Cloning and Expression of Recombinant Proteins

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May 29, 2013, the question how DNA can be shuffled between organisms for biotech purposes caused a lot of speechlessness... I concluded that it is helpful to review the process systematically.
Natural plasmid are huge and contain a multitude of genes. E.g., the plasmid associated with the entero-hemolytic *E. coli* strain O157 is more than 90 kb (kilo-basepairs) long and contains genes for its own replication, transduction into an acceptor strain, pili, pathogeneity factors, and other.
Natural plasmid are huge and contain a multitude of genes. For biotechnological purposes, these plasmids have been stripped down to their essentials.
Plasmids use in biotech have been stripped down to their essentials. The minimal requirement comprises:

- the origin of replication (in most cases ColEI, which works by priming of DNA synthesis through RNA II and its counter-acting anti-sense RNA I, regulated by the 4-helix protein rop),

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Bacterial plasmids
DNA isolation
Methods to introduce DNA into bacteria
Restriction-enzyme based cloning
PCR
DNA Sequencing
Yeast genetics
Eukaryotic protein expression systems
Transfection of cell culture cells
Insect cell expression
Summary

Plasmids use in biotech have been stripped down to their essentials. The minimal requirement comprises: the origin of replication, an antibiotics resistance gene (here: the ampicillin resistance, based on a protein called beta-lactamase),
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Mittwoch, 19. Juni 2013

Plasmids use in biotech have been stripped down to their essentials. The minimal requirement comprises: the origin of replication, an antibiotics resistance gene (here: the ampicillin resistance, based on a protein called beta-lactamase), a multiple cloning (sometimes also called „polycloning“) site,

2686 bp
< 5 coding sequences

β-lactamase
(confering ampR)

multiple cloning site

origin of replication

ampl (beta-lactamase)

2686 bp

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- The origin of replication,
- An antibiotic resistance gene (here: the ampicillin resistance, based on a protein called beta-lactamase),
- A multiple cloning (sometimes also called „polycloning“) site,
- And (optional) selection markers that help identifying correct clones.
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- and (optional) selection markers that help identifying correct clones.

Plasmids typically vary in size, but the plasmid shown here is 2686 bp long and contains:

- < 5 coding sequences
- β-lactamase (confering ampR)
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Plasmids
(symbiotic, see right)
$10^3$-$10^5$ bp size
multiple, 1-200/cell)

Chromosome
~$5 \times 10^6$ bp size
(symbiotic; 1/cell)
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Membrane Lipids

Proteins

Carbohydrates
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Membrane Lipids

Proteins

Carbohydrates

SDS
sodium docecyl sulfate

SDS
NaOH, pH 10
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http://www.embl.de/pepcore/pepcore_services/cloning/cloning_methods/index.html
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