RapGene

Zampini, Massimiliano; Rees Stevens, Pauline; Pachebat, Justin; Kingston-Smith, Alison; Mur, Luis; Hayes, Finbarr

Published in:
Scientific Reports

DOI:
10.1038/srep11302

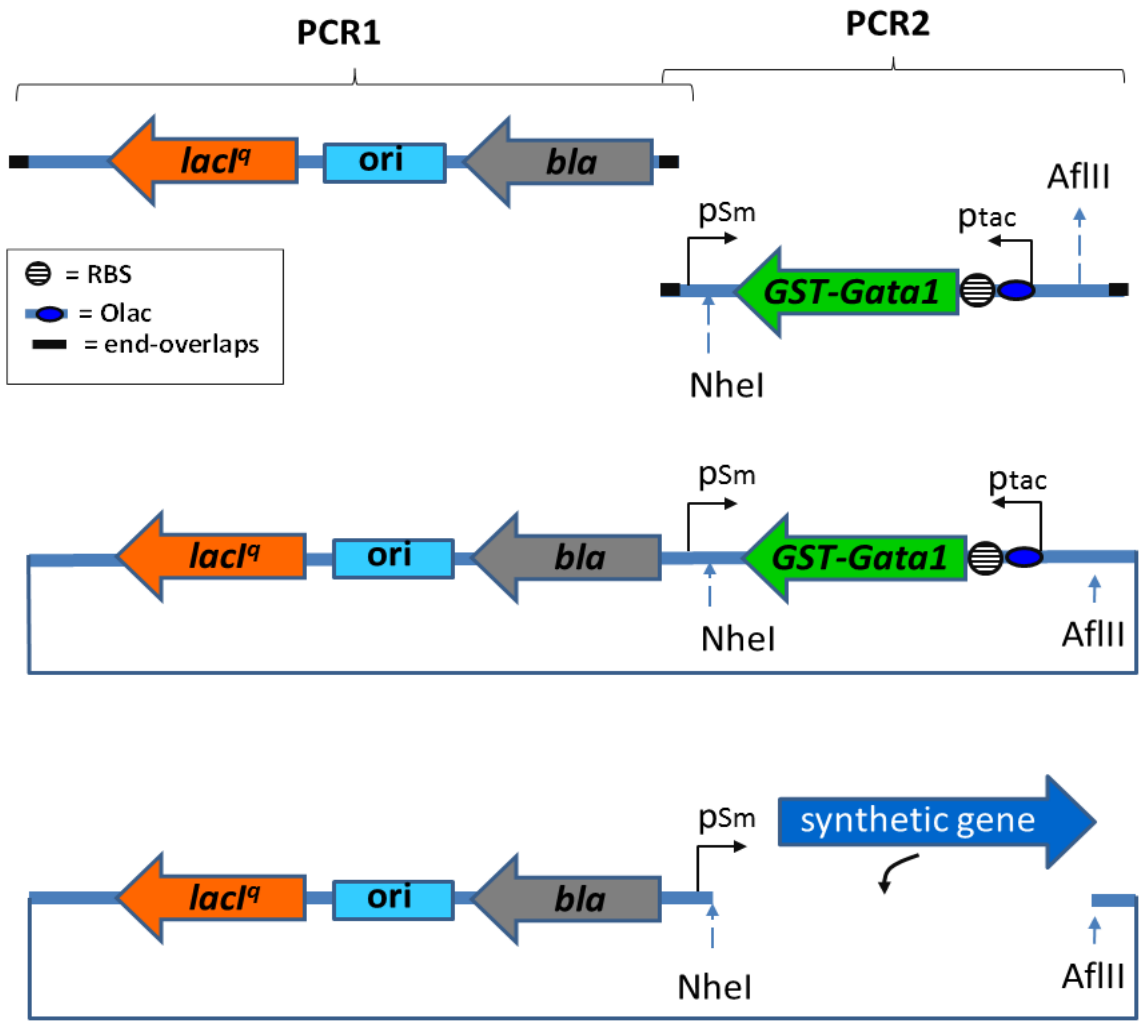
Publication date:
2015

Citation for published version (APA):
Zampini, M., Rees Stevens, P., Pachebat, J., Kingston-Smith, A., Mur, L., & Hayes, F. (2015). RapGene: a fast and accurate strategy for synthetic gene assembly in Escherichia coli. *Scientific Reports*, *5*, [11302]. https://doi.org/10.1038/srep11302

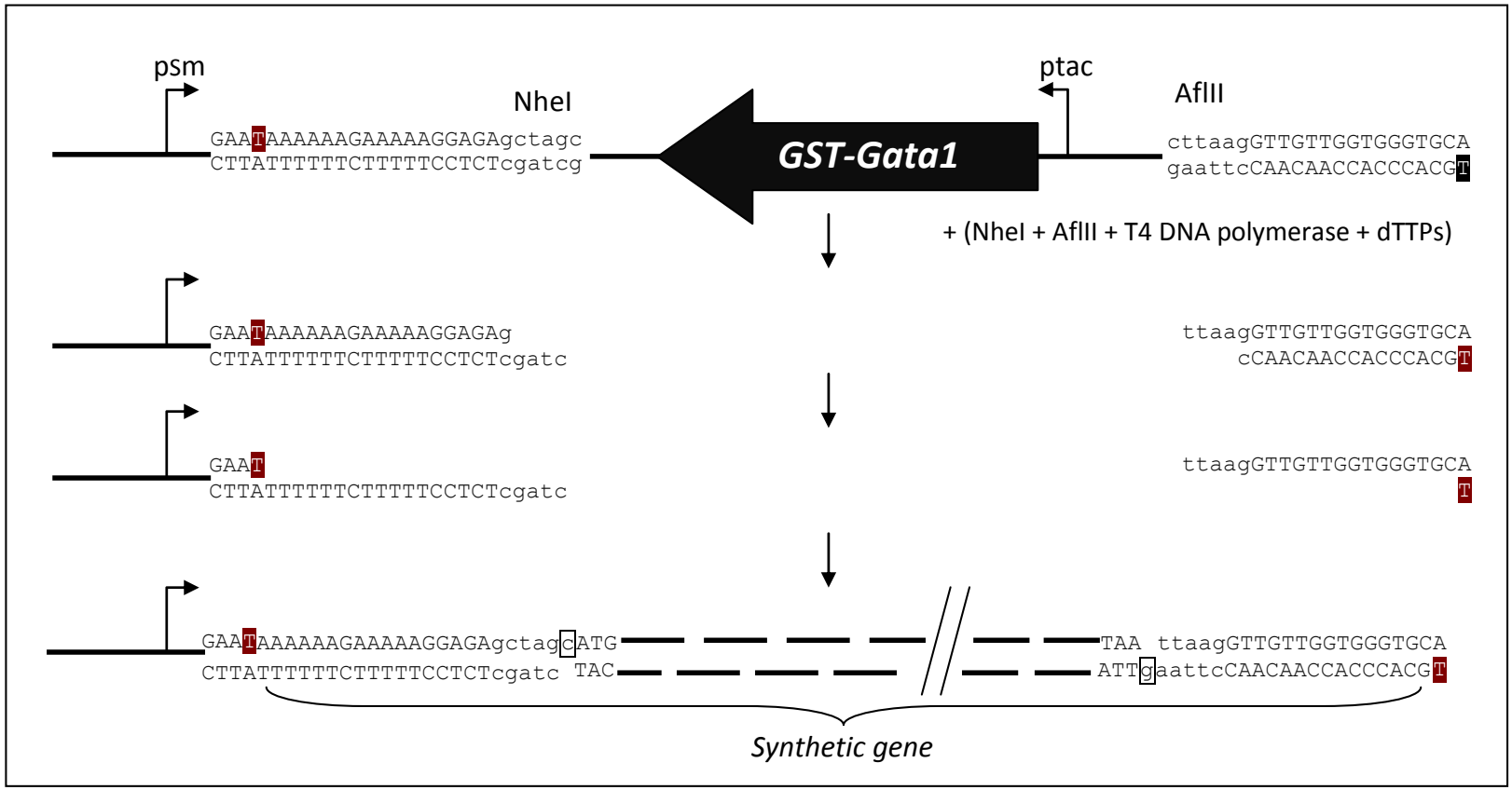
Supplementary Information

RapGene: a fast and accurate strategy for synthetic gene assembly in *Escherichia coli*

Massimiliano Zampini, Pauline Rees Stevens, Justin A. Pachebat, Alison Kingston-Smith, Luis A. J. Mur & Finbarr Hayes



Supplementary Figure S1. pRG1.0 assembly and structure (not to scale). Plasmid pRG1.0 was assembled from two PCR products derived from pGATA using the Gibson Assembly Cloning Kit. pSm, streptomycin/spectinomycin promoter from pCDFDuet-1. ptac, tac promoter. RBS, ribosome binding site. Olac, lac operator. *bla*, beta-lactamase gene. ori, origin of replication. *lacI*^{q}, lac repressor gene with a modified promoter. NheI, restriction site for NheI. AflII, restriction site for AflII. End-overlaps, homology regions required by the Gibson assembly cloning kit.



Supplementary Figure S2. pRG1.0 cloning region (not to scale). Plasmid pRG1.0 is prepared to generate long 3'-recessed cohesive ends by digesting with AflII and NheI in the presence of T4 DNA polymerase and 1 mM dTTPs. The reaction is stopped by spin-column purification. The single-nucleotide gap (boxed on the complementary strand) between the assembled synthetic insert and the vector, produced in all inserts after annealing, is filled *in vivo* in *E. coli*. Dashed lines represent the single oligonucleotides within the synthetic gene.

Name	Sequence (5'-3')	Restriction sites	Signals	Notes
PCR1f	ACAGCTCActtaagGTTGTTGGTGGGTGCAGCCGGAAGCATAAAGTGTAAAGCC	AflII		Template for PCR: pGATA
PCR1r	CCTAGGTTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATACCGGTACCTCTGACACATGCAGCTCCCGGAG		- promoter (-35 and -10 regions)	Template for PCR: pGATA
PCR2f	TCATGAGACAATAACCCTGATAAACCTAGGAATAAAAAAGAAAAAGGAGAgctagcTTTCACCGTCATCACCGAAACGCGCGAGGCAGATCGTCAG	NheI	- promoter (-10 region)	Template for PCR: pGATA
PCR2r	TGCACCCACCAACAACcttaagTGAGCTGTTGACAATTAATCATCGGCTCG	AflII		Template for PCR: pGATA
Sm-1	AAAAAAGAAAAAGGAGAgctagcATGAGGGAAGCGGTGATCGCCGAAGTATCGACTCAAC	NheI		top strand
Sm-2	GACGCCAACTACCTCTGATAGTTGAGTCGATACTTCGGCGATCACCGCTTCCCTCAT			bottom strand
Sm-3	TATCAGAGGTAGTTGGCGTCATCGAGCGCCATCTCGAACCGACGTTGCTGGCCGTACA			top strand
Sm-4	CCACTGCGGAGCCGTACAAATGTACGGCCAGCAACGTCGGTTCGAGATGGCGCTCGAT			bottom strand
Sm-5	TTTGTACGGCTCCGCAGTGGATGGCGGCCTGAAGCCACACAGTGATATTGATTTGCTG			top strand
Sm-6	AGCCTTACGGTCACCGTAACCAGCAAATCAATATCACTGTGTGGCTTCAGGCCGCCAT			bottom strand
Sm-7	GTTACGGTGACCGTAAGGCTTGATGAAACAACGCGGCGAGCTTTGATCAACGACCTTT			top strand
Sm-8	AGGGGAAGCCGAAGTTTCCAAAAGGTCGTTGATCAAAGCTCGCCGCGTTGTTTCATCA			bottom strand
Sm-9	TGGAAACTTCGGCTTCCCCTGGAGAGAGCGAGATTCTCCGCGCTGTAGAAGTCACCAT			top strand
Sm-10	TGATGTCGTCGTGCACAACAATGGTGACTTCTACAGCGCGGAGAATCTCGCTCTCTCC			bottom strand
Sm-11	TGTTGTGCACGACGACATCATTCCGTGGCGTTATCCAGCTAAGCGCGAACTGCAATTT			top strand

Sm-12	TCATTGCGCTGCCATTCTCCAAATTGCAGTTCGCGCTTAGCTGGATA ACGCCACGGAA			bottom strand
Sm-13	GGAGAATGGCAGCGCAATGACATTCTTGCAGGTATCTTCGAGCCAG CCACGATCGACA			top strand
Sm-14	CAGCAAGATAGCCAGATCAATGTCGATCGTGGCTGGCTCGAAGATAC CTGCAAGAATG			bottom strand
Sm-15	TTGATCTGGCTATCTTGCTGACAAAAGCAAGAGAACATAGCGTTGCC TTGGTAGGTCC			top strand
Sm-16	CAAAGAGTTCCTCCGCCGCTGGACCTACCAAGGCAACGCTATGTTCT CTTGCTTTTGT			bottom strand
Sm-17	AGCGGCGGAGGAACTCTTTGATCCGGTTCCTGAACAGGATCTATTTG AGGCGCTAAAT			top strand
Sm-18	TTCCATAGCGTTAAGGTTTCATTTAGCGCCTCAAATAGATCCTGTTCA GGAACCGGAT			bottom strand
Sm-19	GAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTGGCGATG AGCGAAATGTAG			top strand
Sm-20	AATGCGGGACAACGTAAGCACTACATTTCGCTCATCGCCAGCCCAGT CGGGCGGCGAG			bottom strand
Sm-21	TGCTTACGTTGTCCCGCATTTGGTACAGCGCAGTAACCGGCAAAATC GCGCCGAAGGA			top strand
Sm-22	TTGCCCAGTCGGCAGCGACATCCTTCGGCGCGATTTTGCCGGTTACT GCGCTGTACCA			bottom strand
Sm-23	TGTCGCTGCCGACTGGGCAATGGAGCGCCTGCCGGCCCAGTATCAG CCCGTCATACTT			top strand
Sm-24	AGATAAGCCTGTCTAGCTTCAAGTATGACGGGCTGATACTGGGCCG GCAGGCGCTCCA			bottom strand
Sm-25	GAAGCTAGACAGGCTTATCTTGGACAAGAAGAAGATCGCTTGGCCTC GCGCGCAGATC			top strand
Sm-26	GTGGACAAATTCTTCCAACTGATCTGCGCGCGAGGCCAAGCGATCTT CTTCTTGTCCA			bottom strand
Sm-27	AGTTGGAAGAATTTGTCCACTACGTGAAAGGCGAGATCACCAAGGTA GTCGGCAAATAA		- *aadA1* stop codon	top strand
Sm-28	GCACCCACCAACAACcttaagTTATTTGCCGACTACCTTGGTGATCTCG CCTTTCACGTA	AflII	- *aadA1* stop codon	bottom strand
Sm-A1	GCACCCACCAACAACcttaagCAGCAAATCAATATCACTGTGTGGCTTC	AflII		bottom strand

	AGGCCGCCAT			
Sm-A2	GCACCCACCAACAACcttaagAAATTGCAGTTCGCGCTTAGCTGGATAA CGCCACGGAA	AflII		bottom strand
Sm-A3	GCACCCACCAACAACcttaagATTTAGCGCCTCAAATAGATCCTGTTCA GGAACCGGAT	AflII		bottom strand
Sm-A4	GCACCCACCAACAACcttaagAAGTATGACGGGCTGATACTGGGCCGG CAGGCGCTCCA	AflII		bottom strand
GFP-1	AAAAAAGAAAAAGGAGAgctagcATGAGTAAAGGAGAAGAACTTTTCAC TGGAGTTGTCCCAATTC	NheI		top strand
GFP-2	TTTGCCCATTAACATCGCCATCTAATTCAACAAGAATTGGGACAACTC CAGTGAAAAGTTCTTCTCCTTTACTCAT			bottom strand
GFP-3	TTGTTGAATTAGATGGCGATGTTAATGGGCAAAAATTCTCTGTCAGTG GAGAGGGTGAAGGTGATG			top strand
GFP-4	AAATAAATTTAAGGGTAAGTTTTCCGTATGTTGCATCACCTTCACCCT CTCCACTGACAGAGAATT			bottom strand
GFP-5	CAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGGAAGC TACCTGTTCCATGGCCAA			top strand
GFP-6	ATTGAACACCATAAGAGAAAGTAGTGACAAGTGTTGGCCATGGAACA GGTAGCTTCCCAGTAGTGC			bottom strand
GFP-7	CACTTGTCACTACTTTCTCTTATGGTGTTCAATGCTTTTCAAGATACC CAGATCATATGAAACAGC			top strand
GFP-8	AACCTTCGGGCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGAT CTGGGTATCTTGAAAAGC			bottom strand
GFP-9	ATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGA ACTATATTTTACAAAGATG			top strand
GFP-10	ACTTGACTTCAGCACGTGTCTTGTAGTTCCCGTCATCTTTGTAAAATA TAGTTCTTTCCTGTACAT			bottom strand
GFP-11	ACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACC CTTGTTAATAGAATCGAGT			top strand
GFP-12	TGTTTCCATCTTCTTTAAAATCAATACCTTTTAACTCGATTCTATTAAC AAGGGTATCACCTTCAA			bottom strand
GFP-13	TAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAAA TGGAATACAACTATAACT			top strand
GFP-14	TTGGTTTGTCTGCCATGATGTATACATTATGTGAGTTATAGTTGTATTC CATTTTGTGTCCAAGAA			bottom strand

GFP-15	CACATAATGTATACATCATGGCAGACAAACCAAAGAATGGAATCAAA GTTAACTTCAAAATTAGAC			top strand
GFP-16	CTGCTAATTGAACGCTTCCATCTTTAATGTTGTGTCTAATTTTGAAGTT AACTTTGATTCCATTCT			bottom strand
GFP-17	ACAACATTAAAGATGGAAGCGTTCAATTAGCAGACCATTATCAACAAA ATACTCCAATTGGCGATG			top strand
GFP-18	ACAGGTAATGGTTGTCTGGTAAAAGGACAGGGCCATCGCCAATTGGA GTATTTTGTTGATAATGGT			bottom strand
GFP-19	GCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCC CTTTCCAAAGATCCCAACG			top strand
GFP-20	CAAACTCAAGAAGGATCATGTGATCTCTCTTTTCGTTGGGATCTTTGG AAAGGGCAGATTGTGTGG			bottom strand
GFP-21	AAAAGAGAGATCACATGATCCTTCTTGAGTTTGTAACAGCTGCTGGG ATTACACATGGCATGGATGAACTATACAAATAA		*gfp* stop codon	top strand
GFP-22	GCACCCACCAACAACcttaagTTATTTGTATAGTTCATCCATGCCATGTG TAATCCCAGCAGCTGTTA	AflII	*gfp* stop codon	bottom strand
RG1.0f	CTCCGGGAGCTGCATG			
RG1.0r	GGCACGACAGGTTTCCCG			

Supplementary Table S1. Oligonucleotides used in this study. Sequences designed to anneal to the DNA template in PCR are underlined. Boxed regions correspond to the homology sequences required by the Gibson Assembly Cloning Kit to produce pRG1.0. Sequences highlighted in black represents signals (i.e. promoters, stop codons). Restriction sites are indicated in lower case.

***aadA1*-assembly**					
Type of assembly	***E. coli* strain**	**sample**	**Cycle 0**	**Cycle 1**	**Cycle 2**
Oligonucleotides annealed simultaneously	NEB5α	*aadA1* (6-oligonucleotides)	100%	100%	90%
Oligonucleotides annealed simultaneously	NEB5α	*aadA1* (12-oligonucleotides)	100%	100%	100%
Oligonucleotides annealed simultaneously	NEB5α	*aadA1* (18-oligonucleotides)	80%	70%	70%
Oligonucleotides annealed simultaneously	NEB5α	*aadA1* (24-oligonucleotides)	-	20%	-
Oligonucleotides annealed simultaneously	NEB5α	*aadA1* (28-oligonucleotides)	-	20%	10%
***gfp*-assembly**					
Type of assembly	***E. coli* strain**	**sample**	**Cycle 0**	**Cycle 1**	**Cycle 2**
Oligonucleotides annealed simultaneously	NEB5α	*gfp* (22-oligonucleotides)	44%	20% 15% 15%	-
Oligonucleotides pre-annealed separately in three groups	NEB5α	*gfp* (22-oligonucleotides)	-	20% 15% 10%	-

Supplementary Table S2. RapGene *aadA1* and *gfp* cloning efficiencies during the first two freezing-thawing cycles of the oligonucleotides used for the corresponding assemblies. Percentages of correct-size inserts as determined by colony PCR are reported. The table also reports yields for the assembly performed with three groups of consecutive oligonucleotides pre-annealed separately.