Synthetic Nucleic Acids: from Biomedical Research to CRISPR Genome Editing

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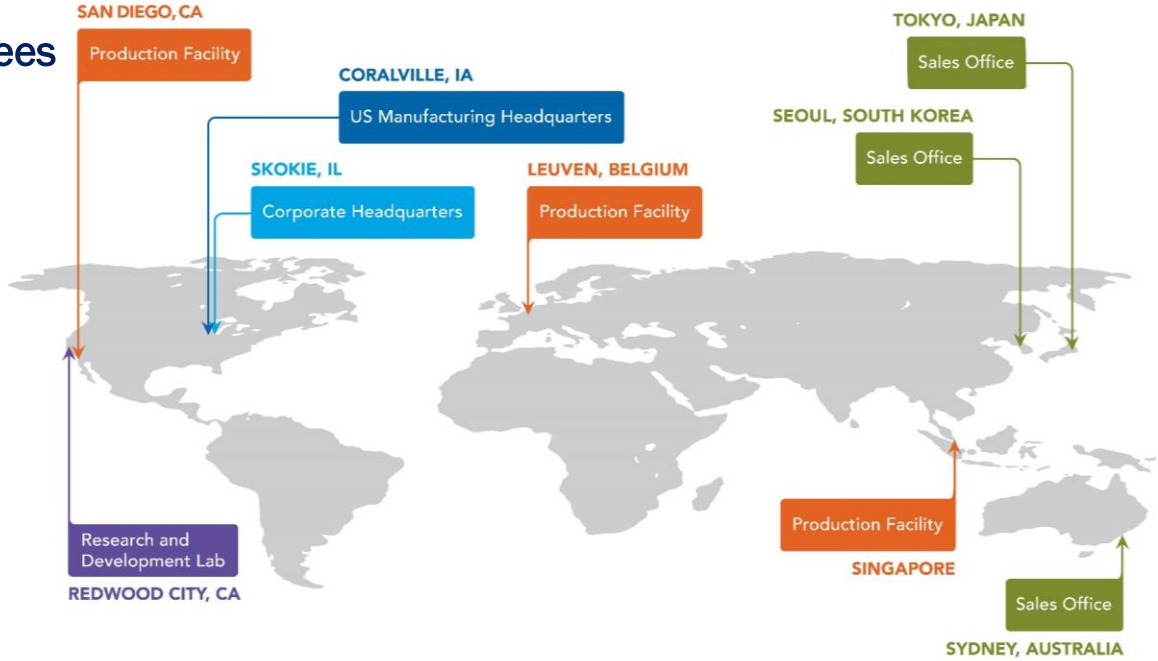
July 20, 2017 Des Moines Rotary Club

IDT INTEGRATED DNA TECHNOLOGIES

IDT – world's largest manufacturer of synthetic nucleic acids

- 9 locations and >1000 employees
- >100,000 active customers
- >65,000 oligonucleotides synthesized per day
- 4,000 orders per day
- >300,000 website visits per month



Founded by Dr. Joseph Walder as a spin-off from the University of Iowa in September 1987 = 30th anniversary!

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Where we make DNA …

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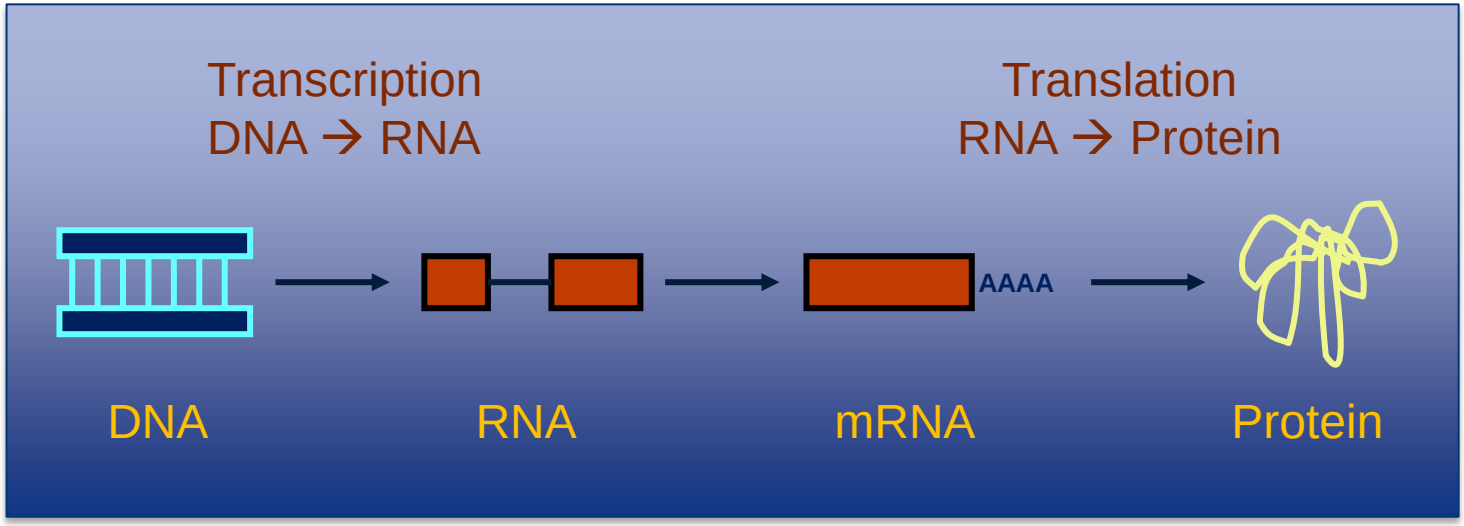
DNA is the blueprint of life



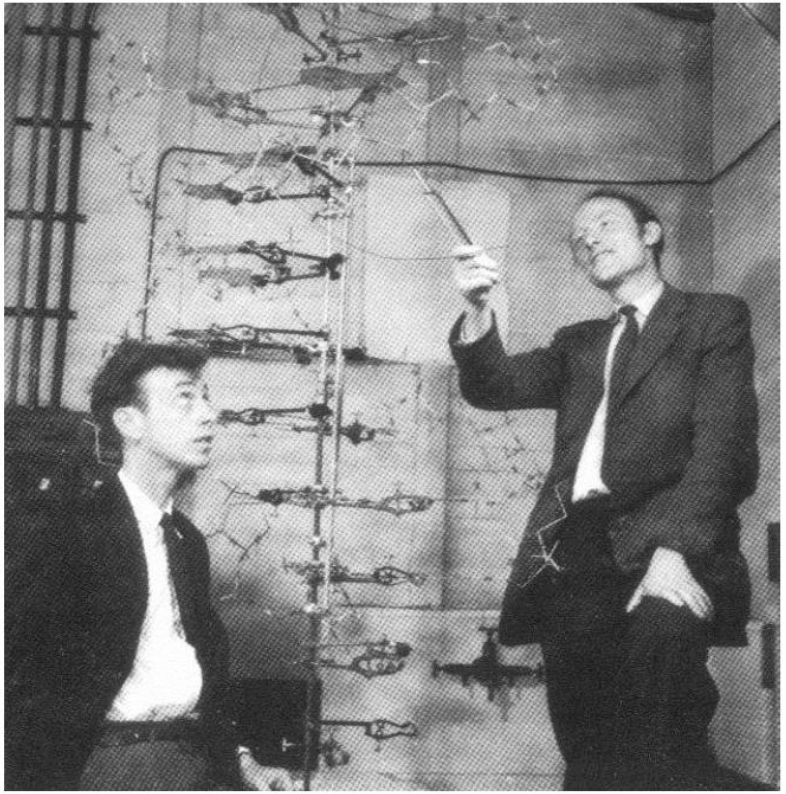
Central Dogma of Molecular Biology

Structure of DNA

Watson and Crick

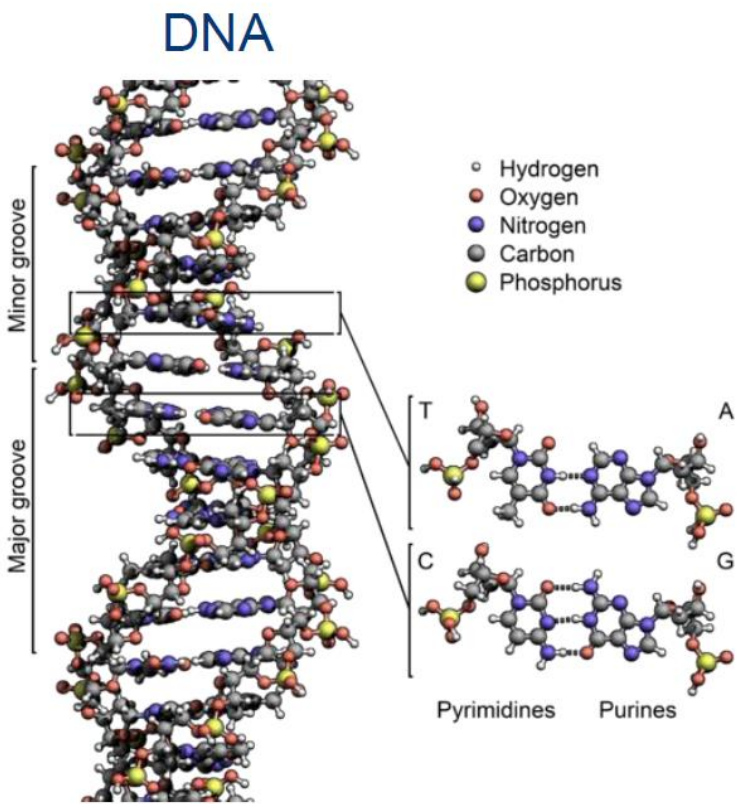
Proposed double helix 1953

Nobel Prize 1962



Structure of DNA

- **DNA is double stranded (“double helix”); one strand codes for protein while the other simply mirrors it**
- **DNA is comprised of 4 types of nucleotides: Adenine, Guanine, Cytosine, Thymine**
- **A binds to T & G binds to C**
- **A single-strand DNA molecule will bind to its mirror complement if it is present and can do so in the presence of thousands of different strands**



Why oligos (synthetic DNA) are useful

ssDNA binds to ssDNA to make dsDNA

Synthetic short known DNA finds it's mirror complement and binds to it:

- Identification (find presence of similar sequence)
- Manipulate, purify, alter, study sequences in the lab

```
GATCCCAGTTAC
||||||||||||
CTAGGGTCAATG
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Amazing specificity …

DNA bases	complexity
1	4
2	16
3	64
4	246
5	2014
6	4096
20	1 trillion

There are 3 billion bases of DNA in every human cell, yet a simple 20 base synthetic oligo can specify a single location in all of your genes!

Like a cell phone number with area code, it will find you wherever you are!

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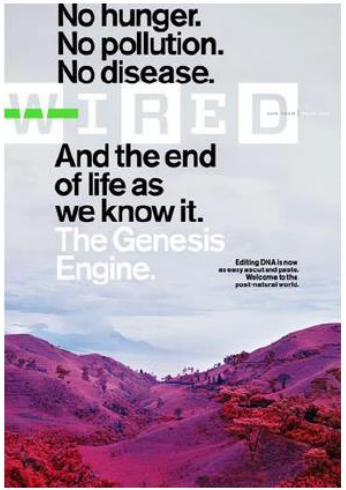
What do we use it for?

- Genetic research
- Medical diagnostics
  - Cancer mutations
  - Infectious diseases
- Biodiversity, herd tracking
- Plant breeding
- Identification
  - Paternity
  - CSI

Newest hot area: Genome Editing (CRISPR)

IDT customers range from the University of Iowa to the NIH to the FBI to Pharmaceutical Companies to the San Diego Zoo!

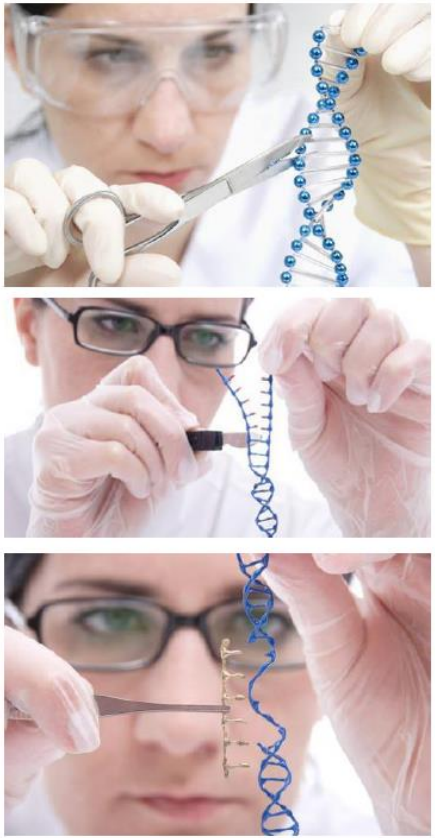
The genome editing revolution…



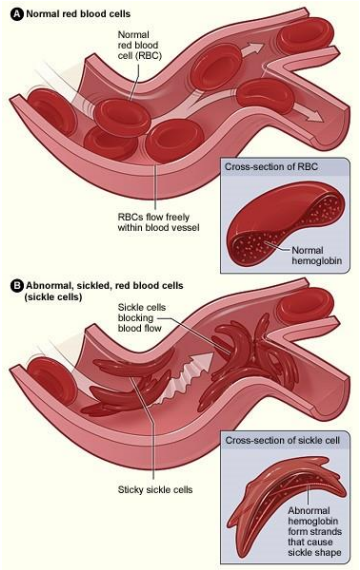
Genome editing is like re-writing a book…

- Add or delete single letters to fix typos
- Delete or move whole words or sentences to change the meaning
- Write in new sentences to add information

Genome editing – fixing errors in genomic DNA in live cells

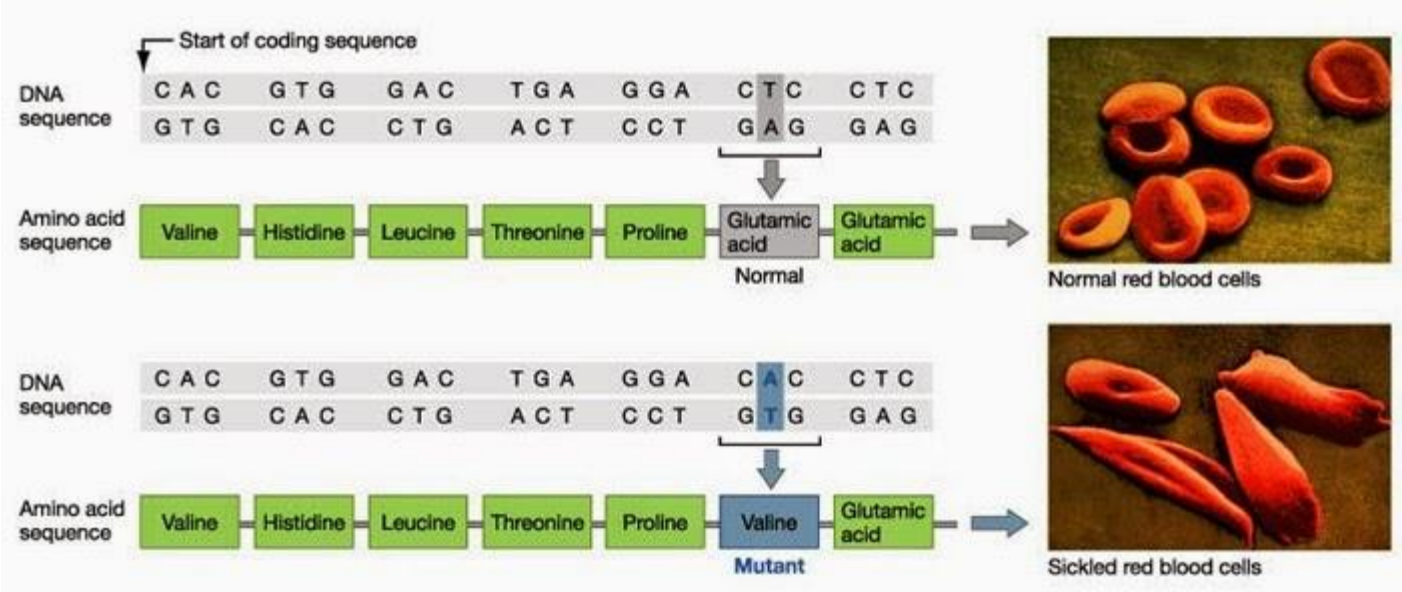


Alter DNA sequence in a living cell and have that change passed on to daughter cells or progeny



Example of medical utility:
Treat Sickle-cell disease (SCD)

Sickle cell disease – single base change in β-hemoglobin



Patient → Blood stem cells → CRISPR gene fix in lab → re-infused into Patient

SCD Rx in mouse models now, human clinical trials 2018!

CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells

Daniel P. Dever[1]*, Rasmus O. Bak[1]*, Andreas Reinisch[2], Joab Camarena[1], Gabriel Washington[1], Carmencita E. Nicolas[1], Mara Pavel-Dinu[1], Nivi Saxena[1], Alec B. Wilkens[1], Sruthi Mantri[1], Nobuko Uchida[3]†, Ayal Hendel[1], Anupama Narla[4], Ravindra Majeti[2], Kenneth I. Weinberg[1] & Matthew H. Porteus[1]

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SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

SICKLE CELL DISEASE

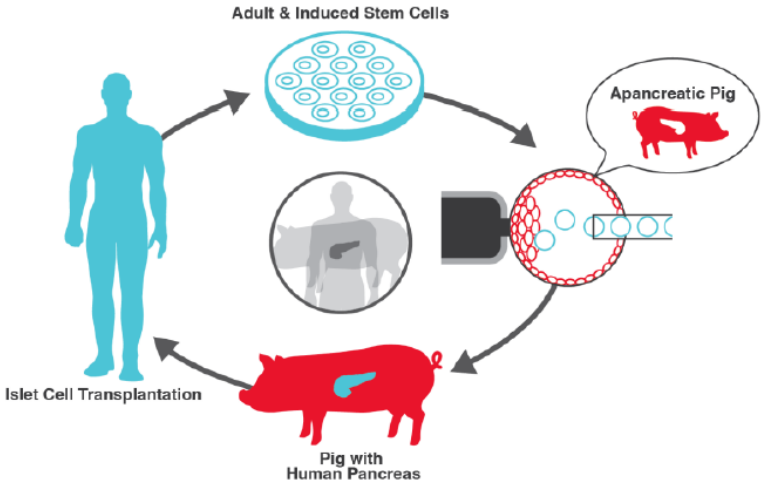
Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells

Mark A. DeWitt,[1,2] Wendy Magis,[3] Nicolas L. Bray,[1,2] Tianjiao Wang,[1,2] Jennifer R. Berman,[4] Fabrizia Urbinati,[5] Seok-Jin Heo,[3] Therese Mitros,[2] Denise P. Muñoz,[3] Dario Boffelli,[3] Donald B. Kohn,[5] Mark C. Walters,[3,6] Dana Carroll,[1,7]* David I. K. Martin,[3]* Jacob E. Corn[1,2]*

DeWitt *et al., Sci. Transl. Med.* **8**, 360ra134 (2016) 12 October 2016

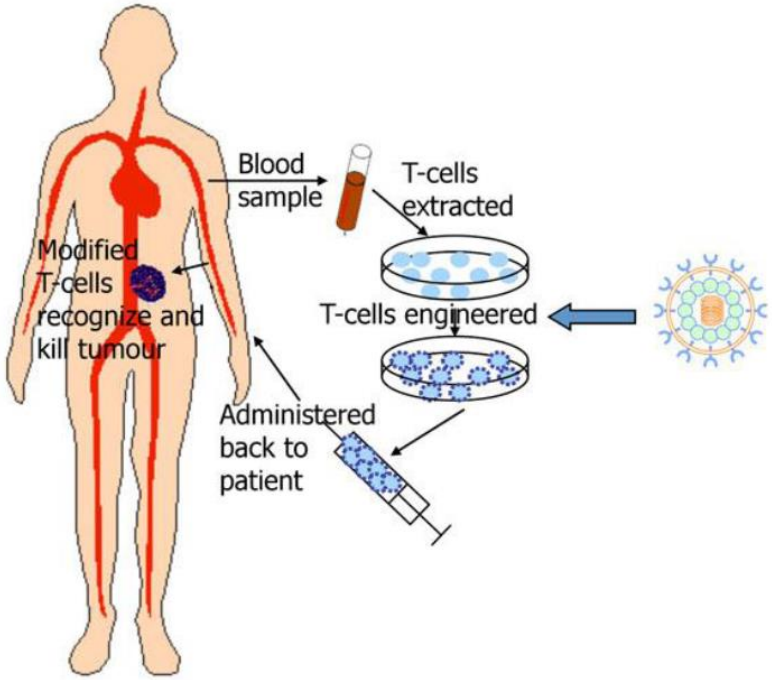
Humanized organs for transplant

- >100,000 people in the US are waiting for a life-saving organ transplant
- Pig organs could make up this shortage but there are problems
  - Rejection due to immune response
  - Risk of porcine endogenous retrovirus (PERV) infection
- Use CRISPR to knockout PERV genes and engineer human compatibility
- eGenesis – start-up company in Boston (founded by George Church, Harvard, 2017) to make pigs suitable for organ transplantation!



Improved T-cell therapy for cancer

- Use CRISPR to engineer a patients T cells (immune cells)
  - Remove T cells from patients and perform CRISPR edits
    - Insert a gene for a protein engineered to detect cancer cells and struct the T cells to target them
    - Remove the gene for a protein that identifies the T cells as immune cells and prevent the cancer cells from disabling them
  - Infuse the edited cells back into the patient
  - First CRISPR clinical trial – approved by NIH in June 2016



Genome editing in animals

- CRISPR/Cas9 has enabled the rapid generation of new model organisms for medical research
  - i.e. transgenic mice disease model systems
- Tuberculosis-resistant cattle
  - Inserted a gene for natural resistance-associated macrophage protein-1 (NRAMP1)
- Creating polled (hornless) cattle
  - Most U.S. dairy animals are dehorned
  - Traditional cross-breeding is slow and very expensive

Genetically dehorned Holstein bull

Genetically modified plants

- Can use genetic engineering to edit the genes of various crops
  - Improve taste or nutrition
  - Better survivors of heat and stress
  - Herbicide/Pesticide resistance
- Herbicide tolerant cassava
  - Cassava is third-largest source of food carbohydrates in the tropics
  - Major staple food in the developing world
- Reduced trans fat soybean oil
  - Soybeans with higher level of monounsaturated fats
- Gluten reduced wheat
- Monsanto and Dupont/Pioneer have both licensed CRISPR technology with the hope of developing valuable new crop varieties

Gene editing – how far is too far?

- CRISPR-Cas9 has the potential to target at unwanted locations – ‘off target effects’
  - We can make changes we aren’t aware we are making (but is clearly better than random mutagenesis, which has been the gold standard for years!)
- Ecological disequilibrium
  - If we eliminate malaria carrying mosquitos, what are the larger ecological consequences?
  - Important to maintain agricultural biodiversity
- Regulations for consumers
  - Will the FDA require special regulation or labeling for CRISPR modified organisms, even if they do not contain any recombinant DNA?
- Editing of human embryos/germline
  - If you edit the germline, this can be passed down to future generations
  - ‘Designer babies’ – regulation prevents this in the US, but what about elsewhere?
  - Chinese group edited a non-viable human embryo (April 2016)

Rodriguez E (2016) Ethical Issues in Genome Editing using Crispr/Cas9 System. J Clin Res Bioeth 7:266. doi:10.4172/2155-9627.1000266

IDT's research team

Integrated DNA Technologies

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- Sarah Jacobi
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- Jessica Lister
- Garrett Rettig
- Bernice Thommandru
- Rolf Turk
- Chris Vakulskas
- Michel Cannieux
- Justin Barr
- Brian Wang