The Tools

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E.-K. Ehmoser-Sinner, C.-W. D. Tan, *Lessons on Synthetic Bioarchitectures*, Learning Materials in Biosciences, https://doi.org/10.1007/978-3-319-73123-0_5

What You Will Learn in This Chapter

The ambitious nature of projects in synthetic biology requires special methods to match them. For those projects that require extensive modification of genes and others perhaps whole genomes, conventional techniques used in molecular biology have to be improved to meet their needs. These needs include the necessity for gene modification methods to be reliable, easy to handle, and compatible between laboratories. This implies the need for certain standards, particularly for the materials and methods used. One approach to achieve this is to reduce the genetic material manipulated to highly interchangeable and interconnectable modules. We will look at how BioBricks allow us to do this. Another requirement is that the techniques used be precise and capable of large-scale changes to the target genetic material. We will see how the CRISPR/Cas9 system was developed to meet this need, as well as the range of DNA modifications it is capable of. Finally, the hazards posed by both technologies, as well as how those concerned have chosen to deal with them, are discussed.

5.1 Modularity and Standardization

As we have seen, synthetic biology hopes to play a significant role in the pharmaceutical industry. There are attempts to create novel antibiotics to tackle the challenge of rising resistance, new kinds of implants that would be more biocompatible, and new ways to target drugs more effectively, among other projects.

However, a technology's quality has to be taken to a higher level whenever it reaches industry. This is for reasons of safety, reliability and, very importantly, compatibility. When whole systems have to be redesigned to produce a new but related product, significant resources are wasted. Compatibility reduces this problem. Consider the Luer taper standard. As long as components contain the standard Luer male part, they can fit any other component that carries the Luer female part. This allows us to mix and match different components, easily giving us a wide range of solutions to a problem.

Synthetic biology tries to address this issue by applying principles of engineering such as abstraction and modulation. Complex systems are gradually built up from simpler systems and these from simpler parts. At each step, the materials, products, and processes have to meet rigorous standards of quality and reproducibility.

5.2 Reliability and Compatibility in Molecule Biology

Synthetic biology began with, and is still largely concerned with, creating novel biological functions in existing organisms. To do this rationally, one needs to use an engineering approach. Very simply put, this involves identifying a problem, designing a solution, modeling the solution mathematically or on a small scale, testing the solution, and identifying any new problems. This is iterated until the original problem is solved. One way to make this process more efficient is to build in certain levels of reliability so that certain tests of quality need not be repeated.

5.3 Establishing Standards

Consider this problem in the case of molecular biology. This technique is critical to those synthetic biology projects that rely heavily on manipulating genes and gene expression, such as pathway engineering. To make such endeavors more reproducible and, generally, less difficult for both veterans and novices alike, it would be useful if standardized parts, tools, and processes were available.

In this case, our products are vectors and the parts in question are DNA fragments. Each fragment would have a specific function, such as a promoter, ribosome binding site, forward transcription start signal, stop signal, and so on. Our tools would be enzymes and host cells. Our processes would include DNA restriction, ligation, transformation, host selection, and so on.

These are, of course, established techniques in molecular biology. However, it is often the case that one lab uses an entirely different set of materials, tools, and processes from another. An example of how this diversity affects compatibility between collaborating laboratories is that often a vector supplied by one partner is not compatible with a downstream process used by the other. As a result, the desired genes often have to be recloned.

On the other hand, if we have a standard set of vector parts that can be combined using a standard set of tools and processes, any two laboratories using these standards will be able to share materials and techniques much more easily. This will mean greater versatility in finding solutions for cloning problems. Most importantly, it will save time and effort.

5.4 The BioBrick Standard

In 2003, Thomas Knight from the Artificial Intelligence Laboratory at the Massachusetts Institute of Technology (MIT) proposed a method of standardizing the structure of DNA fragments in order to standardize their handling and use. He called this the "BioBrick standard for physical composition of biological parts." He proposed flanking DNA fragments with EcoRI and XbaI restriction sites upstream and SpeI and PstI downstream (Fig. 5.1).This constitutes a BioBrick insert. Every insert is carried by a BioBrick vector.

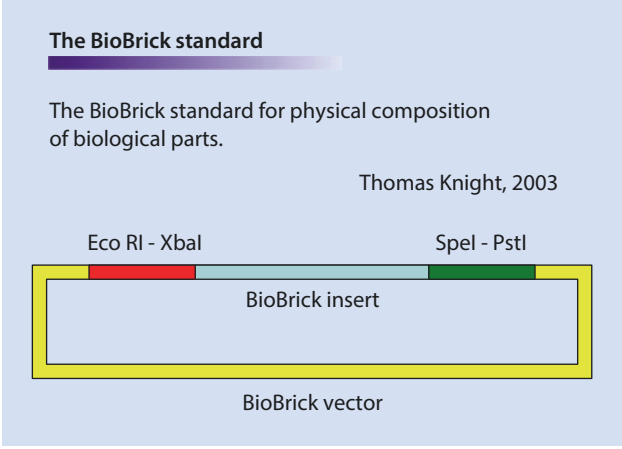

Fig. 5.1 The figure shows the common features of a basic BioBrick. Note the presence of the two sets of paired restriction enzymes. The EcoRI restriction site is just upstream of the XbaI restriction site and, after a stretch of DNA, the SpeI restriction site is found just upstream of the PstI restriction site. Each BioBrick part comprises a segment of DNA flanked by the EcoRI-XbaI and SpeI-PstI restriction site pairs

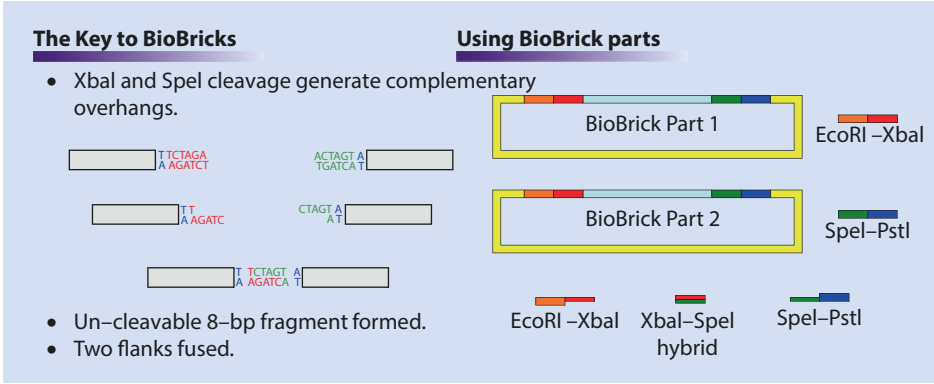

Fig. 5.2 The key to the BioBrick standard – XbaI and SpeI. Restriction of the XbaI (*red*) and SpeI (*green*) recognition sites results in complementary overhangs. This allows a cleaved XbaI site to anneal with a cleaved SpeI site. Once ligated, the resultant hybrid element will no longer be recognised by either XbaI or SpeI

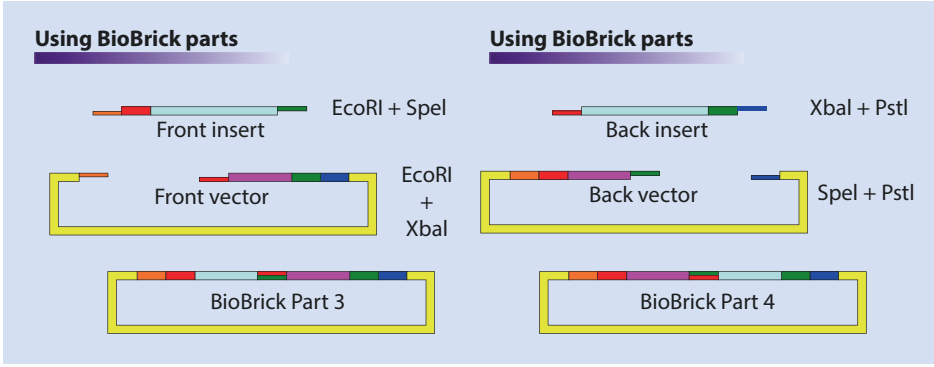

Fig. 5.3 The figure illustrates how BioBricks are used. Basic BioBrick parts can be combined in different ways to produce a more complex BioBrick part, comprising two former BioBrick parts. The choice of whether to use EcoRI or PstI in addition to the special pair of XbaI and SpeI determines whether a BioBrick will be spliced upstream or downstream of the target BioBrick part. Most importantly, the combined parts will be separated by an un-cleavable hybrid XbaI-SpeI element and *still* flanked by the EcoRI-XbaI and SpeI-PstI restriction site pairs. In other words, this new combination of parts is, itself, a BioBrick part

These restriction sites were selected for a special reason—XbaI and SpeI restriction enzymes generate complementary overhangs that can base pair promiscuously. However, once ligated, neither enzyme will be able to cleave the resulting eight-base-pair element (Fig. 5.2).

Use of these restriction enzymes, in specific combinations with either EcoRI or PstI, allows one to insert a foreign BioBrick insert upstream or downstream from an existing BioBrick insert (Fig. 5.3). Most importantly, the new compound insert will *still* carry EcoRI and XbaI restriction sites upstream and SpeI and PstI downstream. So, it too is a BioBrick part.

Restricting BioBrick Part 1 with EcoRI and SpeI will release a front insert from the donor vector. Restricting BioBrick Part 2 with EcoRI and XbaI will create an acceptor vector. The restriction digestions generate complementary overhangs, which will result in the front vector inserting upstream of BioBrick Part 2. This creates a third BioBrick part.

Conversely, restricting BioBrick Part 1 with XbaI and PstI will release a back insert from the donor vector. Restricting BioBrick Part 2 with SpeI and PstI will create an acceptor vector. The restriction digestions generate complementary overhangs, which will result in the back vector inserting downstream of BioBrick Part 2. This creates a fourth BioBrick part.

In this way, a growing collection of BioBrick parts, each compatible with any other, can be obtained. These could be used and modified just as easily by any other laboratory using the same standard—the BioBrick Assembly Standard 10. Such laboratories could form highly versatile and compatible networks.

5.4.1 Parts, Devices, and Systems

Next, Knight went on to establish the Registry of Standard Biological Parts. This is a special bank where BioBrick material of all kinds is deposited either as sequence information or as physical DNA fragments. All deposits in the registry are organized in different ways. They can be grouped according to function, species, encoded content, and so on. The simplest means is to rank them by complexity into parts, devices, and systems.

A part is basically a fragment of DNA with a defined function, such as a promoter, protein coding sequence, or terminator. One could recombine specific parts to create a higher function. An example would be to recombine BioBrick promoters and coding sequences to create genes. Such a combination of parts is called a BioBrick device. BioBrick devices can, in turn, be recombined to create a collection of related functions, such as in an expression vector where several tasks including host selection, protein expression, and replication are performed. Such a recombination of devices is called a system.

The important thing is that each BioBrick can be combined with any other BioBrick in the registry since they are all produced using the same standards. In this way, even BioBrick devices will be compatible with each other and BioBrick systems will have extremely exchangeable components for increased versatility.

Registered users can freely use the BioBrick parts from the registry so long as the contributor is acknowledged. In turn, registered contributors can add parts to the registry, so long as they adhere to the BioBrick standard and promise not to enforce intellectual rights on registered users. They must also ensure that their parts adhere to BioBrick Assembly Standard 10.

5.4.2 The BioBrick Foundation

In 2005, Drew Endy, who had worked closely with Thomas Knight, founded the BioBrick Foundation. This is an organization that allows the synthetic biology community to share ideas, resources, and activities, including teaching and training. It also organizes the annual International Genetically Engineered Machine (iGEM) competition, where undergraduate students are given actual BioBrick parts and challenged to produce something useful from them.

The goal of this foundation is to create a community wherein synthetic biology is conducted ethically and with sincere aims, in a free, safe, and effective manner. They hope

that this will result in work that will benefit mankind and our world in general. It brings together engineers, scientists, lawyers, students, teachers, and laypersons, encouraging them to work toward these ideals.

5.4.3 iGEM

This activity is best represented by the annual iGEM students' course, and later competition, begun at MIT in 2003. Teams are formed and each is given a package of BioBrick parts, which they must use to create novel BioBrick systems. In this way, the students will contribute, through high-quality work, to the development and future of synthetic biology.

Summary

The BioBrick standard embodies the synthetic biology ideal of applying engineering rigor to biology. Ultimately, the aim is to know as much as possible about the materials used, the tools needed, and the processes employed. This knowledge allows us to control the quality of the work done and will allow standards to be set and met on reliability, ease, versatility, ethical research, and safety.

There are other similar attempts to standardize molecular biology. Just like the BioBrick Assembly Standard 10, each has its advantages and disadvantages. Casini et al. provided a review of these methods in their 2015 article titled "Bricks and Blueprints: Methods and Standards for DNA Assembly."

5.5 Discovery of the CRISPR/Cas Immune System

It was 1987 when Japanese scientist Yoshizumi Ishino stumbled across a very interesting gene locus in *Escherichia coli*. This is a region made up of a series of DNA repeats. These were also observed by Spanish scientist Francisco Mojica, who decided to call the locus SRSR for "short regularly spaced repeats." He later suggested calling it CRISPR, which stands for "clustered regularly interspaced short palindromic repeats" (Fig. 5.4).

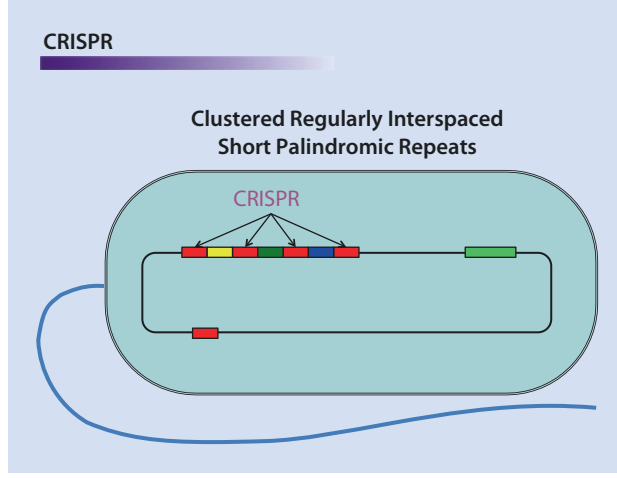

Fig. 5.4 The figure shows how short palindromic repeats (*red*) are clustered in bacterial genomes to form CRISPR loci. CRISPR is the acronym for "clustered regularly interspaced short palindromic repeats"

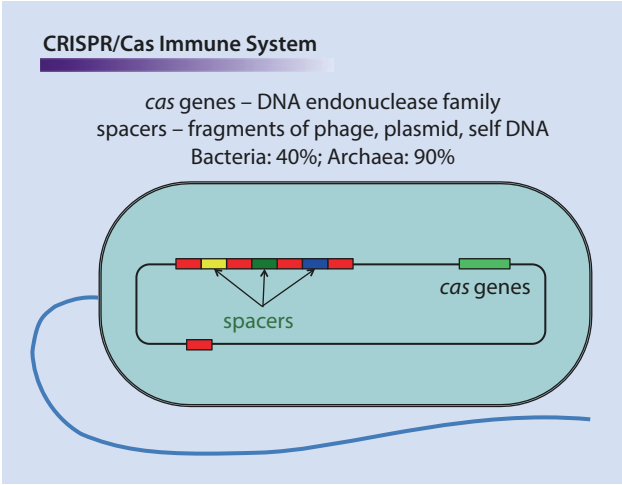

Fig. 5.5 The clustered palindromic repeats in the CRISPR loci are separated by same-sized stretches of distinct DNA elements, called spacers. Although the short palindromic repeats were what first caught the attention of scientists, it is the spacers between them which have come to be of greater interest. Spacer DNA typically comprises fragments of foreign DNA, such as phage genetic material. Associated with the CRISPR locus is another cluster of genes which encode the Cas proteins

Each of these repeats is 24–48 nucleotides long, and they are spaced 20 nucleotides apart. When scientists began looking at sequenced prokaryotic genomes, they found CRISPR loci in about 40% of bacterial samples and in almost 90% of archaeal samples. However, they still did not quite know what its function was.

Then, they started studying the sequences in detail and found that each repeat has a dyad symmetry. These are similar to the DNA recognition sites for type I restriction enzymes. What are even more interesting are the 20 nucleotide sequences *between* these repeats. These are called spacers and are identical to parts of phage DNA or foreign plasmids (Fig. 5.5). The idea quickly developed that CRISPR loci might be the heart of some sort of prokaryotic immune system. But how does it work?

By this time, it was also known that near the CRISPR locus is a group of genes encoding enzymes that can unwind and cleave DNA. These are the *cas* genes. The fact that they are associated suggested that the Cas proteins somehow work with the CRISPR locus to defend the prokaryote against reinfection.

What happens, in fact, is that when an invader, such as a phage or another bacterium, injects its DNA into the prokaryote, Cas proteins attack this DNA by binding to it and cleaving it into short fragments (Fig. 5.6). These fragments are then carried back to the CRISPR locus and *added to it*. In this way, a new spacer is created between the repeat sequences and a bit of the invader is added to the prokaryote's CRISPR library. A single invader may contribute more than one spacer to the locus. The prokaryote will now be able to recognize the invader in future attacks (Fig. 5.6).

It is necessary for the Cas proteins to be able to differentiate foreign DNA, introduced by an invader, from that in its CRISPR library. Otherwise, they would cleave the spacers from the CRISPR locus, leading to considerable genome damage. This is prevented by the need for a 3- to 5-nucleotide signal, found only on the foreign DNA, for the Cas proteins to be active. This signal is the protospacer adjacent motif (PAM). The Cas enzyme will cleave the DNA only if it finds a PAM sequence nearby. This model was confirmed when a group managed to make *Streptococcus thermophilus* immune to phage invasion, using spacer DNA derived from a phage.

Later, when the prokaryote is attacked by the same invader, the CRISPR locus becomes active again. At first, the entire locus is transcribed, so we have a single long messen-

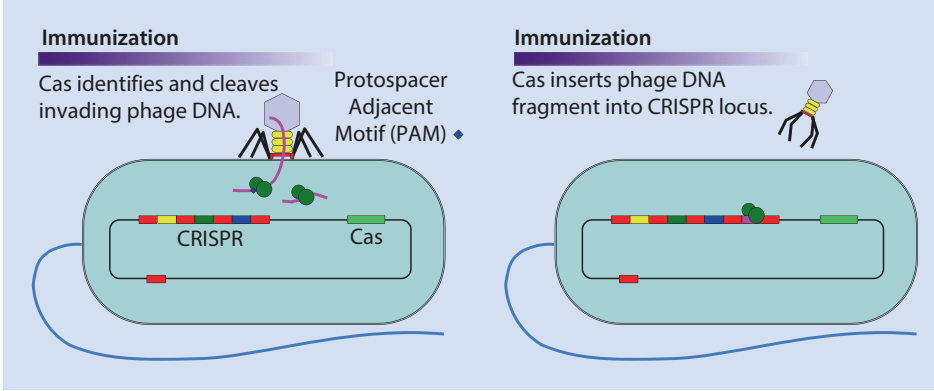

Fig. 5.6 The figure illustrates how the CRISPR-Cas system responds to an invasion of foreign genetic material. Novel phage material is cleaved and incorporated into the CRISPR loci as spacers. These would now serve as identifiers of the invader in the next encounter and primes the bacterium to respond in defence

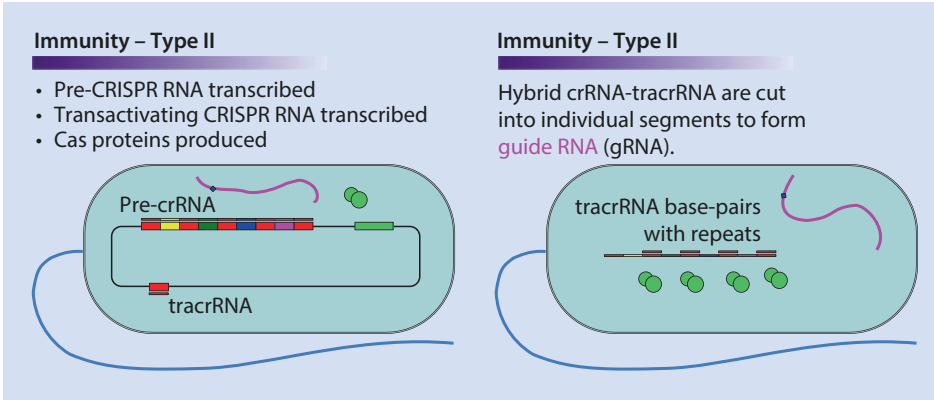

Fig. 5.7 The figure shows a Type II CRISPR-Cas response to a second invasion by the same phage DNA. The entire CRISPR locus is transcribed into pre-crRNA, the tracrRNA is similarly produced and the Cas genes are expressed. Hybridisation of tracrRNA to the pre-crRNA repeats and subsequent cleavage of the double-stranded junctions create guide RNA (gRNA) fragments

ger RNA encoding the repeats as well as the spacers between them (Fig. 5.7). This is called pre-CRISPR RNA (pre-crRNA). A second RNA molecule called transactivating crRNA (tracrRNA) is also produced. This is complementary to, and base pairs with, the pre-crRNA repeat sequences. The result is a hybrid single- and double-stranded RNA complex where the single-stranded pre-crRNA segments code for a spacer sequence and the double-stranded segments are the repeat sequences base paired with the tracrRNAs.

This complex carrying multiple spacers will then be cleaved into individual spacer–duplex units (Fig. 5.8). Each unit will then act as a guide for other Cas proteins. The latter will unwind and scan the invading DNA for sequences complementary to the guiding spacer sequence. If these are found, and if a PAM signal is at hand, the crRNA–tracrRNA–Cas complex will proceed to cleave the DNA. In this way, the invading DNA will be degraded.

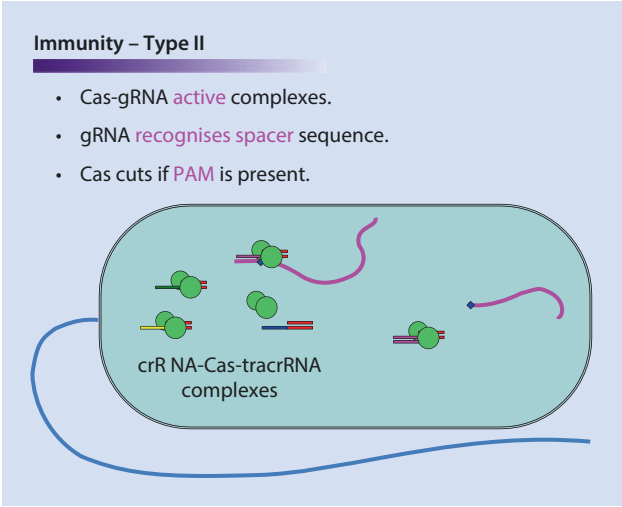

Fig. 5.8 Each gRNA then forms a complex with a Cas protein. Each of these complexes then scans the invading DNA for sequences complementary to their gRNA spacer sequence. When these are encountered, the Cas protein may cleave the invading genetic material. In the Type II CRISPR-Cas system, the Cas protein will only cleave a target if a protospacer adjacent motif (PAM) signal is present. (Modified from CtSkennerton, 2014)

Three classes of such CRISPR/Cas immune systems have been defined: types I, II, and III. These three classes differ in how the crRNA–tracrRNA complex guides Cas to deal with the foreign DNA. The type II system would later be developed into the CRISPR/Cas9 technology.

This entire process involves considerable DNA cleavage and ligation. Foreign DNA is first cleaved into fragments, and for these fragments to be inserted into the CRISPR locus, so must the CRISPR locus be. Insertion of the new spacer sequences involves ligation of the DNA material. Cleavage and ligation of DNA are processes at the heart of molecular biology. Can the CRISPR/Cas system be used, then, for genetic engineering? Jennifer Doudna, Emmanuelle Charpentier, and their co-workers, decided to see if this was possible using the type II CRISPR/Cas system (Fig. 5.9).

5.6 Adapting the CRISPR/Cas9 System for Molecular Biology

In the type II CRISPR/Cas system, Cas9 and RNaseIII cut the crRNA–tracrRNA complexes to produce the guide complexes or guide RNAs (gRNAs). These gRNAs then each form an active complex with Cas9. As this complex scans foreign DNA, it will search for sequences complementary to its crRNA. When it encounters the target, it will scan the vicinity of the target for a PAM signal. Having identified such a signal, Cas9 will cleave the target DNA. Up to this point, the important components for this process to work are (1) the target sequence; (2) a PAM signal near the target; (3) crRNA; (4) tracrRNA; (5) RNaseIII; and (6) Cas9.

Suppose you want to cleave the DNA of a cell at a specific site. Suppose, also, that this cell does not carry the CRISPR locus. For any target sequence to be cleave specifically, all one needs to supply are the crRNA, tracrRNA, and Cas9. Of these three, only the crRNA is unique, since it is unique to the target sequence. If one could produce active crRNA–Cas9–tracrRNA complexes carrying different crRNA, one could target multiple sequences simultaneously. What is important is that each sequence must be accompanied by a PAM signal. The most commonly exploited PAM signal is NGG, where N is any nucleotide.

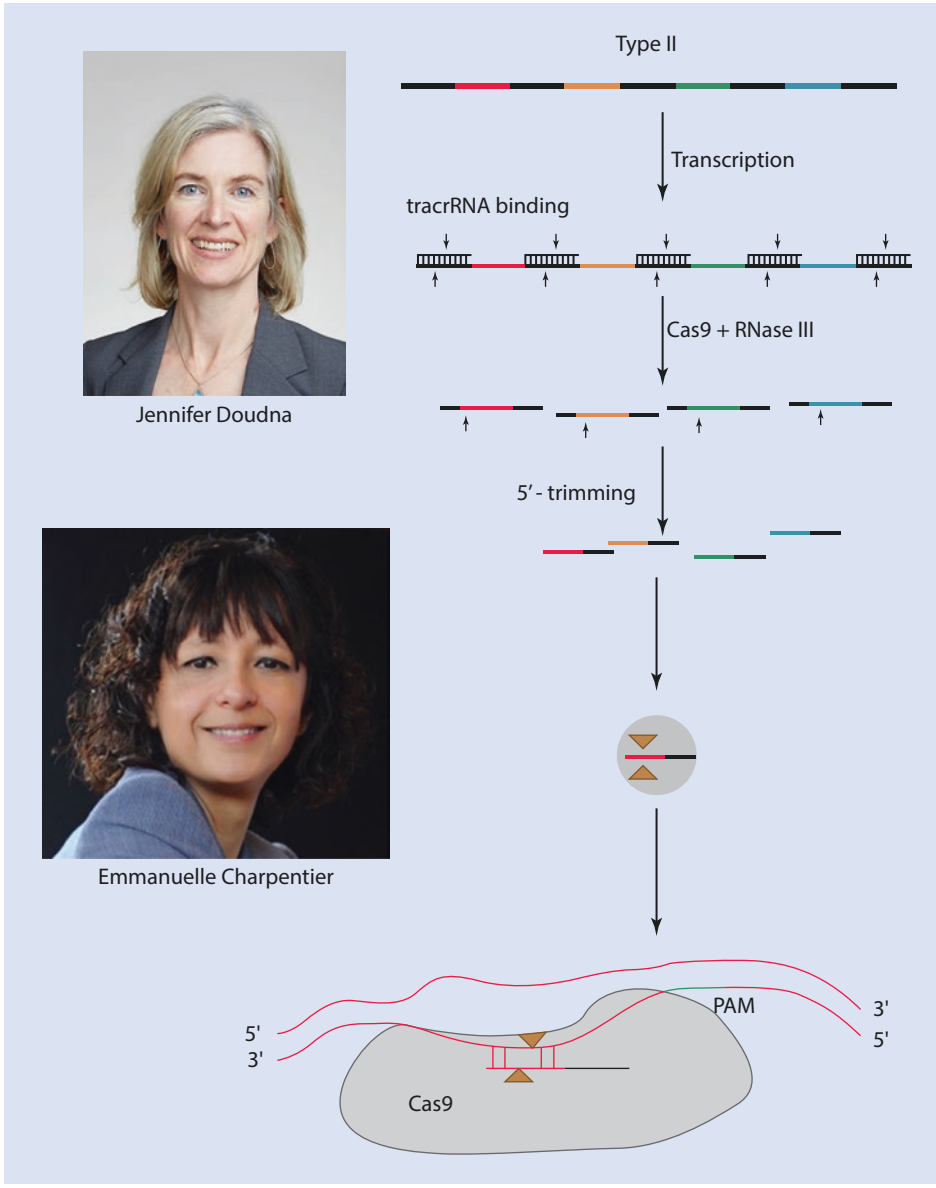

Fig. 5.9 The figure illustrates the basic features of the Type II CRISPR-Cas system in greater detail. Of note is the Cas9 protein, which was the first to be specifically developed as a CRISPR/Cas tool for molecular biology. Inset, are two of the scientists who guided this development

Since PAM signals are short and not very specific, it is likely that a target would have one associated with it.

In 2014, Doudna and Charpentier showed that one could introduce the crRNA, tracrRNA, and Cas9 genes into a target cell with a single plasmid encoding all three components. Instead of having the crRNA and tracrRNA separately encoded, one could

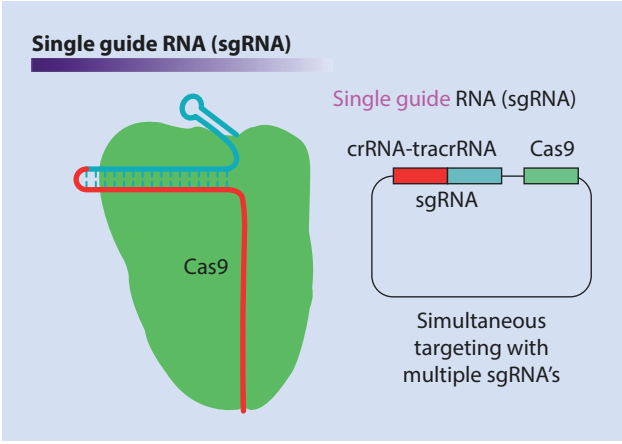

Fig. 5.10 To make the CRISPR-Cas9 system amenable for molecular biology, it had to be streamlined for efficiency. Instead of an entire CRISPR locus, researchers might use only a single spacer for a specific target as the pre-crRNA (now the crRNA). Instead of expressing the tracrRNA separately from the crRNA, the tracrRNA sequence might be spliced downstream of the crRNA. In this way, crRNA and tracrRNA would be inextricably linked, forming a single guide RNA (sgRNA), and ensuring more efficient formation of the active complex. The final component for a working system would be the Cas9 protein. Genes encoding all three components can be carried on a single vector, which can then be transformed into the cell to be modified

also concatenate these two genes to form a single construct—single guide RNA (sgRNA) (Fig. 5.10).

In fact, multiple crRNAs can be included in the plasmid so that the resultant crRNA–tracrRNA–Cas9 complexes can simultaneously target multiple sequences. To demonstrate this, a team in Boston cleaved 62 retroviral sequences simultaneously from the genome of a porcine kidney cell line, without the need to remove and replace this genetic material. This work will be discussed in detail later. Clearly, CRISPR/Cas9 is a powerful new tool for genome editing.

5.7 What Is It Capable Of?

It should be noted, however, that the mainstay of the CRISPR/Cas9 system is not only the ability to cleave specific DNA sequences. It so happens that the Cas9 protein has variants with unique functions apart from cleaving DNA. Some cleave only one strand of their targets, while others cleave both. Some Cas9 variants do not cleave DNA at all. Each variant can be exploited for specific needs. In this way, the CRISPR/Cas9 system can be used for a wide range of genetic manipulations. The following are some examples.

5.7.1 Knock-In and Knock-Out Mutations

When a Cas9 variant capable of cleaving DNA is used, either homology-directed recombination or nonhomologous end joining will be used to repair the damaged DNA (Fig. 5.11). If repair template DNA with flanking homology is provided, this template

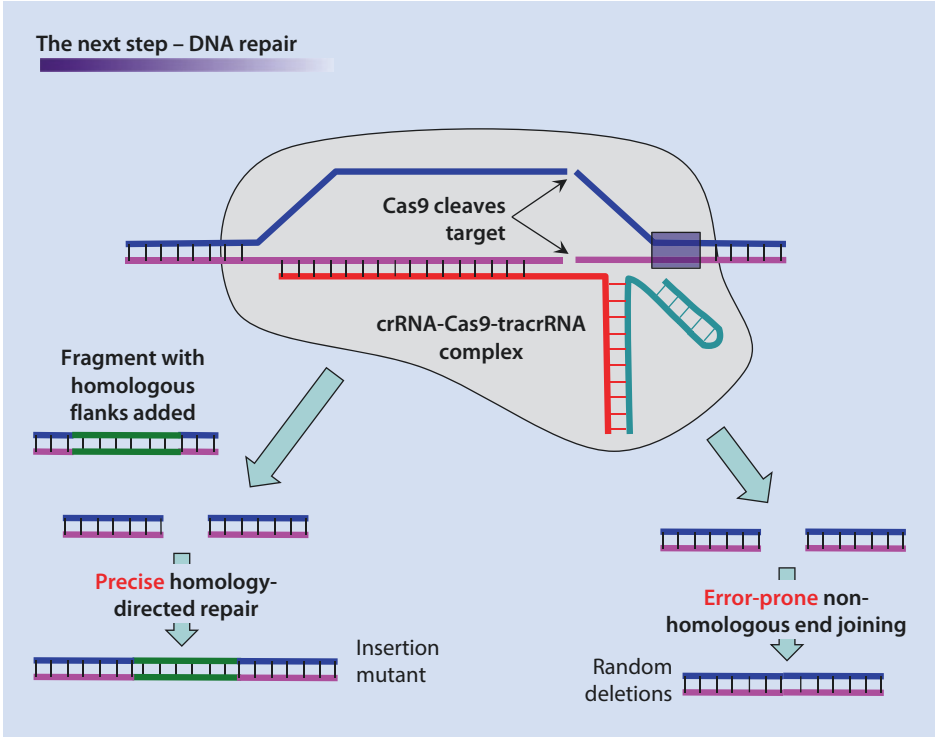

Fig. 5.11 When used to cleave genetic material, the CRISPR-Cas9 system has to be supported by a DNA repair mechanism. Homology-directed repair can be exploited to create controlled insertion mutations in the target genome. In contrast, non-homologous end joining is error-prone and would result in random mutations when the cleaved DNA is repaired

will be incorporated into the cleaved site precisely. This approach can be used to insert a gene into a target genome. This strategy can also be used to permanently disable a target gene by inserting a disrupting nonsense fragment therein.

5.7.2 Gene Regulation

One might even disable a gene only temporarily, if a Cas9 variant which does not cleave target DNA at all is used. An interesting phenomenon observed is that crRNA–Cas9–tracrRNA activity somehow triggers methylation of the target DNA. This will silence the gene but will not destroy it. Once this methylation is removed, the gene will be active again. One can use the same variant of Cas9 but modify it so that it now carries a trans-activating factor. In effect, the complex now has a DNA binding domain and a transactivating domain, just like a transcription factor. Such complexes have been used to upregulate gene expression. Such a system has similarities with the yeast two-hybrid system.

5.7.3 Applying CRISPR/Cas9 to Genome Editing

CRISPR/Cas technology has proven to be a powerful tool in biological research. Several studies have tried to systematically knock out every gene in the human genome, one at a time. This has allowed us to understand the function of each gene as well as its importance, such as whether its function is compensated for by other genes or whether it is resistant to mutation.

In medicine, CRISPR/Cas9 has been used to delete specific genes in order to artificially create known diseases. This has been done in cells as well as in animals, providing us with disease models for study. Use of this technology on cancer cells is allowing us to understand how drug resistance develops in cancer treatment. It was used to disable 62 genes in porcine kidney cells known as porcine endogenous retroviruses (PERVs). PERVs are a major concern when considering the use of pig organs for transplantation. Finally, it is hoped that this technique might someday be a powerful treatment for diseases such as acquired immune deficiency syndrome (AIDS), where in situ gene regulation of the viral genome might be possible.

5.8 What Makes the CRISPR/Cas9 System Unique?

There has been an explosion of publications describing the use of CRISPR/Cas9. However, neither the technology to target DNA nor that to cleave and insert DNA is new. Conventional systems such as Zn-finger nucleases and transcription activator-like effector nucleases (TALENS) can emulate CRSPR/Cas function. Homologous recombination technology is almost as old as the internet. In fact, the Cre–lox recombination system, developed in 1992, is a very similar technology to CRISPR/Cas9, although it has not been as successful. These technologies have been limited largely by the fact that we have to engineer proteins, such as Zn-finger nucleases and TALENS, in order to target a gene. Manipulation of protein structure and function is more difficult than manipulation of nucleic acids. The Cre–lox recombination system requires one to insert *loxP* target sequences on either side of the target in order for the system to recognize it. This means genetically modifying the host *in order to genetically modify it*. In comparison, CRISPR/Cas9 only requires one to produce the guide RNA, and there is no need to modify the target DNA beforehand. To help researchers design gRNAs, developers have created various online software for gRNA design. All of this renders the CRISPR/Cas9 system an attractive alternative for genetic manipulation.

5.9 What Dangers Does It Pose?

The fact that components of CRISPR/Cas9 are so easily created, inexpensive, and easy to use is also what makes the technology a matter for concern. More people will have access to it, and that means more variety in motivations for doing so. So far, we have discussed what biologists and medical researchers hope to achieve with CRISPR/Cas9 technology. What will others, perhaps those outside the scientific mainstream, want to use CRISPR/

Cas9 for? Will it always be for benign purposes? What if CRISPR/Cas9 is used on humans?

CRISPR/Cas technology makes changes that may be permanent in the individual, but only in the cells that have been modified with the crRNA–tracrRNA–Cas9 plasmid. However, if this mutation is made in an important gene locus, it will be preferentially passed on to dividing cells. If this occurs in the sperm or egg cells—the germline—the mutation becomes inheritable. Again, if this mutation is coupled to a critical gene locus, it could be evolutionarily preserved and quickly spread throughout the species, making it a species mutation. This might accelerate human evolution in unpredictable ways. Once again, the concern is human motivation. CRISPR/Cas9 is a very real and—for the first time—very practical tool for creating humans with specific biological advantages. Those who control this technology might become a powerful factor in how society is organized. Humans modified in such ways might also have an unprecedented impact on the environment and existing life on earth. Such hazards will be much harder to manage, since the threat would be human.

Furthermore, powerful as it is, CRISPR/Cas9 is not perfect. In 2015, a group of Chinese scientists caused panic when they reported a successful modification of human embryonic stem cells. What is just as important is the fact that CRISPR/Cas9 made unexpected mutations elsewhere, besides the target site. This happened because of how frequently one finds PAMs in the human genome, and how many genes share sequence homology. At this stage, if this technology is used to modify a germline, we might create offspring with unintended disabilities. Unlike natural disabilities, these would be the result of willful human tampering.

5.10 How Are These Dangers Addressed?

What can be done to manage the hazards of CRISPR/Cas9? To deal with the emerging risks, we need (1) understanding of what CRISPR/Cas9 can or cannot do; (2) knowledge of what has already been tried and what is possible; (3) understanding of what dangers CRISPR/Cas9 could create; and (4) guidelines regulating the application of CRISPR/Cas9, especially to humans. These processes should involve both scientists and nonscientists. Discussions such as this, involving people of diverse backgrounds, have recently become more frequent and more urgent. This is especially so since genome editing was first reported to have been performed on human cells. These discussions would hopefully result in clear guidelines on how CRISPR/Cas9 is used, and for what purpose. Some groups have already firmly advised against the use of CRISPR/Cas9 for specific cases, such as germline engineering.

However, whatever decision is reached regarding the use of CRISPR/Cas9, or indeed any powerful tool, it will only have meaning to those who partake of the agreement. How should we prepare to address the work of those who do not?

Take-Home Messages

1. Projects in synthetic biology that call for genetic engineering require methods that are powerful, precise, reliable, and safe.
2. The use of BioBricks allows the process of cloning to be more reliable, simple, and compatible between laboratories.
3. This can be achieved by making functional fragments of DNA, such as promoters and coding sequences, easily interchangeable.
4. Each BioBrick is a fragment of DNA with defined structures at the flanks.
5. Using a specific combination of enzymes, one can ligate a BioBrick upstream or downstream of another BioBrick, as desired.
6. A repository exists for BioBricks, which is accessible to all registered users.
7. The BioBrick Foundation aims to encourage the use of BioBricks among its members.
8. It also aims to develop a community, comprising scientists and nonscientists, which discusses and uses this technology for the betterment of mankind.
9. This work is extended to the annual International Genetically Engineered Machine (iGEM) competition. Here, teams of students and mentors are provided with a collection of BioBrick parts and challenged to produce novel BioBrick devices and systems.
10. The CRISPR/Cas system is thought to constitute the immune system in prokaryotes.
11. This system involves Cas enzymes, which have varying functions ranging from DNA helicase activity to single-stranded or double-stranded DNA cleavage.
12. The Cas enzymes are guided to their target by guide RNAs, which are transcribed from fragments of previously encountered foreign phage or plasmid DNA.
13. The CRISPR/Cas9 system has been exploited for in situ site-specific DNA modification in target cells.
14. These modifications include knock-in and knock-out mutations, gene up- and downregulation, and massive deletions and insertions into target genomes.
15. Unlike conventional methods of in situ genome modification, the CRISPR/Cas9 system does not require the design and production of specialized proteins, nor prior modification of the target genome.
16. Furthermore, CRISPR/Cas9 requires only a plasmid encoding the necessary Cas enzymes as well as the target sequences that would form the guide RNAs.
17. The ease of production and use of components of the CRISPR/Cas9 system makes it readily available, and an attractive alternative, to most researchers, including those who may not be affiliated to official laboratories.
18. As CRISPR/Cas9 has the potential to make significant changes to target genomes, its misuse by any user is of great concern.
19. Modification of genomes in germlines and other important targets might lead to hereditary modifications. This is of concern since it could potentially accelerate evolution of the target species, with unpredictable consequences for human health and the environment.
20. To deal with this risk, there should be public discourse about CRISPR/Cas9, for the purpose of helping other scientists and laypeople to understand the technology and what it is capable of. There should be a conscious effort, involving people of diverse backgrounds, to create guidelines and regulations regarding the use of CRISPR/Cas technology.

Further Reading

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