Rewriting genes with CRISPR/Cas9

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Biology’s IT Toolbox

• DNA structure/sequencing
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- Restriction enzymes
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• DNA structure/sequencing
• Restriction enzymes
• DNA synthesis/PCR
• Genome engineering
Reading DNA is much faster than writing.

![Graph showing the productivity in DNA synthesis and sequencing](image_url)

- **Reading DNA**
- **Computer speeds**
- **Writing DNA**

The graph illustrates the productivity in DNA synthesis and sequencing using commercially available instruments. The productivity of reading DNA grows exponentially faster than the productivity of writing DNA and computer speeds.
Reading is also much cheaper than writing.

We are approaching the “$1,000 genome”
Writing vs. re-writing

We can synthesize genes cheaply and easily

Synthetic DNA
- fast, inexpensive, easy

Genome editing
- slow, expensive, hard
How are genomes edited?

1) Cut genome at a specific site

2) Cut is repaired by the cell
   a. NHEJ repair $\rightarrow$ disrupt gene
   b. HDR repair $\rightarrow$ change gene
Genome editing used to be slow and expensive

Protein-based modules recognize target DNA
Mature technology, recognition is very specific

But...
Modules are slow to make and test
Costs $3,000 and takes ~1 year for each edit
**CRISPRs: Hallmarks of acquired immunity in bacteria**

Clusters of Regularly Interspaced Short Palindromic Repeats (CRISPRs)

Bacterial/archaeal chromosome

Bolotin et al. 2005
Mojica et al. 2005
Pourcel et al. 2005
CRISPR/Cas9 is a revolution in genome targeting

RNA-guided Cas9 recognizes target DNA
Cas9 protein makes the cut
Uses well-understood rules of base pairing

Costs <$500 and takes ~ 1 week
Easily multiplexed
Supply single-guide RNA (sgRNA) and Cas9 protein
Target DNA must contain special “PAM” sequence

Jinek et al., Science 2012
Jinek et al., ELife 2013
CRISPR/Cas9 is generally applicable
CRISPR/Cas9 research is exploding

Number of CRISPR/Cas9 scientific papers by year
CRISPR/Cas9 can be used for more than editing

Transiently turn genes on or off

Watch genes in living cells in real time
Technology marches on

What should we do with cheap, fast genome editing?
Reading vs writing