Rapid hierarchical assembly of medium-size DNA cassettes

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Supplementary Material

Supplementary Table 1. Description of templates used for the assemblies

Amplicon short identification	Full name of template	Function of the amplified fragment	Source of template
I-A	pTet-On Advanced	CMV-driven Constitutive expression of rtTA transactivator	Clontech
I-B	BAC CTD-3169N4	Inert spacer	BACPAC
I-C	TRE-dsRed-miR FF3	pTRE-driven synthetic microRNA FF3 fused in an intron to a DsRed fluorescent protein	(16)
I-D	BAC CTD-3169N4	Inert spacer	BACPAC
I-E	CMV-LacIKrab-FF3	CMV-driven repressor LacI-Krab fused with a target for a synthetic microRNA FF3	(17)
I-F	BAC CTD-3169N4	Inert spacer	BACPAC
I-G	CMV-LacO-ZsYellow1-FF4x3	LacI-controlled expression of ZsYellow fluorescent protein with a target for a synthetic microRNA FF4	(17)
I-H	BAC CTD-3169N4	Inert spacer	BACPAC
I-I	pAmCyan-C1	CMV-driven constitutive expression of AmCyan fluorescent protein	Clontech
BB2	BAC-FRT	An inducible copy-number back bone with an FRT site for potential site-specific integration in mammalian cells	This paper
BB1.1, BB1.2, BB1.3	pcDNA5/FRT	Bacterial origin of replication and antibiotic resistance	Invitrogen
R1-A, R3-A	pTet-On Advanced	CMV-driven Constitutive expression of rtTA transactivator	Clontech
R1-B, R3-B	p3513	Modified OriP for efficient episomal replication in eukaryotic cells	(14)
R1-C, R3-C	pTRE-Tight-EBNA1	Dox-inducible expression of EBNA1 which is responsible to replicate the OriP plasmid in eukaryotic cells	In preparation
BB-B	pRK5-GFP	Backbone for bacterial	(18)

		amplification	
R1-E, R3-D	pMACS Kk.II	Mouse MHC gene for surface expression and MACS enrichment of transfected cells	Miltenyi GmbH
BB-D (uses only the origin and antibiotic resistance), R2-D, R3-F	PL-SIN-EOS-C(3+)-EGFP	iPS-cell-specific expression of puromycin resistance and GFP for selection of reprogrammed cells	(12)
R3-E	pCAG2LMKOSimO	Expression of four factors Oct-4, Sox-2, Klf-4 and c-Myc as well as mOrange for reprogramming cells to an iPS state	(19)

Supplementary table 2. Templates and primer sequences for the Inverter Circuit.

Amplicon/ Template	Primer	Sequence
I-A/**pTet-On Advanced**	I-A_fwd	TTGTCTCTTGCTGGTGTTCGAAAAACTCGAGGAGCTTGGCCCATTGC
	I-A_rev	GCCTTTCTTGTTCCGGTGTTAAAAACTCCCCGCGCGTTGGC
I-B/**BAC CTD-3169N4**	I-B_fwd	AACACCGGAACAAGAAAGGCTTTTTTTCAAATTCAAATTCGACTCCACCTTTAAATGG
	I-B_rev	ACGCCCAACGAAAAAGAACCTTTTT GTCAGCTATAAAACTAGTTTTGTCA TCATTGTG
I-C/**TRE-dsRed-miR FF3**	I-C_fwd	GGTTCTTTTTCGTTGGGCGTAAAAAGCTTGATATCGAATTCCTGCAGCC
	I-C_rev	TTCGTTGCTTGCTGCCTCTCAAAAAGCCGCTCTAGAACTAGTGGATC
I-D/**BAC CTD-3169N4**	I-D_fwd	GAGAGGCAGCAAGCAACGAATTTTTGTAGAATTGTAGTCAAAGGGAACTG
	I-D_rev	CGAACACCAGCAAGAGACAATTTTTGTCAGTAGTCTTCCAGCCATTCAAC
I-E/**CMV-LacIKrab-FF3**	I-E_fwd	TTGTCTCTTGCTGGTGTTCGAAAAAGGATAACCGTATTACCGCCATGC
	I-E_rev	GCCTTTCTTGTTCCGGTGTTAAAAAGACAAACCACAACTAGAATGCAGTG
I-F/**BAC CTD-3169N4**	I-F-fwd	AACACCGGAACAAGAAAGGCTTTTTGGATAGATCTGCTCTCAAAAGATTG
	I-F_rev	ACGCCCAACGAAAAAGAACCTTTTTCTTGCACTAGACAGGGTTCTCC
I-G/**CMV-LacO-ZsYellow1-FF4x3**	I-G_fwd	GGTTCTTTTTCGTTGGGCGTAAAAACGTAATACGACTCACTATAGGGC
	I-G_rev	TTCGTTGCTTGCTGCCTCTCAAAAAGACAAACCACAACTAGAATGCAGTG

I-H/**BAC CTD-3169N4**	I-H_fwd	GAGAGGCAGCAAGCAACGAATTTTTGTCTAAACATAACTTCCCATGAAGC
	I-H_rev	CGAACACCAGCAAGAGACAATTTTTGAAACAGCACGAATACACAGTCAGG
I-I/**pAmCyan-C1**	I-I_fwd	same as E_fwd
	I-I_rev	same as E_rev
BB1.2/**pcDNA5/FRT**	BB1.2_fwd	GGTTCTTTTTCGTTGGGCGTAAAAGCAGGTGCTATGGCTTCTGAGGCGGAAAG
	BB1.2_rev	TTCGTTGCTTGCTGCCTCTCAAAAGCAGGTGGCGTATATCTGGCCCGTACATC
BB1.3/**pcDNA5/FRT**	BB1.3_fwd	AACACCGGAACAAGAAAGGCTTTTGCAGGTGCTTCTGAGGCGGAAAGAACCAG
	BB1.3_rev	ACGCCCAACGAAAAAGAACCTTTTGCAGGTGGCGTATATCTGGCCCGTACATC
BB2/**BAC-FRT**	BB2_fwd	AACACCGGAACAAGAAAGGCTTTTGCAGGTGCGAATTCGAGCTCGGTACCC
	BB2_rev	CGAACACCAGCAAGAGACAATTTTGCAGGTGCAATTCACTGGCCGTCGTTTTACAAC
BB1.1/**pcDNA5/FRT**	BB1.1_fwd	GAGAGGCAGCAAGCAACGAATTTTTCTTCTGAGGCGGAAAGAACCAG
	BB1.1_rev	CGAACACCAGCAAGAGACAATTTTTGCGTATATCTGGCCCGTACATC

Supplementary Table 3. Templates and primer sequences for the Reprogramming Circuit 1.

Amplicon/ Template	Primer Name	Sequence
R1-A/pTet-On Advanced	R1-A_fwd	CCACTCTCCATCAACACCTAGGGGCTCGAGGAGCTTG GCCCATTGC
	R1_A_rev	ATTCCCTCCACCTTAACACCGGGGCTCCCCGCGCGTTG GC
R1-B/p3513	R1-B_fwd	GGTGTTAAGGTGGAGGGAATCCCCGATCTCTCGAGTC TAGAGGCCTGACTG
	R1-B_rev	GTATTTGGTAGGGAGAGGTTCCCCGTATCACGAGGCC CTTTCGTCTTCAAG
R1-C/pTRE-tight-EBNA1	R1-C_fwd	AACCTCTCCCTACCAAATACGGGGCGCCACCTCGACA TACTCGAG
	R1-C_rev	CCTACCATCCATCATTCTCTGGGGGAGTCAGTGAGCGA GGAAGCTCG
BB-B/pRK5-GFP	BB-B_fwd	AGAGAATGATGGATGGTAGGCCCCGCAGGTGCACTCT CAGTACAATCTGCTCTGATGC
	BB-B_rev	TAGGTGTTGATGGAGAGTGGCCCCGCAGGTG CCACACCCTAACTGACACACATTCC
BB-D/PL-SIN-EOS-C(3+)-EGFP	BB-D fwd	TAGGTGTTGATGGAGAGTGGCCCCGAAAGAACATGTG AGCAAAAGGCCAG
	BB-D rev	GTATTTGGTAGGGAGAGGTTCCCCCAGGTGGCACTTTT CGGGGAAATG
R1-D/ pCAG2LMKOSimO	R1-D_fwd	AGAGAATGATGGATGGTAGGCCCCGAAGAATCTGCTT AGGGTTAGGCGTTTTGC
	R1-D_rev	GGTGTTAAGGTGGAGGGAATCCCCGCTGGTTCTTTCCG CCTCAGAAGC
R1-E/pMACS Kk.II	R1-E_ fwd	AACCTCTCCCTACCAAATACGGGGGCTAGCTTATCGGC CCATGACATTAAC
	R1-E_rev	ATTCCCTCCACCTTAACACCGGGGGCATTGGTAACTGT CAGACCAAGTTTACTC

Supplementary Table 4. Templates and primer sequences for the Reprogramming Circuit 2.

Amplicon/ Template	Primer name	Sequence
R2-D/PL-SIN-EOS-C(3+)-EGFP	R2-D_fwd	AGAGAATGATGGATGGTAGGCCCCCTGGGGATTTGGGGTTGCTCTG
	R2-D_rev	GTATTTGGTAGGGAGAGGTTCCCCCAGGTGGCACTTTTCGGGGAAATG
R2-E/pMACS Kk.II	R2-E_fwd	AACCTCTCCCTACCAAATACGGGGGCTAGCTTATCGGCCCATGACATTAAC
	R2-E_rev	ATTCCCTCCACCTTAACACCGGGGGCATTGGTAACTGTCAGACCAAGTTTACTC
R2-F/ pCAG2LMKOSimO	R2-F_fwd	TAGGTGTTGATGGAGAGTGGCCCCGAAGAATCTGCTTAGGGTTAGGCGTTTTGC
	R2-F_rev	GGTGTTAAGGTGGAGGGAATCCCCGCTGGTTCTTTCCGCCTCAGAAGC

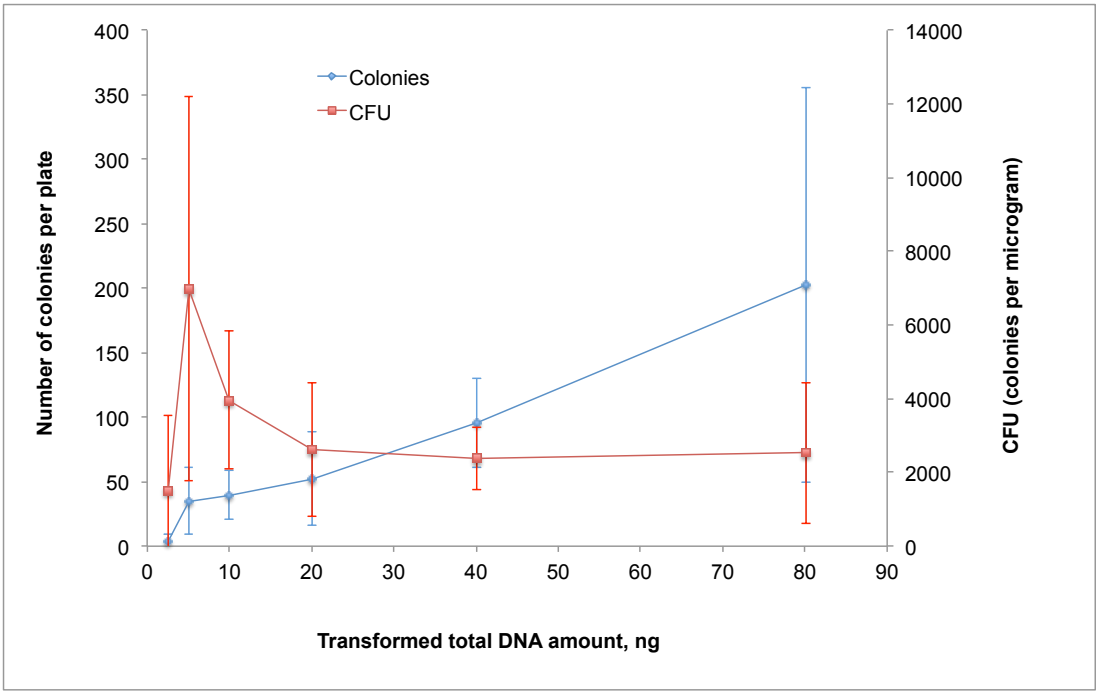
Supplementary Table 5. Templates and primer sequences for the Reprogramming Circuit 3.

Amplicon/ Template	Primer Name	Sequence
R3-A/pTet-On Advanced	R3-A_fwd	AACCTCTCCCTACCAAATACGGGTTAATTAACTCTCCCTACCAAATACGGGCTCGAGGAGCTTGGCCCATTGC
	R3-B_rev	CCTACCATCCATCATTCTCTGGGCTCCCCGCGCGTTGGC
R3-B/p3513	R3-B_fwd	AGAGAATGATGGATGGTAGGCCCTGGCTTAACTATGCGGCATCAGAG
	R3-B_rev	TAGGTGTTGATGGAGAGTGGCCCGATCTCTCGAGTCTAGAGGCCTGACTG
R3-C/pTRE-Tight-EBNA1	R3-C_fwd	CCACTCTCCATCAACACCTAGGGTATTACCGCCTTTGAGTGAGCTGATAC
	R3-C_rev	ATTCCCTCCACCTTAACACCGGGACCGGTGAATTCTCCAGGCGA
R3-D/ pMACS Kk.II	R3-D_fwd	GGTGTTAAGGTGGAGGGAATCCCATGGCACCCTGCATGCTG
	R3-D_rev	GTATTTGGTAGGGAGAGGTTCCCTTAATTAACTCTCCATCAACACCTAGGGCTAGCTTATCGGCCAATTCGGATC
Spacer	Sense oligo	AGAGAATGATGGATGGTAGGCCCCGAAGAATCTGCTTAGGGTTAGGCGTTTTGC
	Antisense oligo	GTATTTGGTAGGGAGAGTTAGCAAAACGCCTAACCCTAAGCAGATTCTTCGGGG
R3-E/ pCAG2LMKOSimO	R3-E_fwd	TAGGTGTTGATGGAGAGTGGCCCCGAAGAATCTGCTTAGGGTTAGGCGTTTTGC
	R3-E_rev	GGTGTTAAGGTGGAGGGAATCCCCGCTGGTTCTTTCCGCCTCAGAAGC
R3-F/PL-SIN-EOS-C(3+)-EGFP	R3-F_fwd	ATTCCCTCCACCTTAACACCGGGGCTGGGGATTTGGGGTTGCTCTG
	R3-F_rev	CCTACCATCCATCATTCTCTGGGCACACAGGAACAGCTATGACCATGATTAC

Supplementary Table 6: Sequencing analysis

Clone	Sequencing coverage [%]	Mutations	Mutated Sequence	Comment
1	98.26	0		
2	99.32	1	CGCTCACTGACTCCC T CAGAGAATGATGGAT	Stop sequence, probably primer mutation
3	98.84	0		
4	98.20	0		
5	99.92	0		
6	99.57	1	ACAGCAGAAACATAC G AGCTGTCAGCTTTGC	Probably due to PCR mutation
7	97.95	0		
8	99.20	0		
9	94.22	0		
10	96.78	0		

10 clones of the first-level assembly of Reprogramming circuit 1 were subject to Sanger sequencing using 16 different sequencing primers. The resulting sequence files were aligned to an *in-silico* assembled sequence of the construct with an in-house alignment software written in C++, which is available upon request. Shown are the percentages of the expected sequence covered by one or more resulting sequences. Also shown are the number and details of encountered mutations.



Supplementary Figure 1. Cloning efficiency data. The first-level assembly of Reprogramming circuit 1 was conducted using different concentrations of the individual fragment DNAs in the chewback reactions. The experiment was done in quadruplicates. Shown are the mean colony numbers +/- the standard deviation of this number observed on the agar plates after 16h (blue) and the calculated cloning efficiency in colonies per microgram of transformed DNA (red).