFHLEAGFAVMAEDIPVAAAT 495
MeA  LIENDEPGFSLPHMHLHGHDVLVGRSPDVAASQQRVFPDPAVDLARLNGDNP-----PRRDTM---LPAGGALLA-FRTDNPSPWFLHCHIAWVSGLSVDFLERPADLRQR 577
PyC  SFPATA-NAPGTHPFPFHLHGHTFAVRSAGS-----SEYNYDNP-----IFRDVVS-TGQP--GDNVTIR-FQTNPPSPWFLHCHIDFHLEAGFAVLAEDTPDTAAV 496
PeO  SMPAL--AVGGPFPFHLHGHTFDVRSAGS-----TTYNYDNP-----ARRDVVN-TGTV-ANDNVTIR-FVTDNPPWFLHCHIDWHLEIGLAVFAEDVTSIS-- 506
TrV  SFPITATNAPGAPHPFHLHGHTSIVRTAGS-----TDTNFPNP-----VRRDVVN-TGTV--GDNVTIR-FVTDNPPWFLHCHIDFHLEAGFAVVFSEDTADVSNT 498
TrV2 SIPGG---VTGGPFPFHLHG-----TDNPPSPWFLHCHIDFHLEAGFAVLAEDQDTIKLV 451
CoH  SFPATA-AAPGAPHPFHLHGHAFAVRSAGS-----TVYNYDNP-----IFRDVVS-TGTPAAGDVTIR-FRTDNPSPWFLHCHIDFHLEAGFAVFAEDIPDVASA 498
CotA SII---NPTRGTHPIHLHVSFRVLDLRRPFDIARYQ-----ESGELSYTPAVPPPPSEGWKDTIQAHAGEVLRIAATFGPYSGRYVWHCHLEHEDYDMMRPMDI-----TDR 511
ArT  VFQGT-S-FLNIENHPLHVGHGNFVVGGRGFG-----NFDPEKDPKRYNLVDP-----PERNTFA---VPTGGAAIR-INADNPPWFLHCHLEQHTSWGLAGFIVKDGPLPSQ 569

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CyB  N--P-PDAWDLNLCPAYATQPTGT----- 513
CoC  N--NPPVEWAQLCEIYDDLPEATS-----IQTVVRAEPTGFSAKFRREG 539
TrVr N--PVPQAWSDLCPYDARDPSDQ----- 520
TrT  N--PVPQAWSDLCPYDALSPDDQ----- 517
MeA  ISQEDDDFNRCDEWRAYWPTNPYPKIDSLKRRRVEESEWLVR----- 623
PyC  N--PVPQAWSDLCPYDALDPSDL----- 518
PeO  ---APPAWDLCPYINALSDNDKG-----GIVPS----- 533
TrV  T--TPSTAWDLCPYINALDSSDL----- 520
TrV2 N--PVPEDWNKLCPTFDKAMNITV----- 473
CoH  N--PVPQAWSDLCPYDALDVNDQ----- 520
CotA HK----- 513
ArT  TLLPPHDLPGC----- 581

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