## Supporting Information

## Automating cloning by natural transformation

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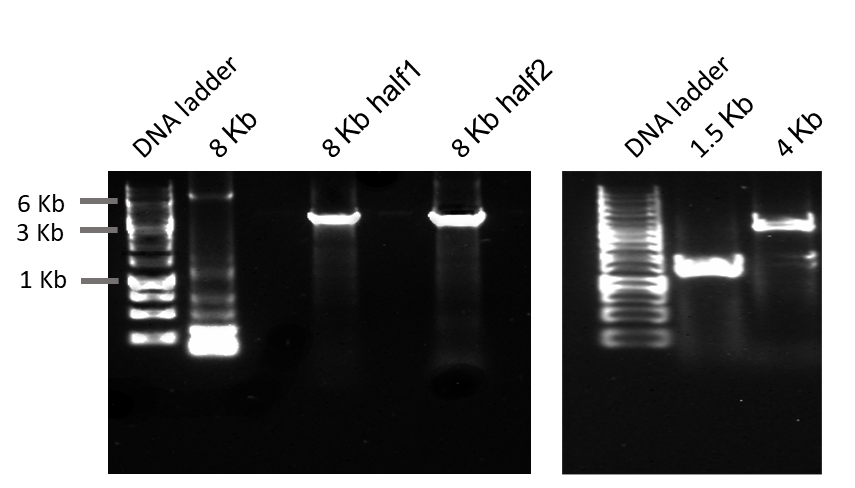
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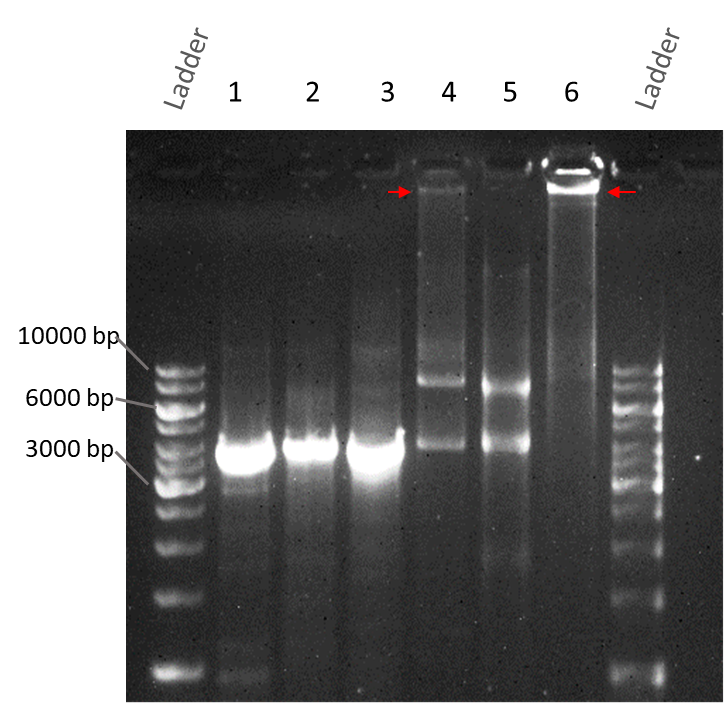
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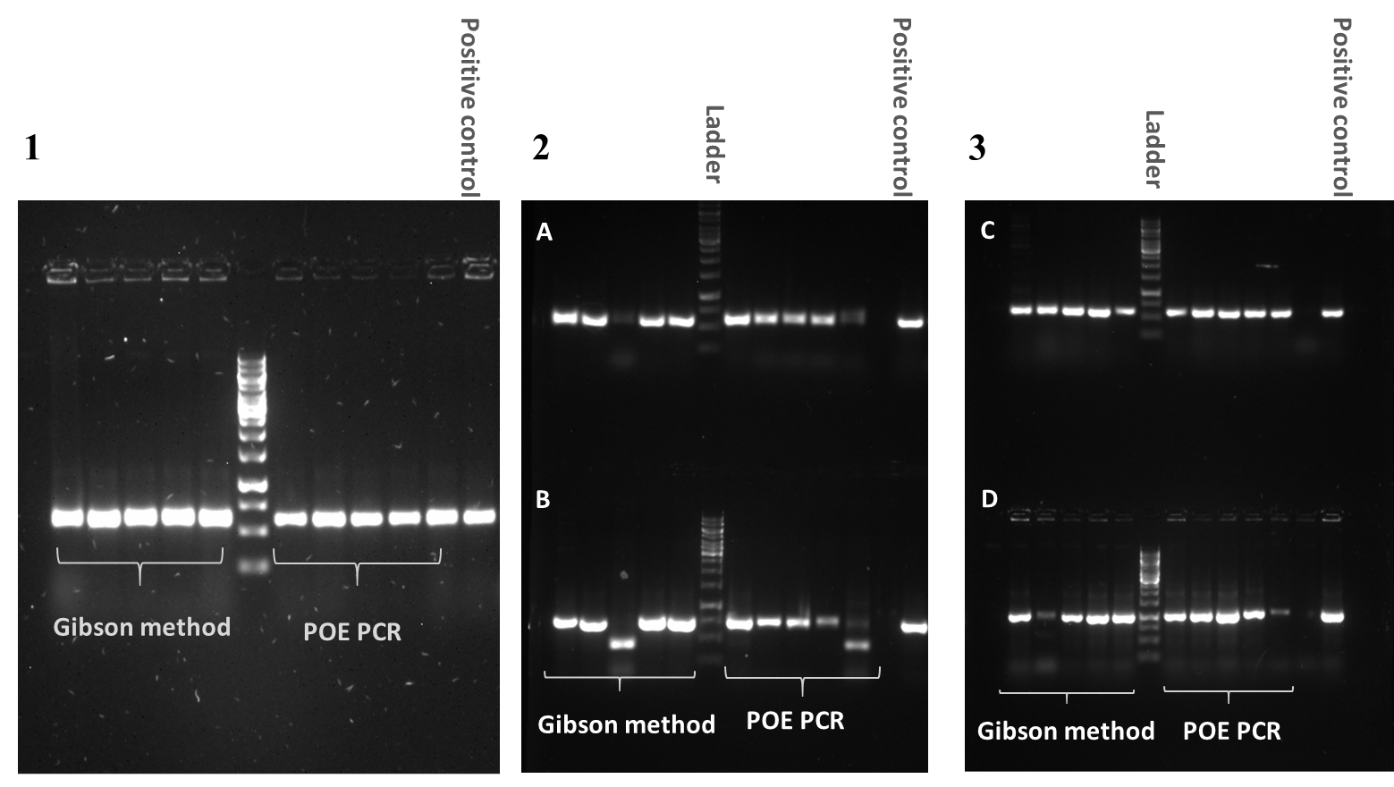
## KEYWORDS: *automated cloning, bench-top robot, natural transformation, Acinetobacter baylyi ADP1, biosynthetic gene clusters*



**Figure S1.** PCR results of the DNA fragments used in the melanin BGC cloning. Genomic DNA of *Streptomyces coelicolor* was used as template. 1M betaine were used as PCR enhancer. Amplification of the 8 kb fragment was not successful, as there was only a weak band at 8 kb. All the other 4 fragments were amplified successfully.



**Figure S2.** DNA assembly products used in the melanin BGC cloning. Lane1, vector backbone; lane2, 8 kb half1; lane3, 8 kb half2; lane 4, Gibson reaction from vector backbone 8 kb half1 and 8 kb half2; lane 5, OE-PCR product from vector backbone and 8 kb half1; lane 6, OE-PCR product from vector backbone 8 kb half1 and 8 kb half2. The multimers are marked by red arrows.



**Figure S3.** Colony PCR result for the melanin BGC cloning: 1)1.5K construct, 2)4K construct; A. refers to 1.5 k primers set, B. refers to 4 k primer set, 3) 8K construct; C. refers 1.5 k primers set, D. refers to 8 k primer set.

**Table S1** **Antibiotic susceptibility test of *A. baylyi ADP1*, *Vibrio natriegens* and *E.coli*.** 20 µl of fresh overnight cultures were spread on LB agar plates with antibiotics. After incubation at 30 °C for 24 hours, colony numbers were counted.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Colony number | 100 µg/ml ampicillin | 50 µg/ml kanamycin | 25 µg/ml chloramphenicol | 25 µg/ml gentamicin |
| *A. baylyi ADP1* | Grew into a lawn | 0 | 0 | 0 |
| *V. natriegens* DSM 759 | 3 ± 3 | 69± 36 | 0 | Grew into a lawn |
| *E. coli* DH5a | 0 | 0 | 0 | 0 |

**Table S2 Summary of the plasmids constructed by *Acinetobacter* natural transformation in this study.**

\*DNA fragments were prepared by PCR as in Table S2 or by chemical synthesis as Table S3

|  |  |  |  |
| --- | --- | --- | --- |
| **Constructed plasmid** | **Vector** | **Insertion fragments\*** | **brief** |
| pUCP-1.5k | pUCP24 | 1.5k | Type III PKS gene cluster (partial) from *Streptomyces* |
| pUCP-4k | pUCP24 | 4k | Type III PKS gene cluster from *Streptomyces* |
| pUCP-8k | pUCP24 | 8k half1 + 8k half2 | Type III PKS gene cluster (extended) from *Streptomyces* |
| pUCP-Pspls | pUCP24 | Pspls | NRPS gene cluster from *Pseudomonas* |
| pUCP-PPcluster1 | pUCP24 | PPcluster1 | None typical gene cluster from *Pseudomonas* |
| pXJ100-RiPP1.1 | pXJ100 | RiPP1.1 | RiPP gene cluster from *Streptomyces* |
| pXJ100-RiPP1 | pXJ100 | RiPP1 | RiPP gene cluster from *Streptomyces* |
| pXJ1000-RiPP2 | pXJ100 | RiPP2 | RiPP gene cluster from *Streptomyces* |
| pXJ100-RiPP3 | pXJ100 | RiPP3 | RiPP gene cluster from *Streptomyces* |
| pXJ100-RiPP5 | pXJ100 | RiPP5 | RiPP gene cluster from *Streptomyces* |
| pXJ100-RiPP6 | pXJ100 | RiPP6 | RiPP gene cluster from *Streptomyces* |
| pXJ100-RiPP7 | pXJ100 | RiPP7 | RiPP gene cluster from *Streptomyces* |
| pXJ100-RiPP8 | pXJ100 | RiPP8 | RiPP gene cluster from *Streptomyces* |
| pXJ100-RiPP7.1 | pXJ100 | RiPP7.1 | RiPP gene cluster from *Streptomyces* |
| pXJ100-RiPP7in1 | pXJ100 | ladder of RiPP1+lasso peptide of RiPP7 and CB of RiPP1 | RiPP gene cluster from *Streptomyces,* refactored |
| pXJ100-RiPP1in7 | pXJ100 | ladder of RiPP1+lasso peptide of Caulosegnin I and CBM of RiPP7 | RiPP gene cluster from *Streptomyces,* refactored |
| pXJ100- CaulosegninI in1 | pXJ100 | ladder of RiPP1+lasso peptide of Caulosegnin I and CB of RiPP1 | RiPP gene cluster from *Streptomyces,* refactored |
| pXJ100-RiPP3.1 | pXJ100 | RiPP3 3inI and RiPP3 A | RiPP gene cluster from *Streptomyces,* refactored |
| pXJ100-RiPP3.2 | pXJ100 | RiPP3 3inII and RiPP3 A | RiPP gene cluster from *Streptomyces,* refactored |
| pXJ100-RiPP3.3 | pXJ100 | RiPP3 3inIII and RiPP3 A | RiPP gene cluster from *Streptomyces,* refactored |
| pXJ100-RiPP3.4 | pXJ100 | RiPP3 3inV and RiPP3 A | RiPP gene cluster from *Streptomyces,* refactored |
| pXJ100-RiPP3.5 | pXJ100 | RiPP3 3inI-V and RiPP3 A | RiPP gene cluster from *Streptomyces,* refactored |
| pXJ100-NN1 | pXJ100 | NN1 half1 and NN1 half2 | Potential nucleoside antibiotic biosynthetic gene cluster from *Photorhabdus asymbiotica* |
| pXJ100-nucleoside1 | pXJ100 | nucleoside1 | Potential nucleoside antibiotic biosynthetic gene cluster from *Xenorhabdus szentirmaii* |
| pXJ100- cmr-plac-gfp (pXJ160) | pXJ100 | Cmr-plac-gfp | green fluorescent protein gene as a reporter gene |
| pXJ100-Cmr-plac-mscarlet | pXJ100 | Cmr-plac and mscarlet | Red fluorescent protein gene as a reporter gene |

**Table S3 Summary of DNA fragments generated by PCR**

\*primer sequences were listed in Table S3

|  |  |  |
| --- | --- | --- |
| **Fragment/size** | **PCR template** | **Primers\*** |
| pUCP24 backbone/3941 bp | pUCP24 linearized by BamHI digestion | pUCP24s |
| pUCP24a |
| 1.5k/1537 bp | gDNA of *Streptomyces coelicolor A3(2)* | S1 |
| R1 |
| 4k/4098 bp | gDNA of *Streptomyces coelicolor A3(2)* | S1 |
| R2 |
| 8k/8045 bp | gDNA of *Streptomyces coelicolor A3(2)* | S1 |
| R3 |
| 8k half1/4098 bp | gDNA of *Streptomyces coelicolor A3(2)* | S1 |
| R2.1 |
| 8k half2/3977 bp | gDNA of *Streptomyces coelicolor A3(2)* | S2 |
| R3 |
| pUCP24 backbone1/3879 bp | pUCP24 linearized by BamHI digestion | Xj332 |
| Xj333 |
| Pspls | gDNA of *Pseudomonas syringae* DSM 50252 | Xj330 |
| Xj331 |
| pXJ100 backbone/3519 bp | pXJ100 linearized by EcoRI digestion | Xj210 |
| Xj211 |
| RiPP1.1/2685 bp | gDNA of *Streptomyces* Nai2 | Xj280 |
| Xj281 |
| RiPP1/4682 bp | gDNA of *Streptomyces* Nai2 | xj282 |
| Xj283 |
| RIPP 2 class III lantipeptide/ 6744 bp | gDNA of *Streptomyces* Nai2 | Xj214 |
| Xj215 |
| RIPP 3 unknown class / 6425 bp | gDNA of *Streptomyces* Nai2 | Xj216 |
| Xj217 |
| RIPP 5 (nai33 cluster 3) / 8927 bp | gDNA of *Streptomyces sp.* NRRL F-5053 | Xj220 |
| Xj221 |
| RIPP 6 (nai33 cluster 37)/4434 bp | gDNA of *Streptomyces sp.* NRRL F-5053 | Xj222 |
| Xj223 |
| RIPP 7 (nai33 cluster 38)/5187 bp | gDNA of *Streptomyces sp.* NRRL F-5053 | Xj224 |
| Xj225 |
| RIPP 8 (rimosus cluster 17)  Class I lantipeptide /8836 bp | gDNA of *Streptomyces* *rimosus* ATCC 10970 DSM40260 | Xj226 |
| Xj227 |
| RIPP 7.1 /3996 bp | gDNA of *Streptomyces sp.* NRRL F-5053 | Xj284 |
| Xj285 |
| CB of RiPP1/2552 bp | gDNA of *Streptomyces* Nai2 | Xj286 |
| Xj287 |
| CBM of RiPP7/ 3783 bp | gDNA of *Streptomyces sp.* NRRL F-5053 | Xj288 |
| Xj289 |
| RiPP3 A/2694 bp | gDNA of *Streptomyces* Nai2 | Xj290 |
| Xj291 |
| RiPP3 3inI/  229 bp | gDNA of *Streptomyces* Nai2 | Xj292 |
| Xj293 |
| RiPP3 3inII/  223 bp | gDNA of *Streptomyces* Nai2 | Xj294 |
| Xj295 |
| RiPP3 3inIII/  239 bp | gDNA of *Streptomyces* Nai2 | Xj296 |
| Xj297 |
| RiPP3 3inV/  235 bp | gDNA of *Streptomyces* Nai2 | Xj298 |
| Xj299 |
| RiPP3 3inI-V/  978 bp | gDNA of *Streptomyces* Nai2 | Xj300 |
| Xj301 |
| pXJ100 backbone 1/ 3549 bp | pXJ100 linearized by EcoRI digestion | xj306 |
| xj307 |
| NN1 half1/  10710 bp | gDNA of *Photorhabdus asymbiotica* ATCC43949 | xj302 |
| xj303 |
| NN1 half2/  5437 bp | gDNA of *Photorhabdus asymbiotica* ATCC43949 | xj304 |
| xj305 |
| nucleoside1/18797 bp | gDNA of *Xenorhabdus szentirmaii* DSM 16338 | xj308 |
| xj309 |
| pxj100 backbone 2 /3520 bp | pXJ100 linearized by EcoRI digestion | fbxj100 |
| rbxj100.1 |
| Cmr-plac-gfp /1700 bp | pXJ157 | Xj466 |
| Xj467 |
| Cmr-plac /932 bp | pXJ157 | Xj466 |
| Xj468 |
| mscarlet /1700 bp | pXJ0.1 | Xj469 |
| Xj470 |

**Table S4 Summary of DNA fragments generated by chemical synthesis (purchased from IDT)**

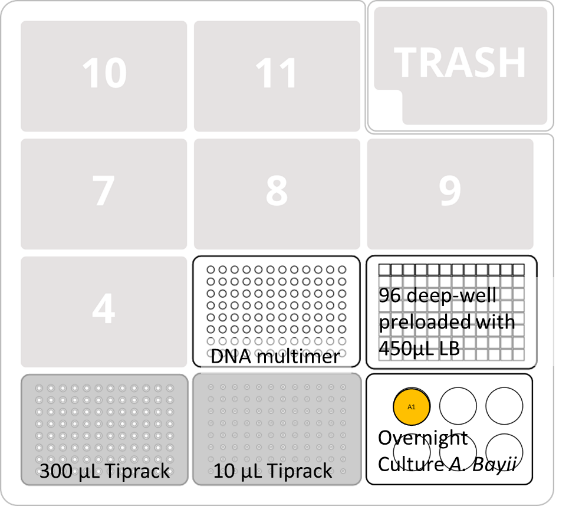
|  |  |
| --- | --- |
| **Fragment name** | **Fragment sequence** |
| leader of RiPP1+lasso peptide of RiPP7 | gatcccgcgaaattaatacgactcactataggggaattgtgagcggataacaattcccctctagaaataattttgtttaactttaagaaggagatataccatgaaaaaagcctatgaggcacccacactggttcgtttaggtacttttcgcaaggaaacgggcaccaataacttcgataccgccgatgacacgcagtacaagaatgcctaatctagagtcacacaggaaagtactagatgactgaactgcacgcaagtgcggggacgggcctcggcgacgcggacttcacggtcttcccggaccgtgcgg |
|
|
| leader of RiPP7+lasso peptide of RiPP1 | gatcccgcgaaattaatacgactcactataggggaattgtgagcggataacaattcccctctagaaataattttgtttaactttaagaaggagatataccatgttgttgacagacggacactctcctgcagaaggtgccggacaagagcgtaaagaggtagcgatggatgcgaacacgacccctgtctatactgctcccttggtgttagatggtggggacgtggtcgaggtcacactgggtttgctgggccgccatgggaacgaccgcttaattttgtcaaagaactaatctagagtcacacaggaaagtactagatgttgagcgccacggccggccccggcaccagaaggcgccgggccaacgcctctctcgtgctggggcacgggg |

**Table S5 summary of the primers used in this study**

|  |  |
| --- | --- |
| **Primer name** | **Primer Sequence** |
| pUCP24s | CCCTGGCGTTACCCAACTTAATCG |
| pUCP24a | GCACAAAGTCGCCATCGTAATCATGGTCATAGCTGTTTCCTG |
| S1 | ATGACCATGATTACGATGGCGACTTTGTGCAGACCCTC |
| R1 | GCAAGGCGATTAAGTTGGGTAACGCCAGGGCCGGCCCTCAGCATGGCG |
| R2 | GCAAGGCGATTAAGTTGGGTAACGCCAGGGGGAGGGGACATGGCCGAGTTC |
| R3 | GCAAGGCGATTAAGTTGGGTAACGCCAGGGGGAGGAGCCGAGGATGGTGCC |
| R2.1 | GGAGGGGACATGGCCGAGTTC |
| S2 | CTCCATGGTGAACTCGGCCATGTC |
| 1,5Vs | TGCTCTGCAACGGCCTCTTCGG |
| 1,5Va | TCTTCAGTCATGCCTGCCTCACCCT |
| 4Vs | GTGTAGATCGCGGAGTCCCGGTG |
| 4Va | TACGACGAGGCCAAGGACGAGTGG |
| 8Vs | CCTCCTGGCTGTGGGACGTGGAC |
| 8Va | ACCTGTGCGGACGGGGCATC |
| Xj210 | CTGCTAACAAAGCCCGAAAG |
| Xj211 | CATGGTATATCTCCTTCTTAAAGTTAAAC |
| Xj214 | CTTTAAGAAGGAGATATACCATGGACAAGCGCTACGAGGTGTACG |
| Xj215 | CTTTCGGGCTTTGTTAGCAGCTCGCTGGGCTTCGGGTTCC |
| Xj216 | CTTTAAGAAGGAGATATACCATGCAATGCCAAGAGGGCCAG |
| Xj217 | CTTTCGGGCTTTGTTAGCAGGCATCCTGCGCGTCGAACTC |
| Xj220 | CTTTAAGAAGGAGATATACCATGTCCGCGCGCTCCAGCT |
| Xj221 | CTTTCGGGCTTTGTTAGCAGAGATCGATCTGCACAGCCTTGCG |
| Xj222 | CTTTAAGAAGGAGATATACCATGGTCTGCCGGTGGGCAGAGTG |
| Xj223 | CTTTCGGGCTTTGTTAGCAGCGGGACGGCACGGAAAGAAAGC |
| Xj224 | CTTTAAGAAGGAGATATACCATGTTGACTGATGGTCACAGCCCG |
| Xj225 | CTTTCGGGCTTTGTTAGCAGCGCGGTGGCCCTCGAAGAC |
| Xj226 | CTTTAAGAAGGAGATATACCATGAAGATGTCGGACGCATTCGACC |
| Xj227 | CTTTCGGGCTTTGTTAGCAGCGGAACCGATGTGGAAGCTGGC |
| Xj280 | tgtttaactttaagaaggagatataccatgAAGAAGGCATATGAAGCGCCGACGC |
| Xj281 | actcagcttcctttcgggctttgttagcagTCATCGTTCGGCCGGCTCGAC |
| xj282 | tgtttaactttaagaaggagatataccatgAAGAAGGCATATGAAGCGCCGACGC |
| Xj283 | actcagcttcctttcgggctttgttagcagTCAGCCGTTCGCCCGACGGC |
| Xj284 | tgtttaactttaagaaggagatataccatgTTGACTGATGGTCACAGCCCGGC |
| Xj285 | actcagcttcctttcgggctttgttagcag**TTAGCGGGTGAGGCGGAGGTTC** |
| Xj286 | **ATGACTGAACTGCACGCAAGTGCGG** |
| Xj287 | actcagcttcctttcgggctttgttagcagTCATCGTTCGGCCGGCTCGAC |
| Xj288 | TTGAGCGCCACGGCCGGC |
| Xj289 | actcagcttcctttcgggctttgttagcagTTAGCGGGTGAGGCGGAGGTTCC |
| Xj290 | TCTAGAGTCACACAGGAAAGTACTAGATGGACATCCGGTCGGTACTGCAC |
| Xj291 | ACTCAGCTTCCTTTCGGGCTTTGTTAGCAGCGGTCAGTAACGGCCGATGAAGGTG |
| Xj292 | ctagaaataattttgtttaactttaagaaggagatataccATGGACACAACCAGAACCCCAGTC |
| Xj293 | CCATCTAGTACTTTCCTGTGTGACTCTAGACTGCGGGCTGTCAGGGTGCT |
| Xj294 | ctagaaataattttgtttaactttaagaaggagatataccATGAACGAGTCGCCTACCCG |
| Xj295 | CCATCTAGTACTTTCCTGTGTGACTCTAGAATGCTGCAGGCGGCCTCTAG |
| Xj296 | ctagaaataattttgtttaactttaagaaggagatataccATGAACAACCAGATCGCGCAG |
| Xj297 | CCATCTAGTACTTTCCTGTGTGACTCTAGACGGAGCTAAGCCCTGCTGGAG |
| Xj298 | ctagaaataattttgtttaactttaagaaggagatataccATGAACGAGGACCTCGAACAGCG |
| Xj299 | CCATCTAGTACTTTCCTGTGTGACTCTAGACCGACACGCGGCCTCACTTG |
| Xj300 | ctagaaataattttgtttaactttaagaaggagatataccATGGACACAACCAGAACCCCAGTC |
| Xj301 | CCATCTAGTACTTTCCTGTGTGACTCTAGACCGACACGCGGCCTCACTTG |
| xj306 | tttctcagctatataccctgcatccttcaaCTGCTAACAAAGCCCGAAAG |
| xj307 | ATAAGGAGGAATTATGATCAATATTTTCATGGTATATCTCCTTCTTAAAGTTAAAC |
| xj302 | ATGAAAATATTGATCATAATTCCTCCTTATATACC |
| xj303 | GCTGCGAGGCGTTTTAAATGTTTCCGGGCCTAATAATGC |
| xj304 | GGCCCGGAAACATTTAAAACGCCTCGCAGCATAACC |
| xj305 | TTGAAGGATGCAGGGTATATAGCTGAG |
| xj308 | AGCATTTTTGAAAGACCCATATTCTACGACACTC |
| xj309 | GCCGACAAACTAACATGAAGATAACCGTTACAC |
| Xj330 | CAATTTCACACAGGAAACAGCTATGAATAACCTGAACCTGTCGACG |
| Xj331 | CCTCTTCGCTATTACGCCAGCGTAAATCCCAATAGCGGAGAGCA |
| Xj332 | GCTGGCGTAATAGCGAAGAGG |
| Xj333 | CATAGCTGTTTCCTGTGTGAAATTG |
| fbxj100 | CACCACCACCACCACCACTGAG |
| rbxj100.1 | GGTCTATCTCCTTCTGGCGGTGCAACAAAATTATTTCTAGAGGGGAATTGTTATC |
| Xj466/65.3 | tgcaccgccagaaggagatagaccCCCTGTTGATACCGGGAAGCC |
| Xj467/60 | ggatctcagtggtggtggtggtggtgTTATTTGTAGAGCTCATCCATGCC |
| Xj466/ | tgcaccgccagaaggagatagaccCCCTGTTGATACCGGGAAGCC |
| Xj468/58.5 | tctCCTTTGCTGACCATGGTATATCTCCTTCTTAAAGTTAAAC |
| Xj469/61.1 | taaGAAGGAGATATACCATGGTCAGCAAAGGAGAAGCAG (overlap tm7.6) |
| Xj470/61.2 | ggatctcagtggtggtggtggtggtgTTACTTGTACAGCTCATCCATGCC |

**Supplementary Method 1**. ***Automatic natural transformation (ANT) using the Opteron OT-2 – system***

For the initial transformation of DNA multimers into *A. Baylyi* using the OT-2 system the setup should be as shown in the following figure. The 96 deep-well plate should be preloaded with 450 µL of LB media. While this loading step could be included in the robotic protocol this would either require a time consuming process of loading individual wells with the p300 single channel tip, or swapping of pipette heads during the experiment. We found both of these options are more cumbersome than preloading the deep well plate. The protocol can be modified to only load and transform a set number of columns by changing the *columns* variable. The default is 12, ie. to transform an entire plate.



Python code:

# -\*- coding: utf-8 -\*-

"""

Created on Wed Mar 11 14:24:07 2020

@author: Lachlan Munro

"""

from opentrons import labware, instruments, robot

metadata = {

'protocolName': 'Step1\_Transform',

'author': 'Lachlan <lajamu@biosustain.dtu.dk>',

'source': 'Lachlan Munro'

}

#Enter number of columns to be transformed

columns = 12

#Load tipracks

tiprack1 = labware.load('opentrons-tiprack-300ul', '1')

tiprack2 = labware.load("geb\_96\_tiprack\_10ul", "2")

#load Pipettes

p10\_Multi = instruments.P10\_Multi(

mount = "left",

tip\_racks = [tiprack2])

p300\_Single = instruments.P300\_Single(

mount="right",

tip\_racks = [tiprack1])

#Overnight culture of Acineto Bayii in 50 mL Falcon tube, placed in an opentrons

#6-tube rack

culture = labware.load("opentrons\_6\_tuberack\_falcon\_50ml\_conical", "3")

#96 Deep-well plate filled with 450 uL of LB media.

media = labware.load("usascientific\_96\_wellplate\_2.4ml\_deep","6")

#PCR producst in 96 well format.

pcr = labware.load('biorad-hardshell-96-PCR', '5')

#Innoculate LB with 50 uL of culture.

well = columns\*8

p300\_Single.distribute(50, culture[0], media[0:well])

#Transfer PCR Products into culture

for col in range(0, columns):

p10\_Multi.transfer(2, pcr.cols(col), media.cols(col), new\_tip="always")