Introduction to Bioengineering
BIOE/ENGR.80
Stanford University

Spring 2020 Class Slides

Day 13
4 May 2020

Week 4 reprise

Abstraction as a tool for **managing** biocomplexity

- Bioengineers can compose higher-level human-defined functions (i.e., devices) from functional biomolecules.
- Common-signal carriers facilitate device reuse.
- Synthetic logic can operate inside living systems.

General system architecture for genetic engineering

- Sense, Compute, Act enables general purpose cell-system programming.

Brain Storm; I Like, I Wish; What If

- Go for quantity (how many ideas can you imagine?)
- Don't be constrained by what exists (> vanilla ice cream)
- Engage others on a constructive basis

Week 5 look ahead

DNA sequencing (reading)

DNA synthesis (writing)

Surfing exponentials

Interconvertibility of matter and information

Team rules & priority setting tools

sequencing
(reading DNA)
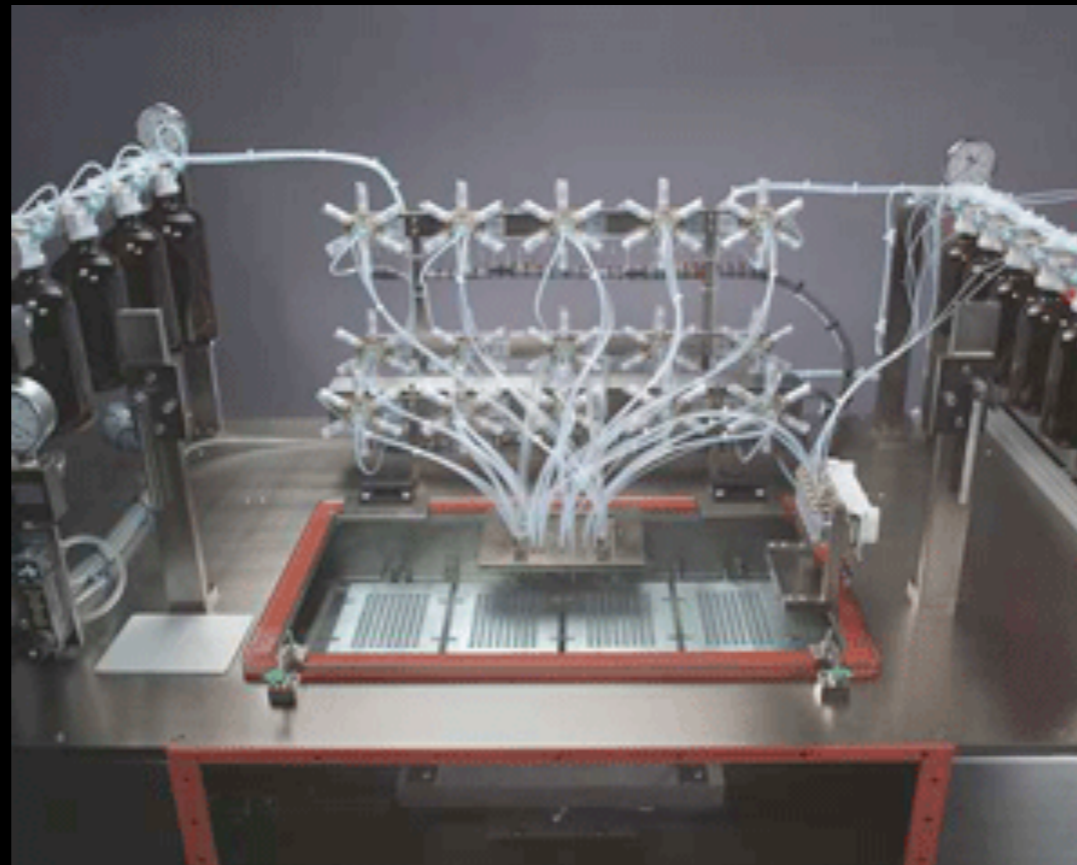((see separate slide deck from Brianna))

synthesis
(writing DNA)
((slides follow in this deck))

DNA synthesis = 4 key keyboard for genetic stuff

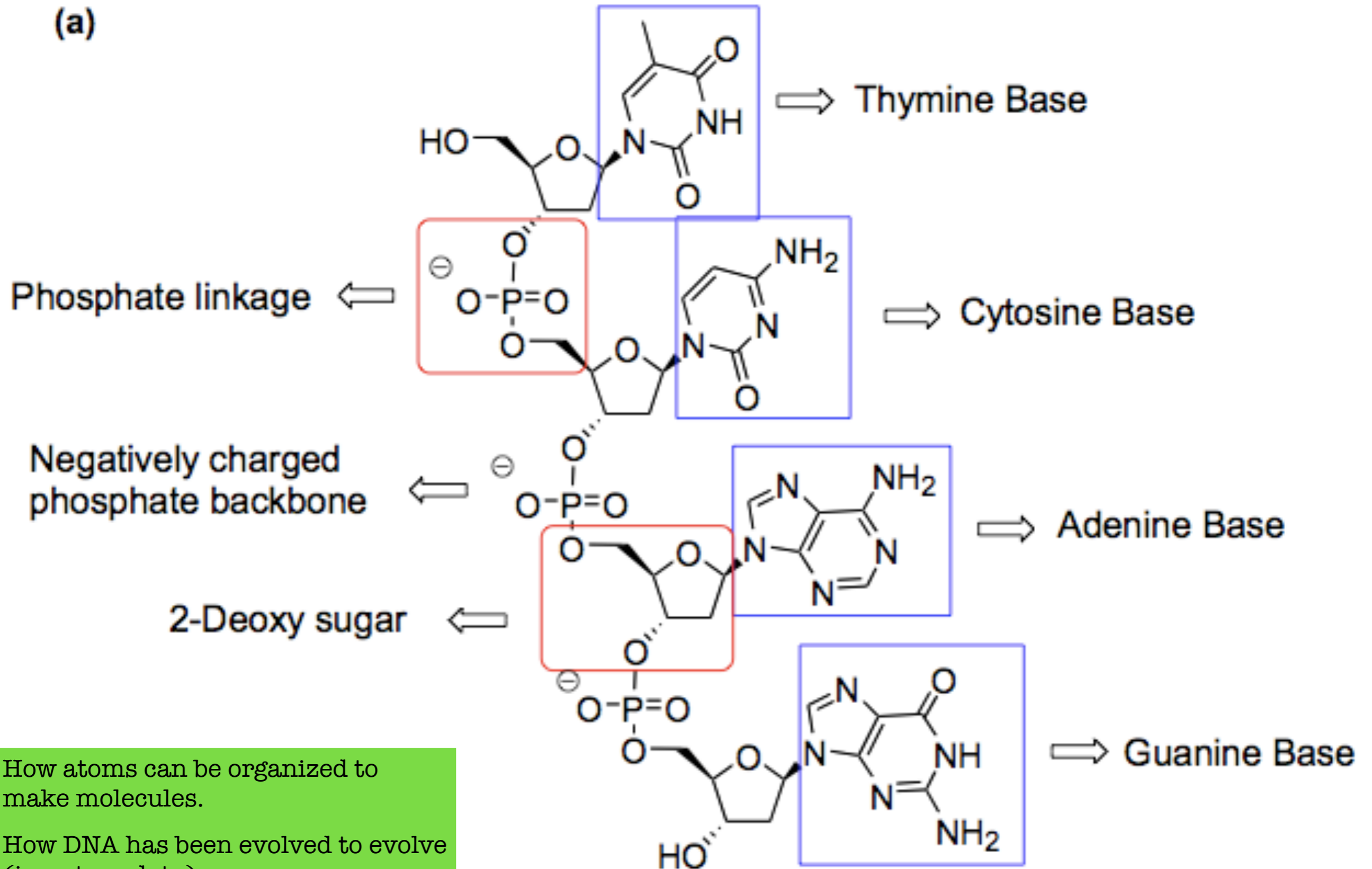Raw chemicals, not derived from existing DNA
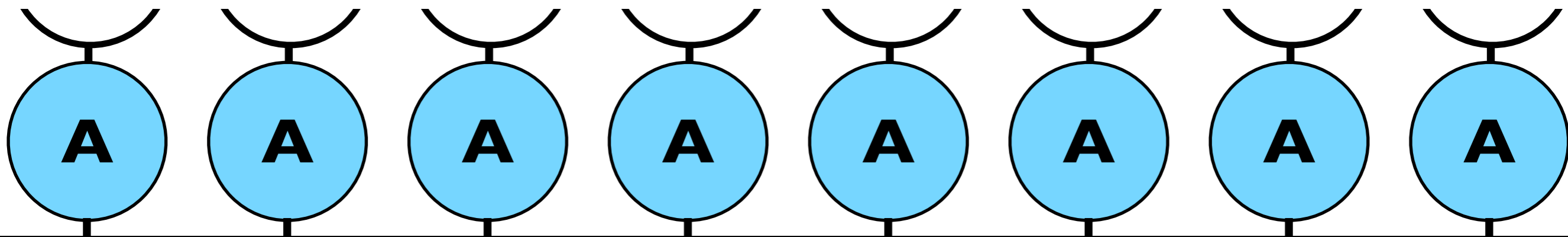
TAATACGACTCACTATAGGGAGA

Play however you like to get the DNA you want, from scratch.

(a)

HO

Phosphate linkage ⟸

Negatively charged phosphate backbone ⟸

2-Deoxy sugar ⟸

⟹ Thymine Base

⟹ Cytosine Base

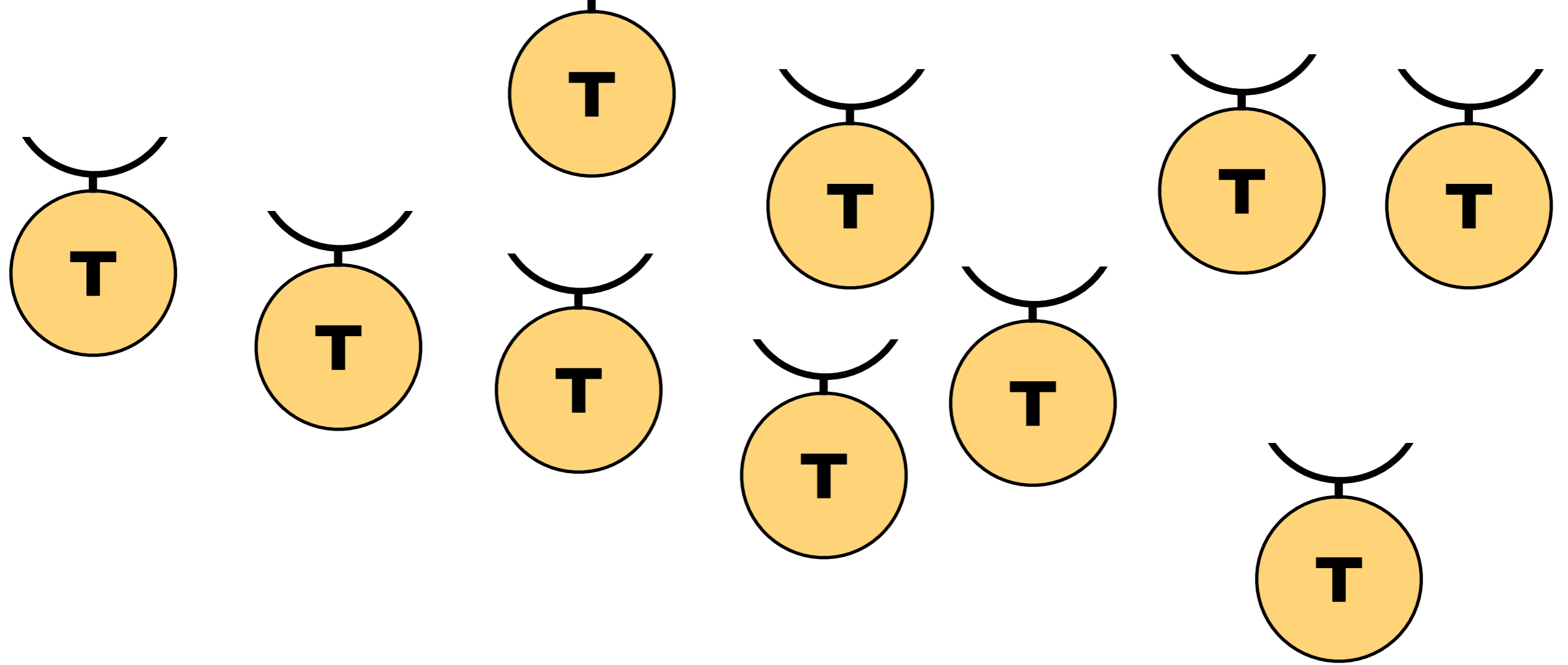⟹ Adenine Base

⟹ Guanine Base

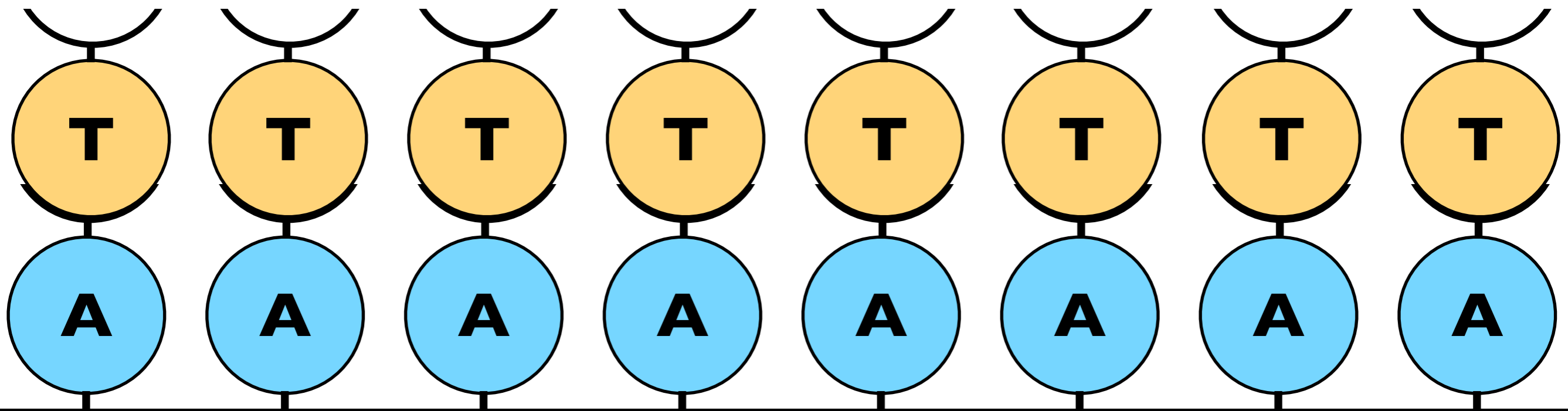How atoms can be organized to make molecules.

How DNA has been evolved to evolve (i.e., store data).
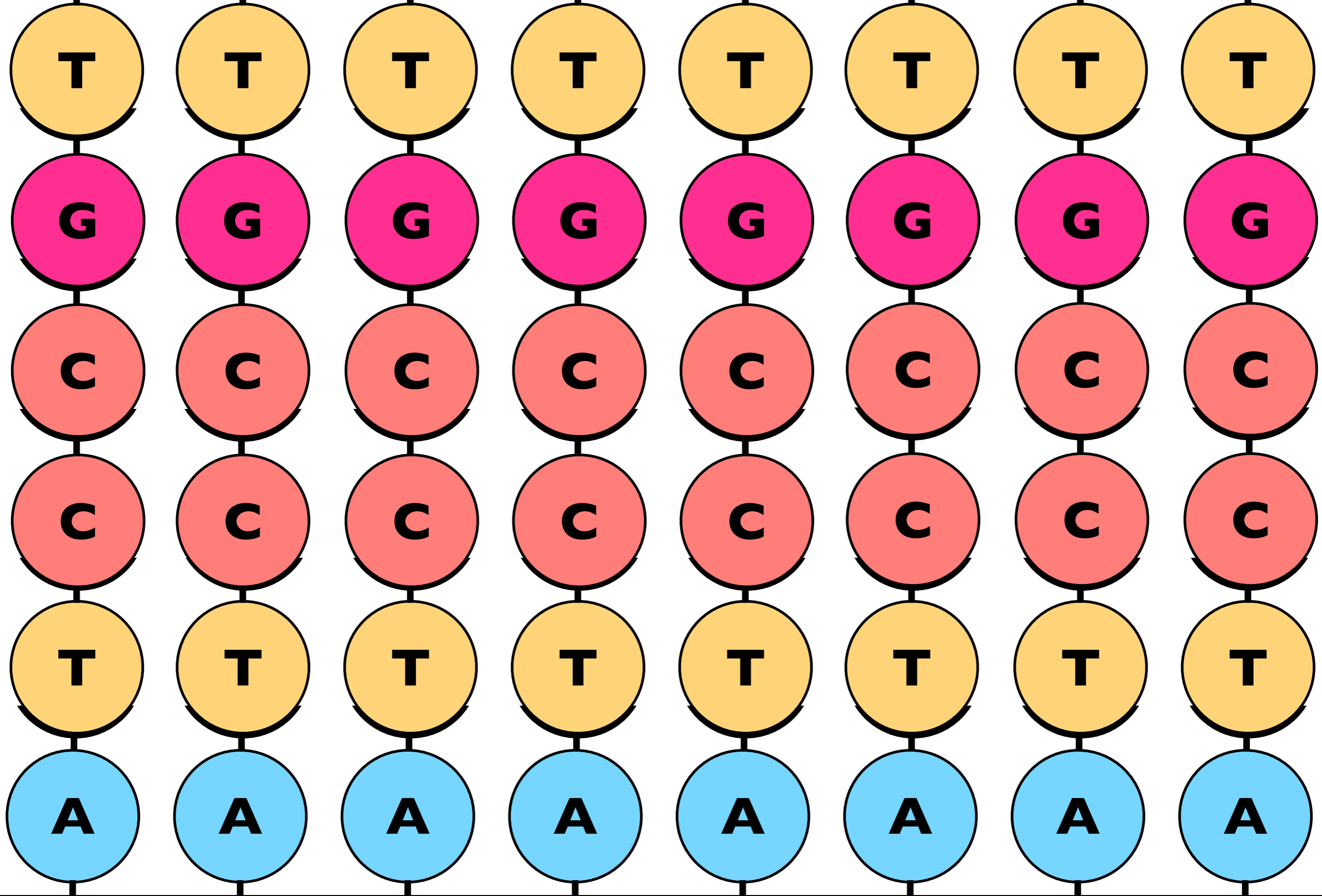
Step 1. Couple 1st base to a surface
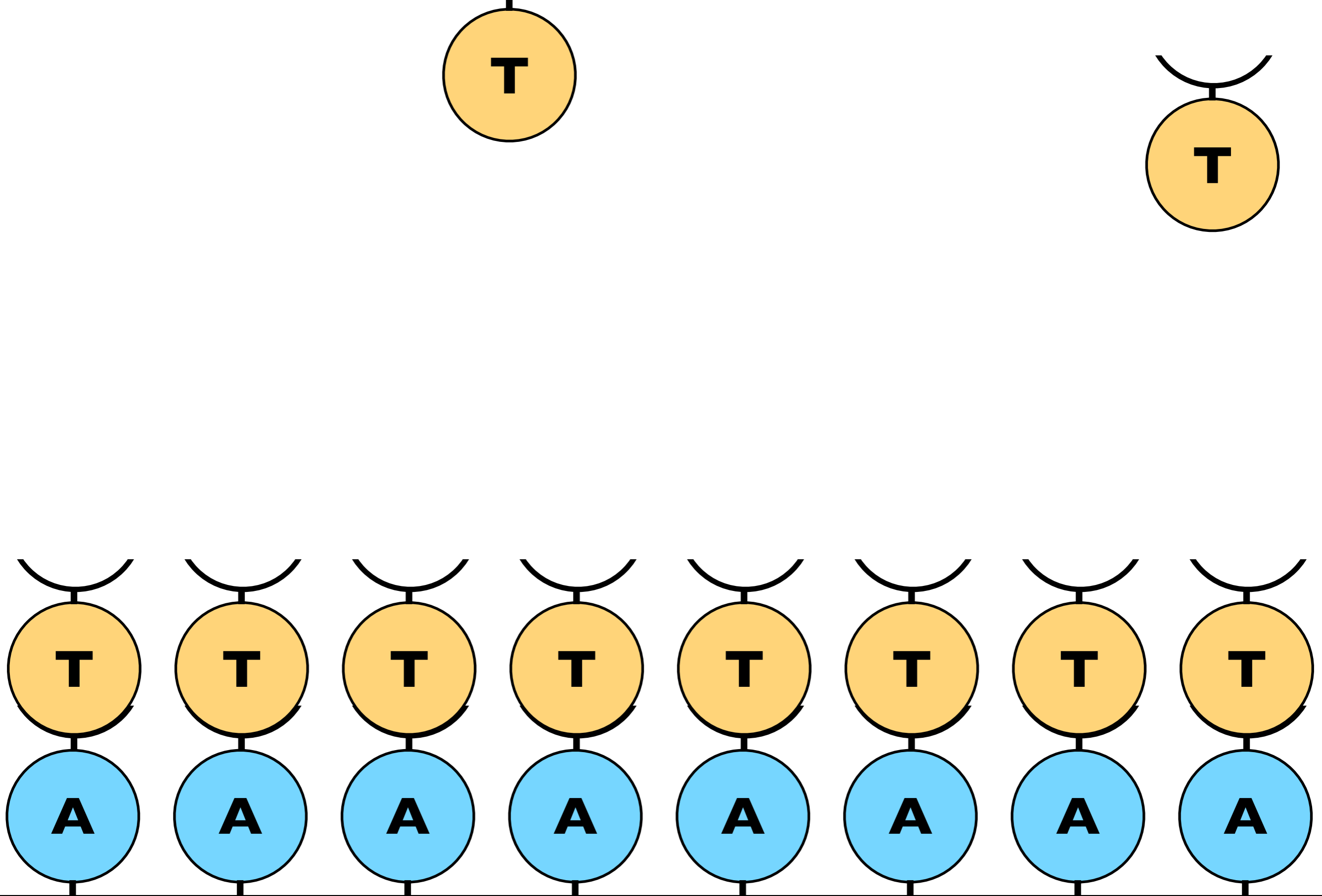
Step 2. Add & attach desired 2nd base

Step 3 (Ideal). Couple 2nd base to 1st

Repeat steps 2 & 3 as you wish! (Ideally)

Unfortunately, things are not so simple...

Fail 1. Extra bases added (insertion mutations)
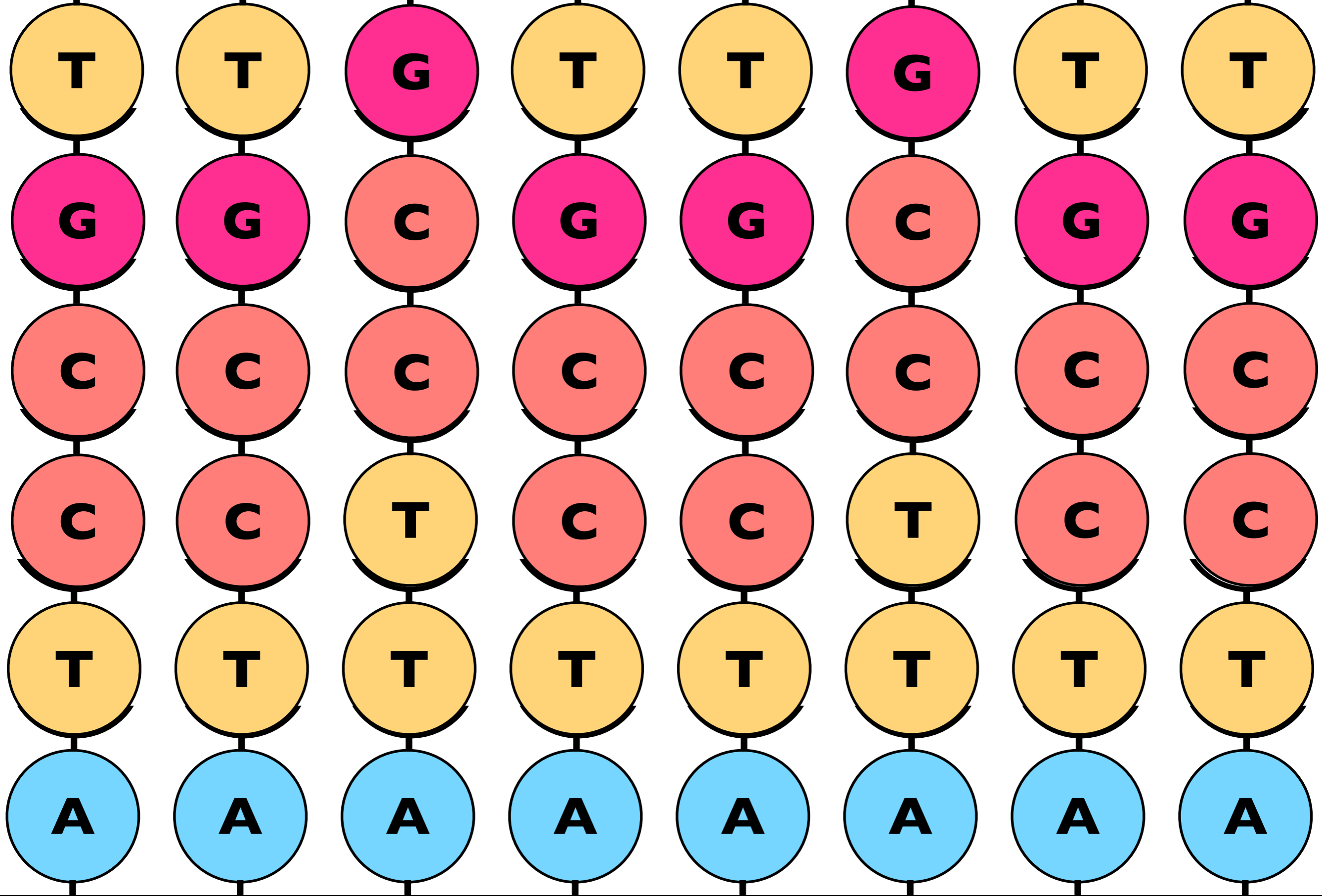
Fail 1. Extra bases added (insertion mutations)

Fail 2. Bases missing (deletion mutations)

Fail 2. Bases missing (deletion mutations)
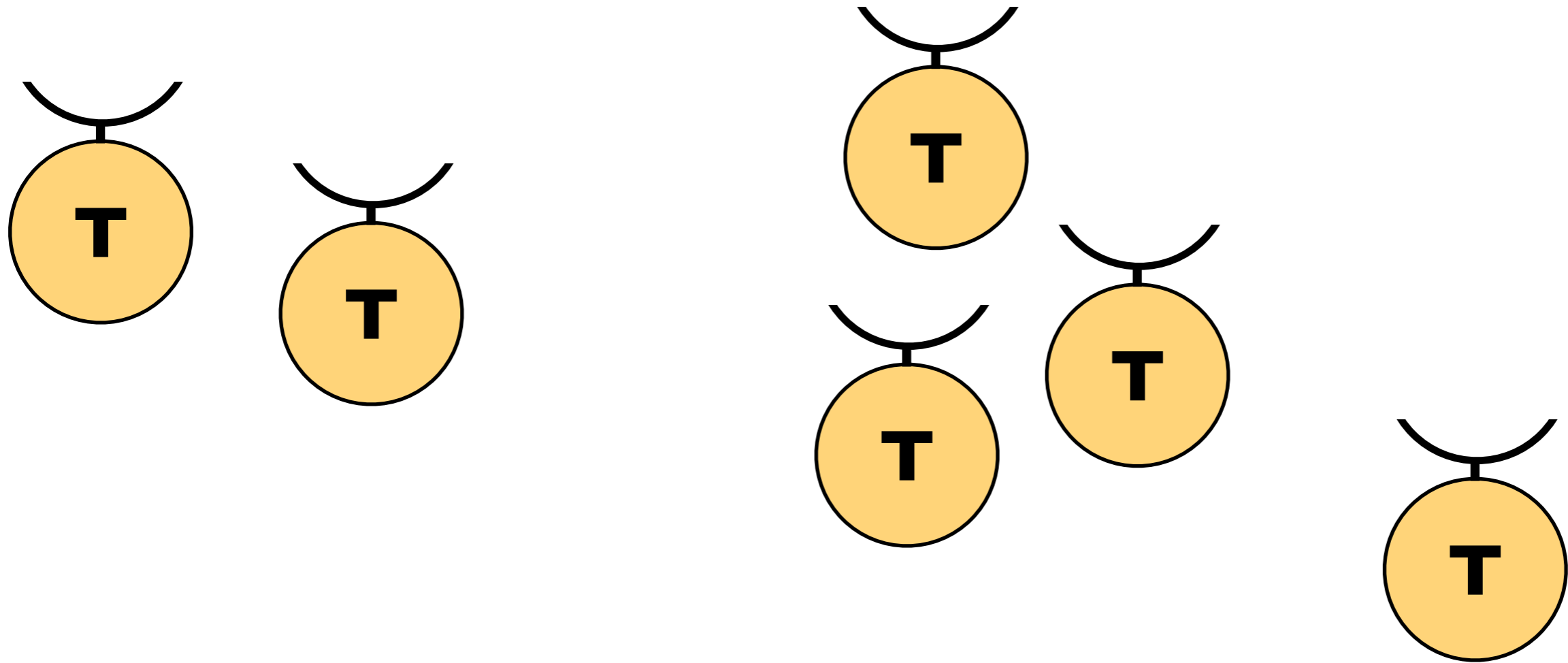
How to print DNA from scratch.
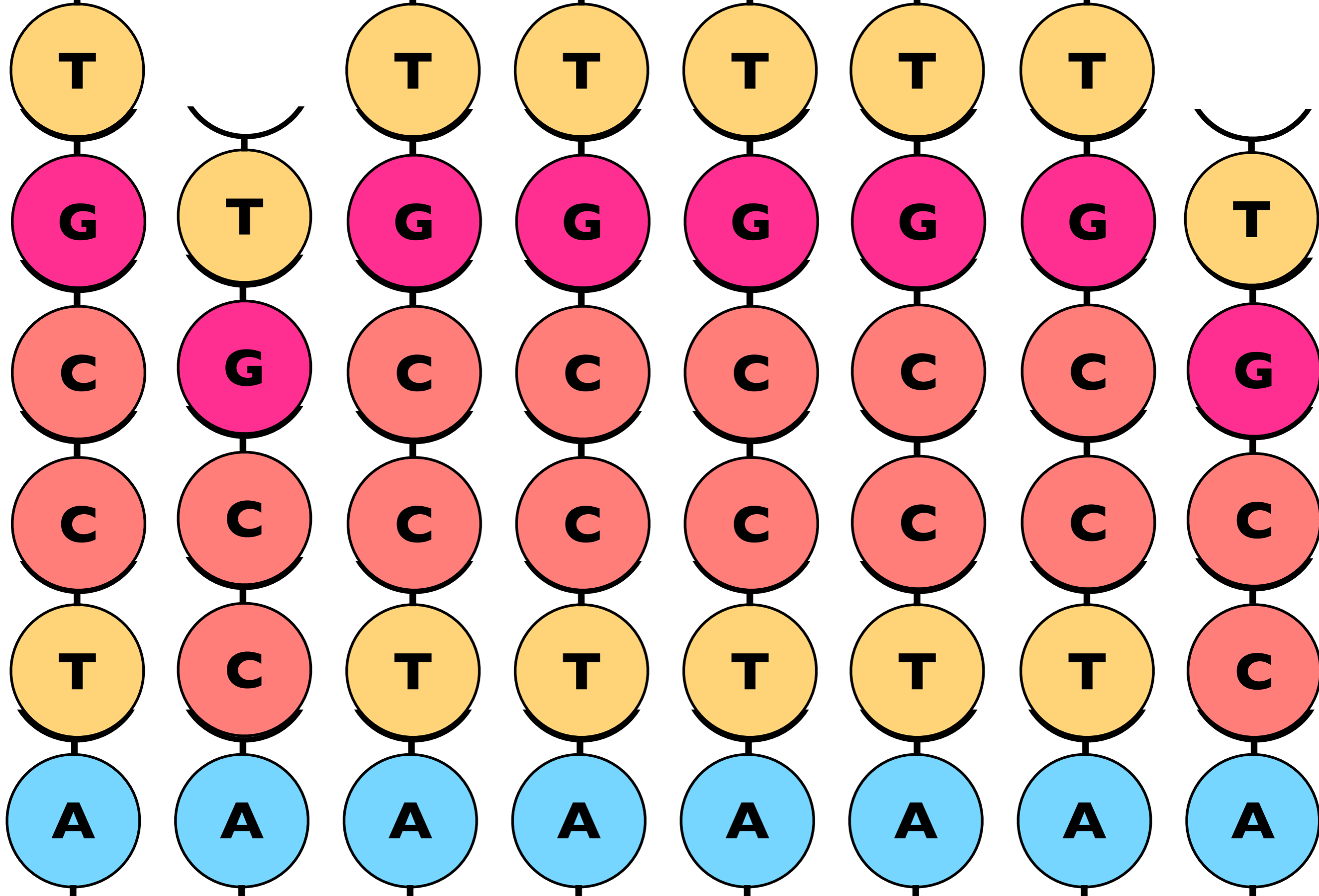
How to optimize synthetic chemistry for accuracy.
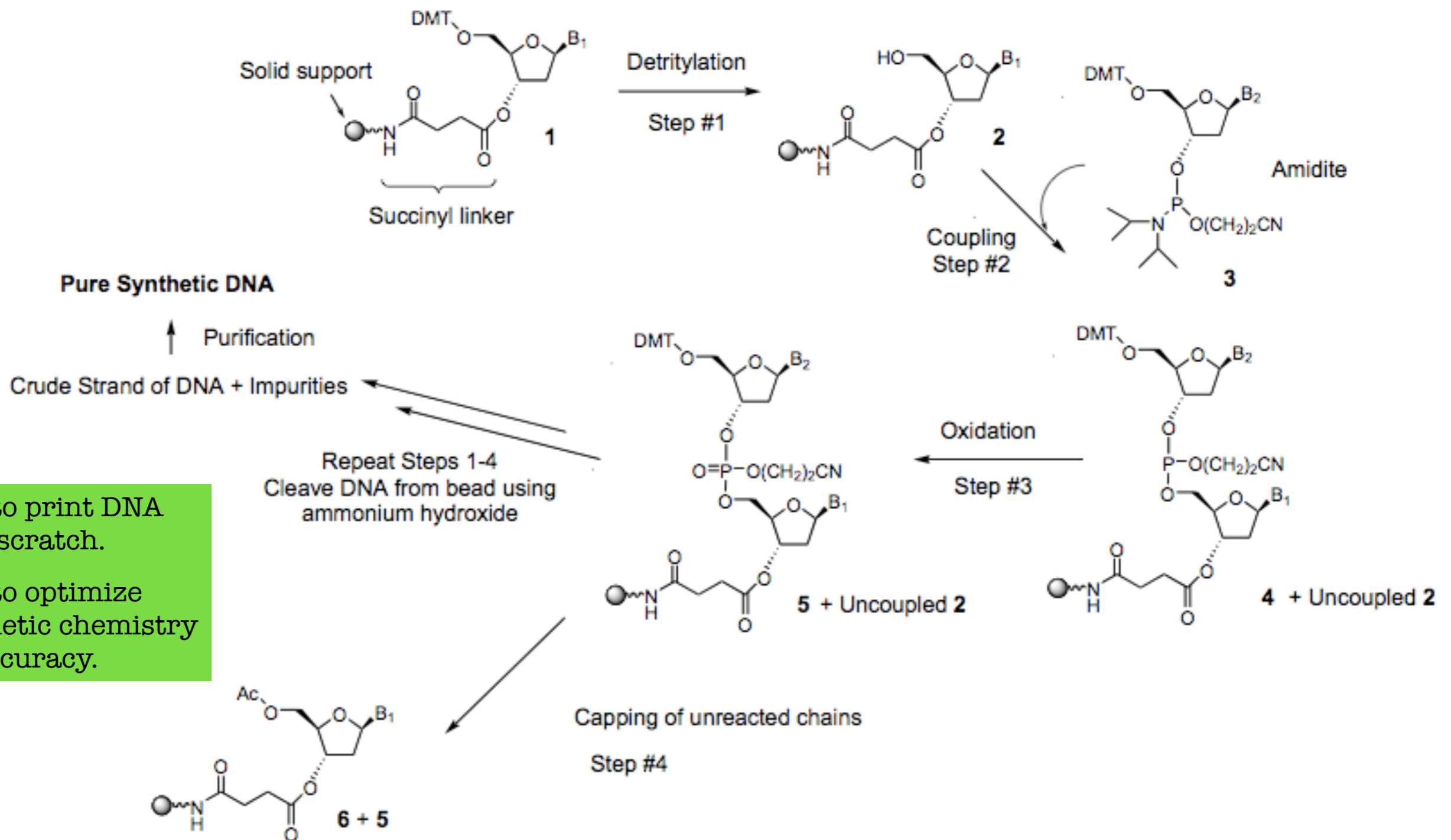
Figure 5: General scheme for automated synthesis of DNA using amidites

from "A Roadmap to the Assembly of Synthetic DNA from Raw Materials" by Sanghvi, Yogesh (http://dspace.mit.edu/handle/1721.1/39657)

Chemistry of DNA defines what must be added (e.g., bases).

Sources of error in synthesis process define added necessary steps:

    - propensity to react leads to <u>blocking</u> group that prevents insertions.

    - failure to couple leads to <u>capping</u> step that prevents deletions.

Cycle efficiency limits length of what can be made from scratch (on conventional DNA synthesizers).

$$\text{yield} = (\text{coupling \%})^{\#bp}$$

$$36\% = (0.99)^{100mer}$$

E.g., how do I make KumaMax?

>KumaMax (amino acid sequence)

MSDMEKPWKEGEEARA VLQGHARAQAPQA VDKGPV AGDERMA VTVVLRRQRAGELAAHVERQAAIAP
HAREHLKREAFAASHGASLDDFAELRRFADAHGLALDRANVAAGTAVLSGP**D**DAINRAFGVELRHFDHPD
GSYRSYLGEVTVPASIAPMIEAVLGLDTRPVARPHFRMQRRAEGGFEARSQAAAPTAYTPLDVAQAYQFPE
GLDGQGQCIAIIELGGGYDEASLAQYFASLGVPAPQVVSVSVDGASNQPTGDPKGPDGEVELDIEVAGALA
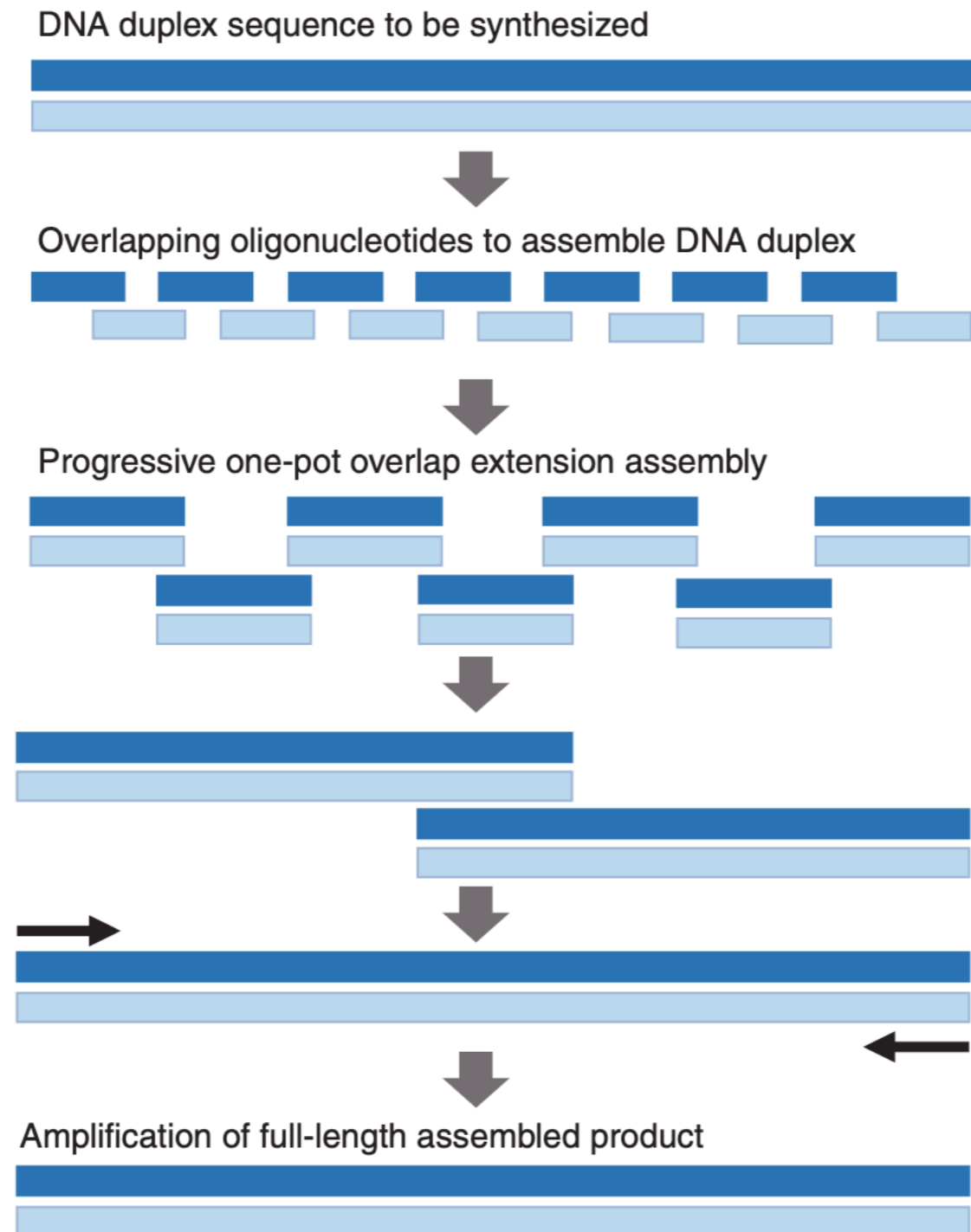PGAKFAVYFAPDTTAGFLDAITTAIHDPTLKPSVVSISWSGPEDSWTSAAIAAMNRAFLDAAALGVTVLAA
AGDSGSTGGEQDGLYHVHFPAASPYVLACGGTRLVASGGRIAQETVWNDGPDGGATGGGVSRIFPLPAW
QEHANVPPSANPGASSGRGVPDLAGNADPATGYEVVIDGEATVIGGTSAVAPLFAALVARINQKLGKAVG YLNPTL
YQLP ADVFHDITEGNNDIANRAQIYQAGPGWDPCTGLGSPIGVRLLQALLPSASQPQPGSTENL YF
QSGALEHHHHHH

>KumaMax (DNA sequence)

atgagcgatatggaaaaaccgtggaaagaaggcgaagaagcgcgcgcggtgctgcagggccatgcgcgcgcgcaggcgccgcaggcggtggataaaggccc
ggtggcgggcgatgaacgcatggcggtgaccgtggtgctgcgccgccagcgcgcgggcgaactggcggcgcatgtggaacgccaggcggcgattgcgccgc
atgcgcgcgaacatctgaaacgcgaagcgtttgcggcgagccatggcgcgagcctggatgattttgcggaactgcgccgctttgcggatgcgcatggcctg
gcgctggatcgcgcgaacgtggcggcgggcaccgcggtgctgagcggcccggatgatgcgattaaccgcgcgtttggcgtggaactgcgccattttgatca
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tggatattgaagtggcgggcgcgctggcgccgggcgcgaaatttgcggtgtattttgcgccggataccaccgcgggctttctggatgcgattaccaccgcg
attcatgatccgaccctgaaaccgagcgtggtgagcattagctggagcggcccggaagatagctggaccagcgcggcgattgcggcgatgaaccgcgcgtt
tctggatgcggcggcgctgggcgtgaccgtgctggcggcggcggggcgatagcggcagcaccggcggcgaacaggatggcctgtatcatgtgcattttccgg
cggcgagcccgtatgtgctggcgtgcggcggcacccgcctggtggcgagcggcggccgcattgcgcaggaaaccgtgtggaacgatggcccggatggcggc
gcgaccggcggcggcgtgagccgcattttccgctgccggcgtggcaggaacatgcgaacgtgccgccgagcgcgaacccgggcgcgagcagcggccgcgg
cgtgccggatctggcgggcaacgcggatccggcgaccggctatgaagtggtgattgatggcgaagcgaccgtgattggcggcaccagcgcggtggcgccgc
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cctgctgcaggcgctgctgccgagcgcgagccagccgcagccgggcagcaccgaaaacctgtattttcagagcggcgcgctggaacatcatcatcatcatc
at

Short synthetic DNA fragments can be assembled into longer and longer fragments

DNA duplex sequence to be synthesized

Overlapping oligonucleotides to assemble DNA duplex

Progressive one-pot overlap extension assembly

Amplification of full-length assembled product

How I make KumaMax!

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DNA = industrial polymer of 21st century.

DNA read/write tools progressing…

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Genomes as a Service…