POLYMERASE CHAIN REACTION

LUCIA DHIANTIKA WITASARI

Polymerase Chain Reaction

Tehnik PCR merupakan metode amplifikasi (penggandaan) fragmen DNA secara *in vitro.*

PCR is an iterative process, consisting of three elements:

1. denaturation of the template by heat
2. annealing of the oligonucleotide primers to the single-stranded target sequence(s)
3. extension of the annealed primers by a thermostable DNA polymerase.

Essential Components of Polymerase Chain Reactions

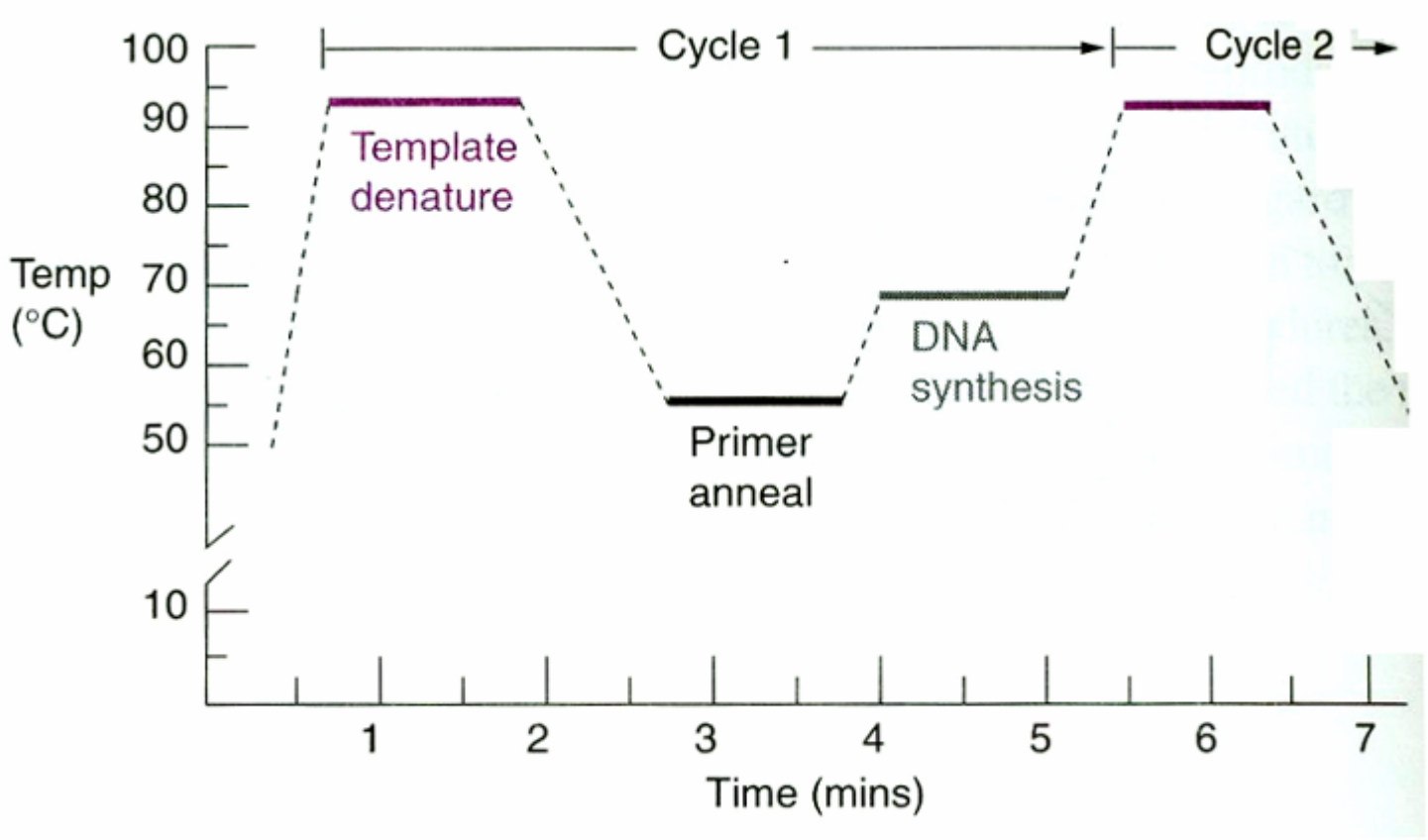
- A thermostable DNA polymerase to catalyze template-dependent synthesis of DNA.
  - Taq polymerase
- Primers : A pair of synthetic oligonucleotides to prime DNA synthesis.
- Deoxynucleoside triphosphates (dNTPs)
  - dATP, dTTP, dCTP, and dGTP
- Divalent cations
  - All thermostable DNA polymerases require free divalent cations — usually Mg^{2+} for activity.
- Buffer to maintain pH
  - Tris-Cl, adjusted to a pH between 8.3 and 8.8 at room temperature,
- Monovalent cations
  - KCl
- Template DNA

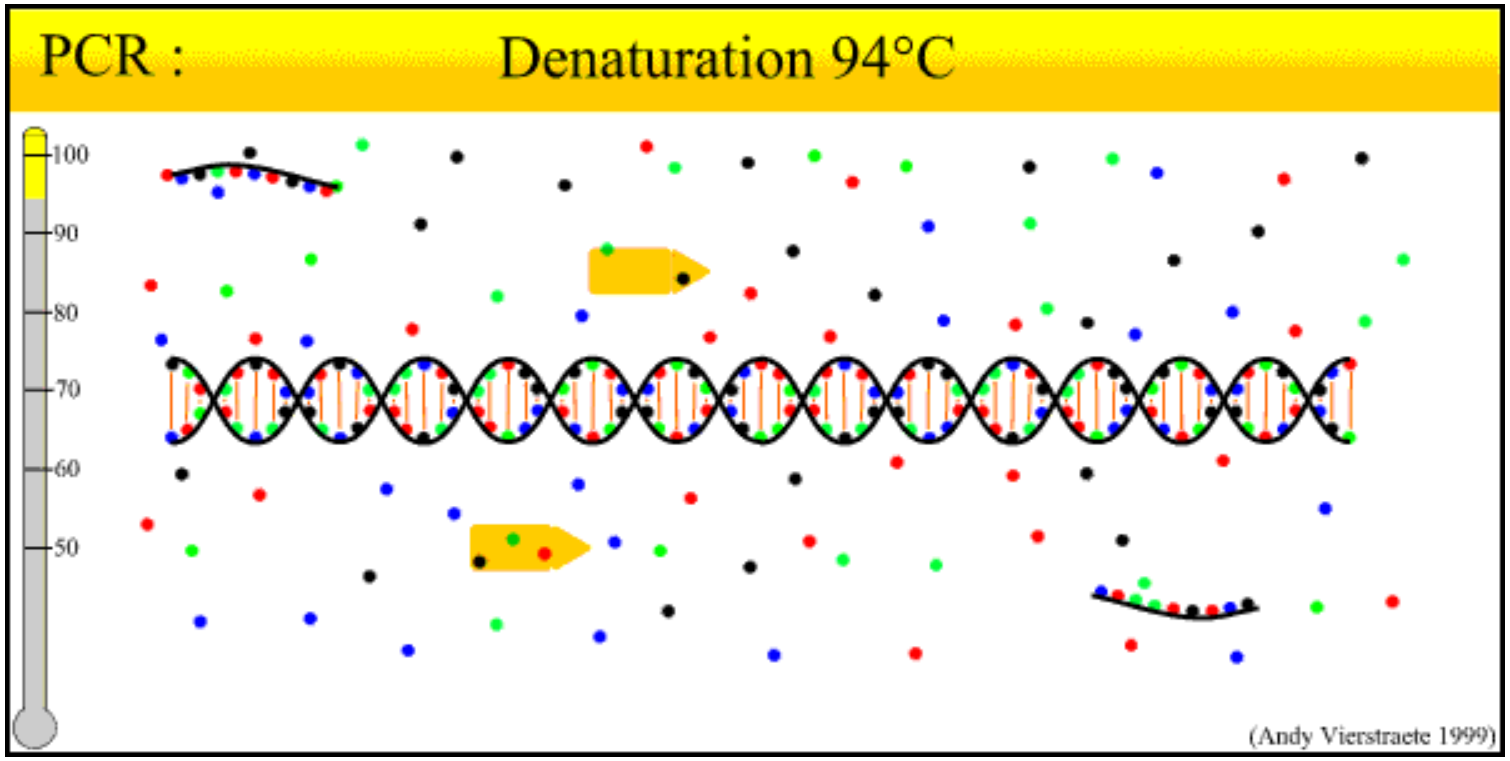
Primer Design

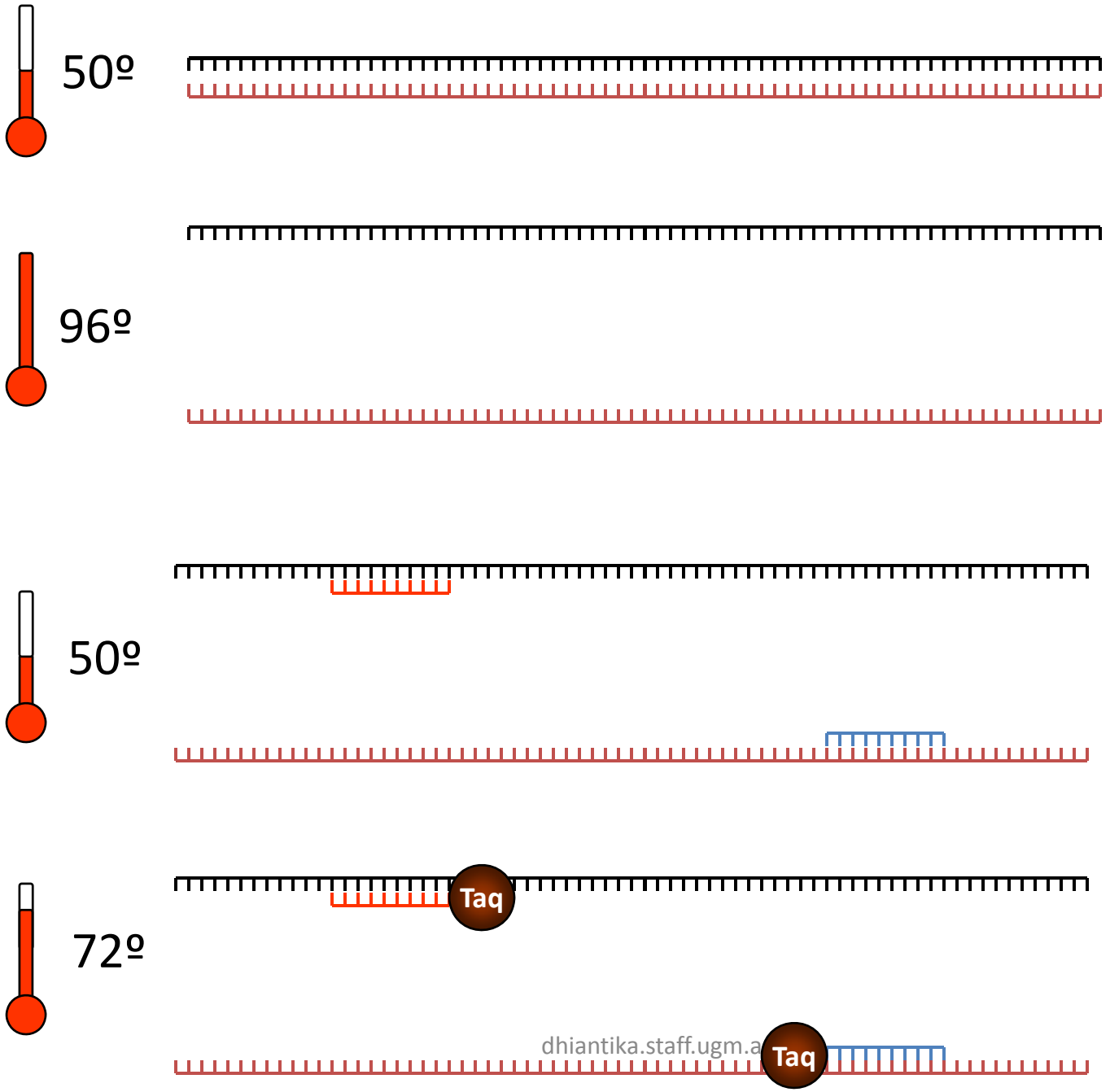
- **Length:** 16 to 25 bases; rarely for efficient amplification is there a need to use longer primers.
- **GC content:** should mirror the content of the amplicon. The 3' end should be "weak"; no G or C as the end base.
- **T_m :** should be balanced, annealing temperatures are usually 5° to 10°C lower than the T_m .
  - The melting temperatures of oligos (generally valid for oligos in the 18–24 base range) can be estimated using the formula:

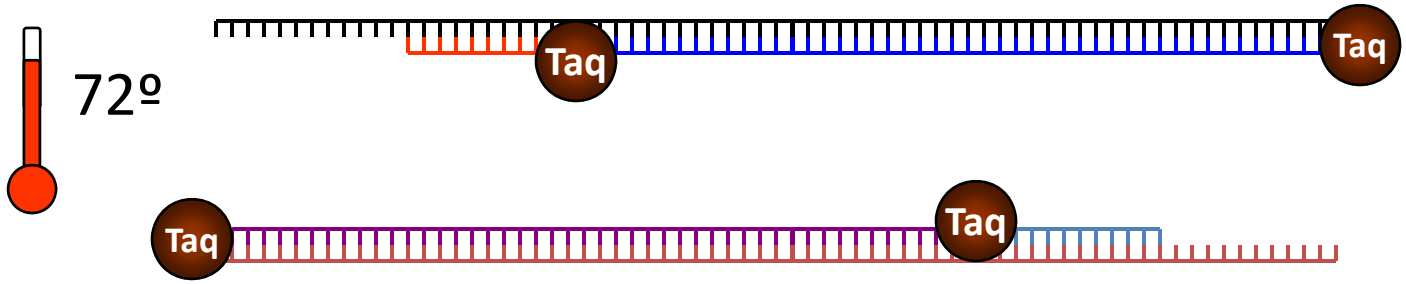
    Tm = 2(A+T) + 4(G+C).
- **Absence of complementarity:** 3'-end, primer-dimer, internal hairpin structures.
- **Orientation and placement:** important more in RT-PCR experiments (placement at the 3' or 5' end, or spanning intron/exon junctions)

PCR cycle

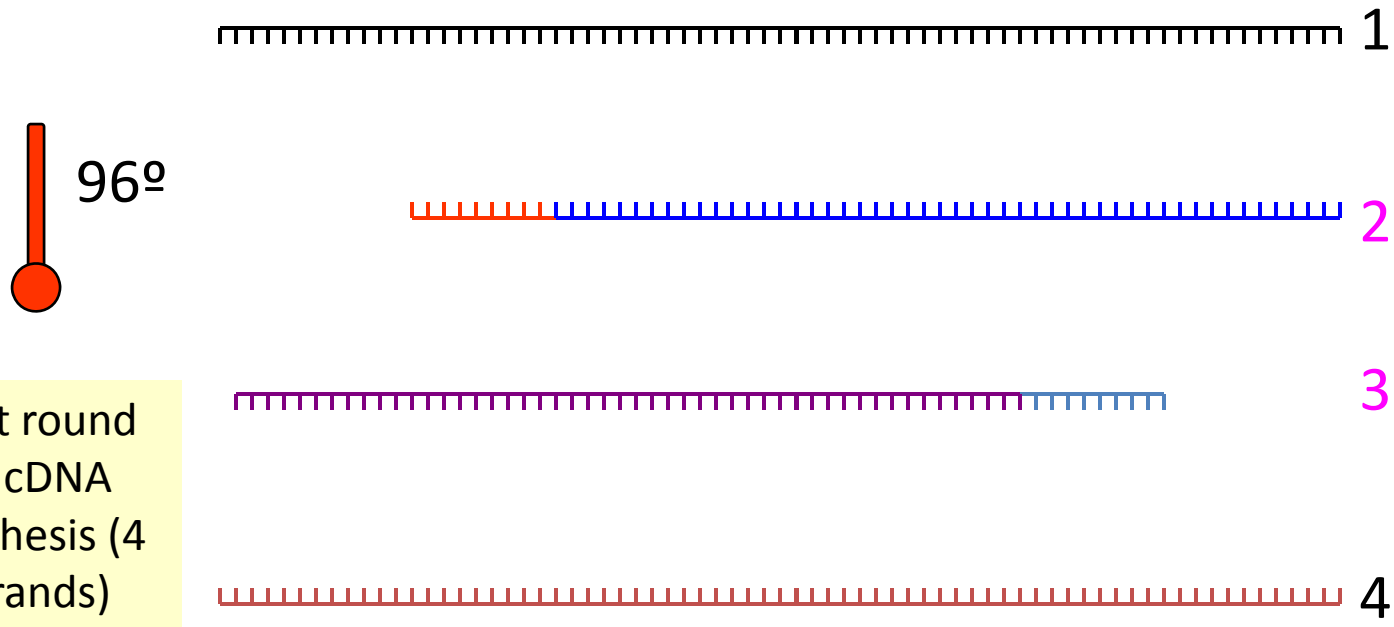

Cycle 1
Cycle 2
100
90
80
70
60
50
10
Temp (°C)
Template denature
Primer anneal
DNA synthesis
1
2
3
4
5
6
7
Time (mins)


PCR :
Denaturation 94°C
100
90
80
70
60
50
(Andy Vierstraete 1999)


50º
A. Double strand DNA
96º
B. Denature
50º
C. Anneal primers
72º
Taq
D. Polymerase binds
Taq

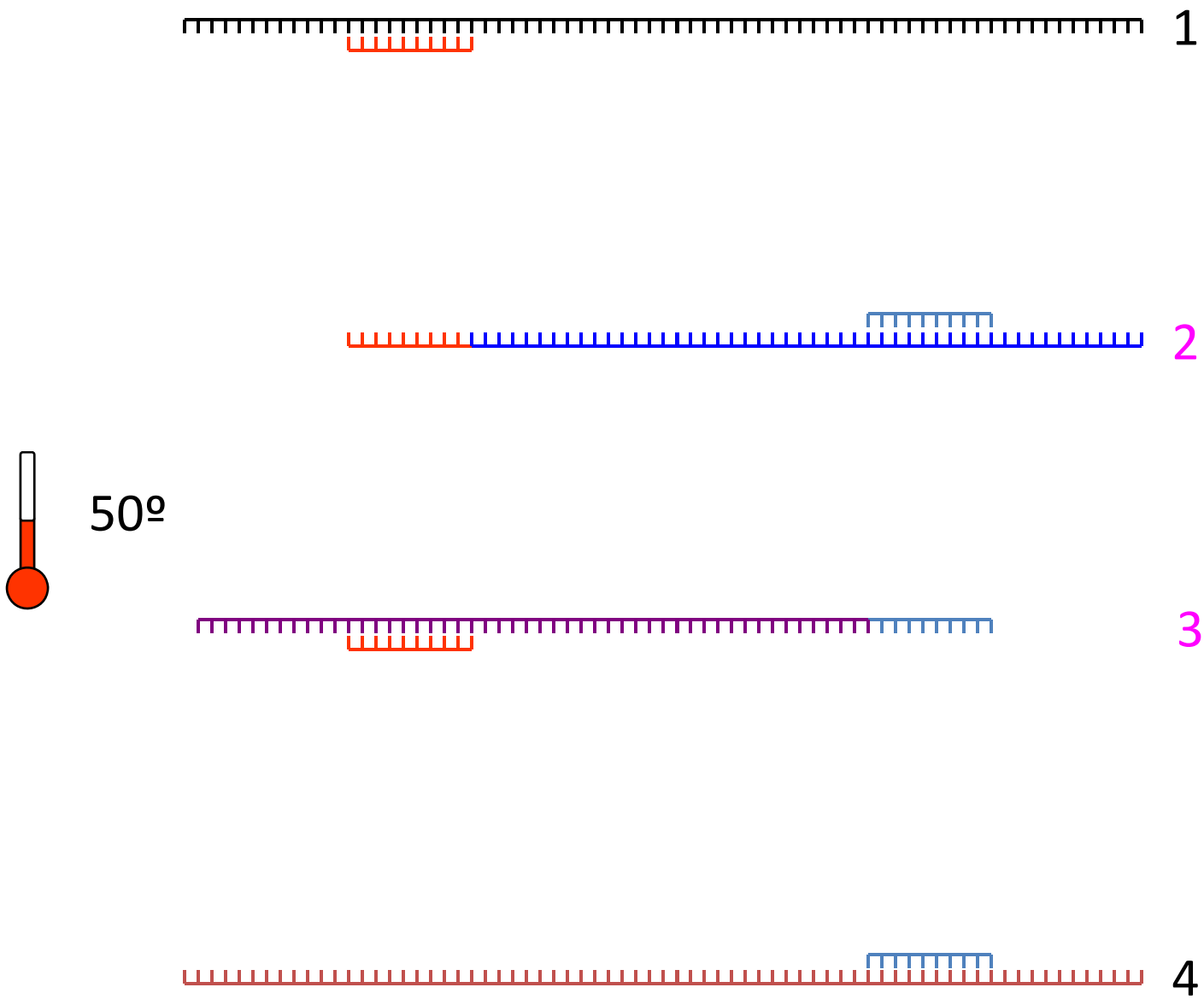


E. Copy strands

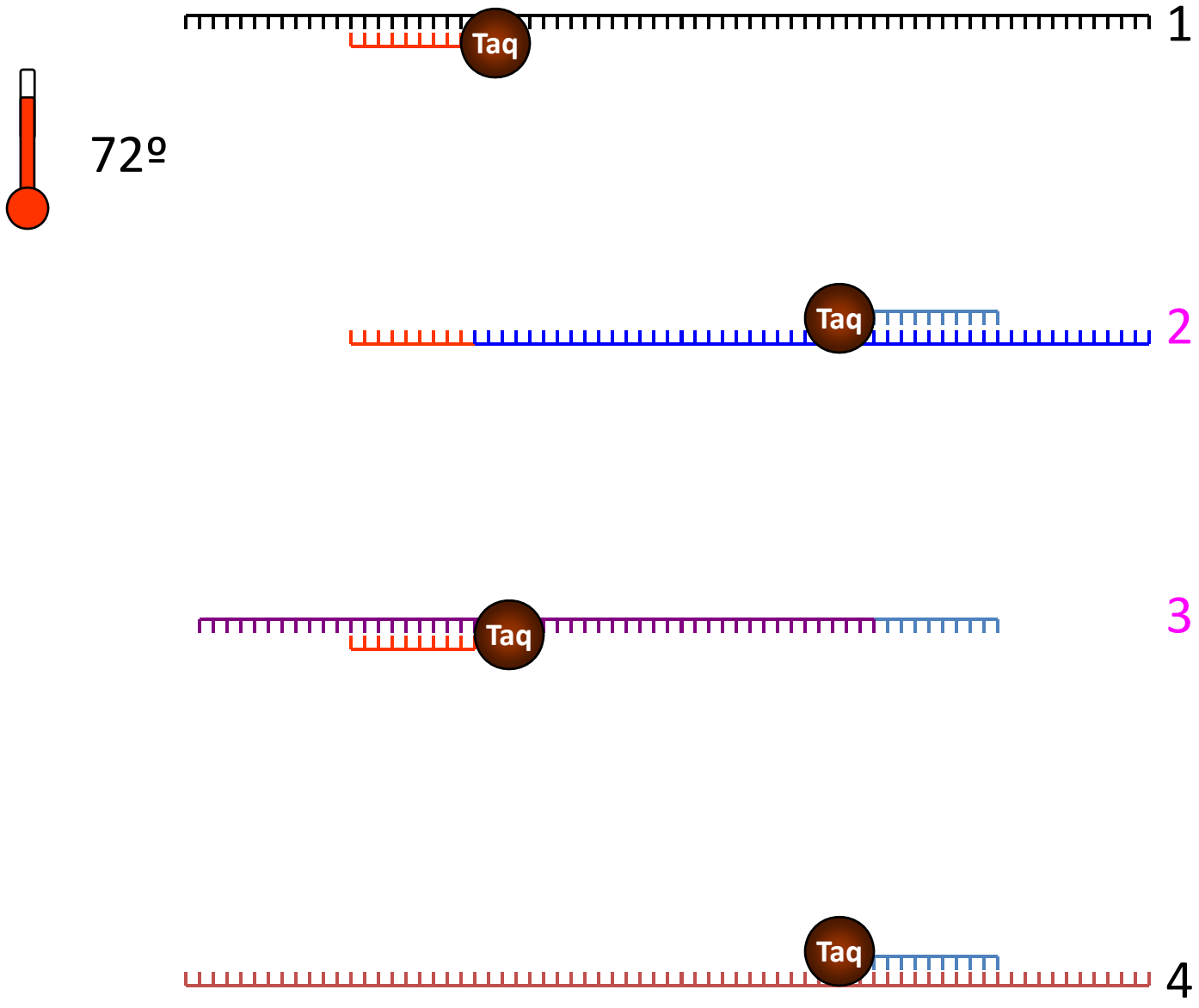


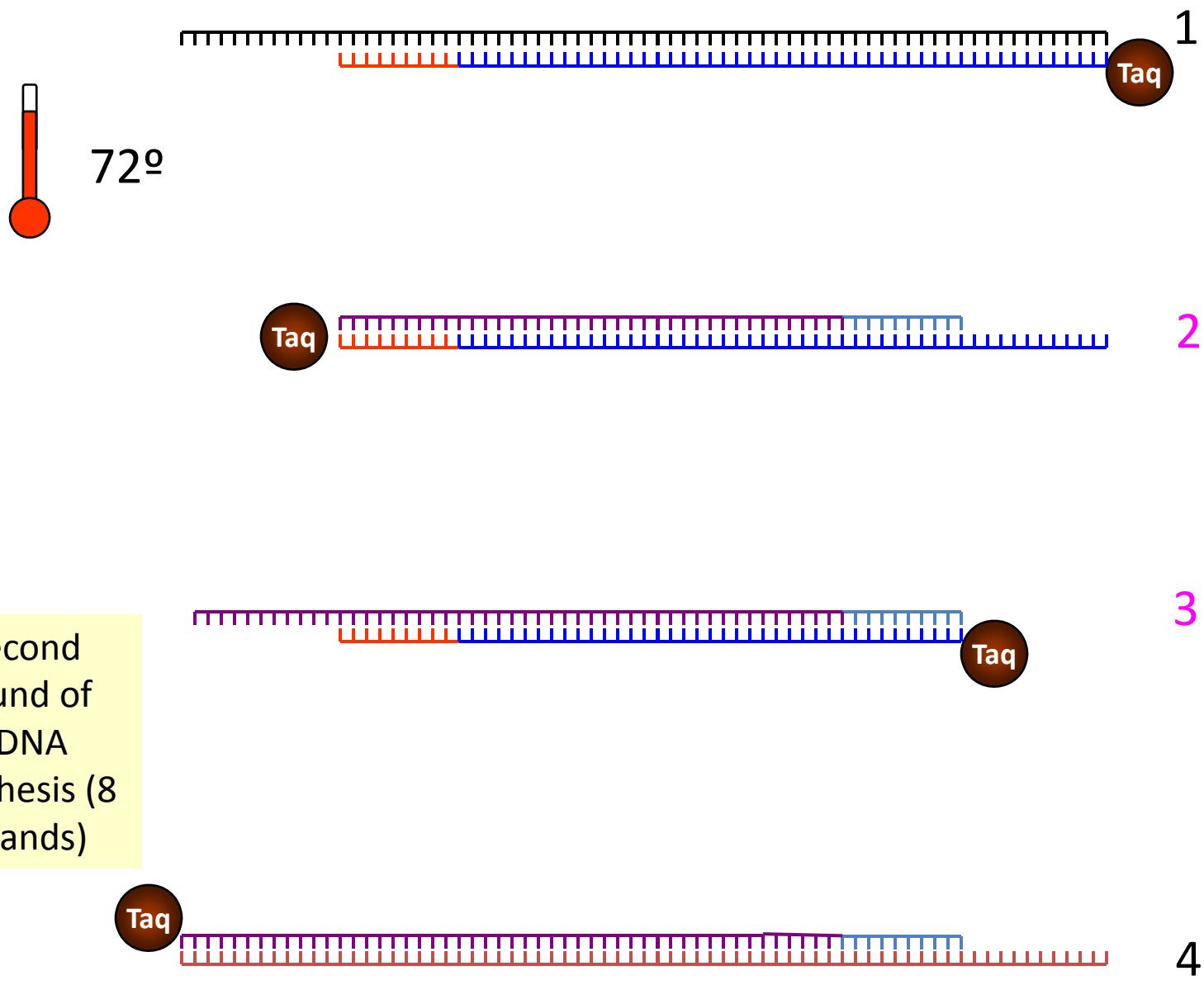
F. Denature

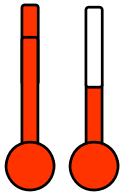
First round of cDNA synthesis (4 strands)

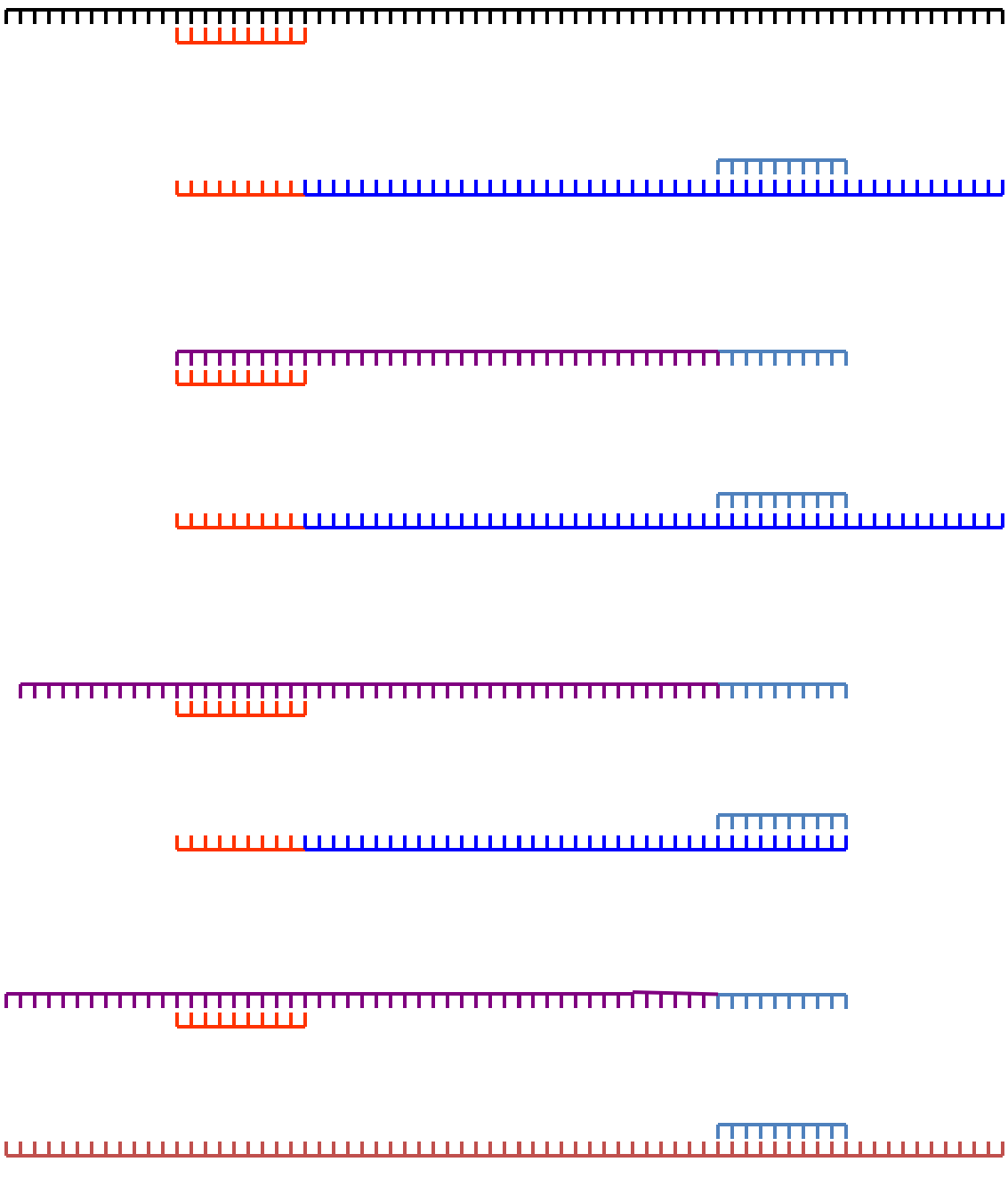


G. Anneal primers

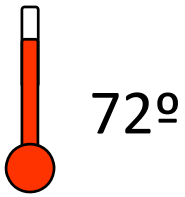

Taq
1
72º
Taq
2
H.
Polymerase
binds
Taq
3
Taq
4


1
Taq
72º
Taq
2
I. Copy strands
3
Taq
Second round of cDNA synthesis (8 strands)
Taq
4

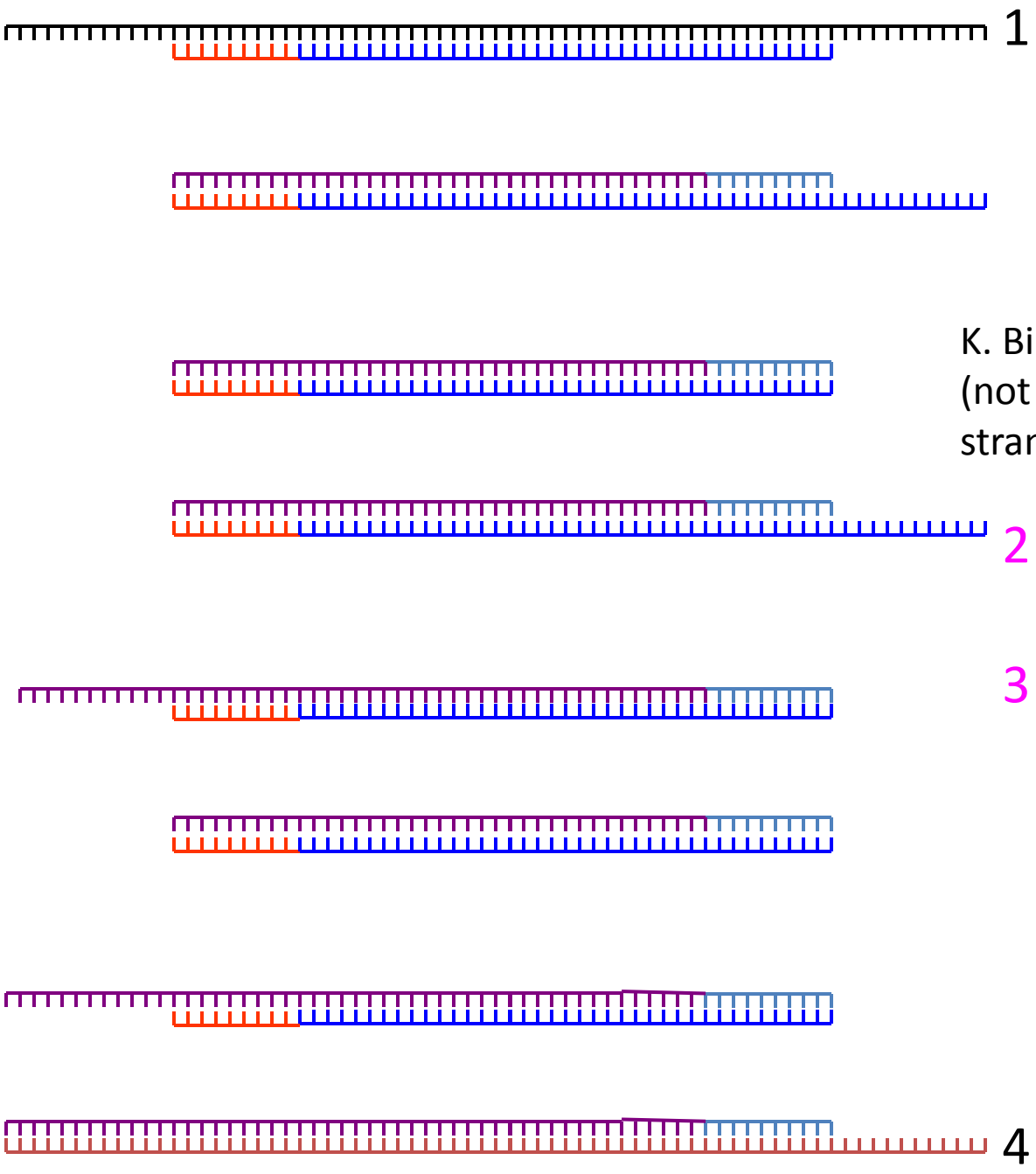




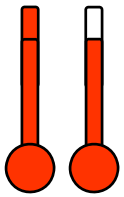
J.
Denature at 96º
Anneal primers at 50º

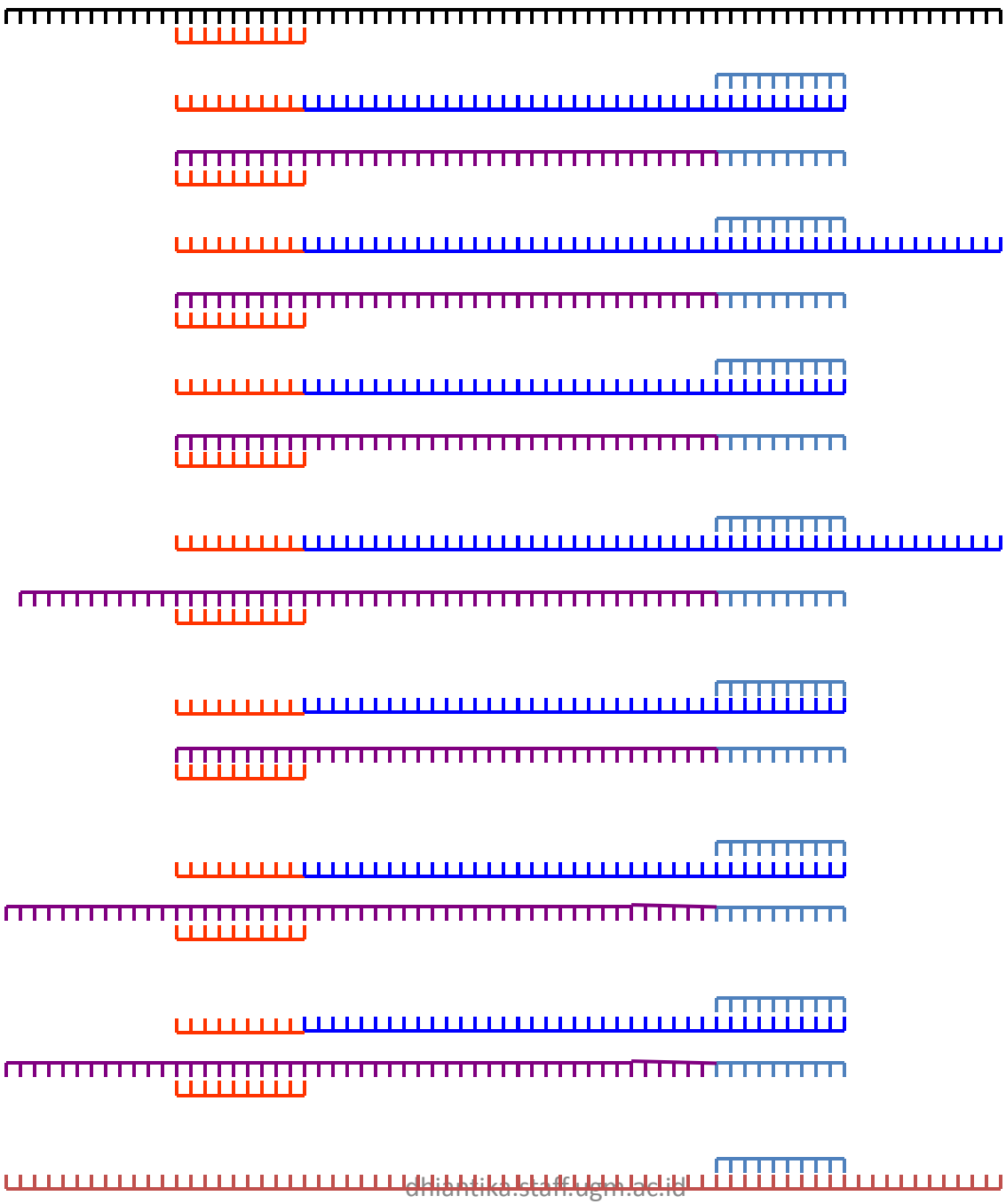


K. Bind polymerase (not shown) and copy strands

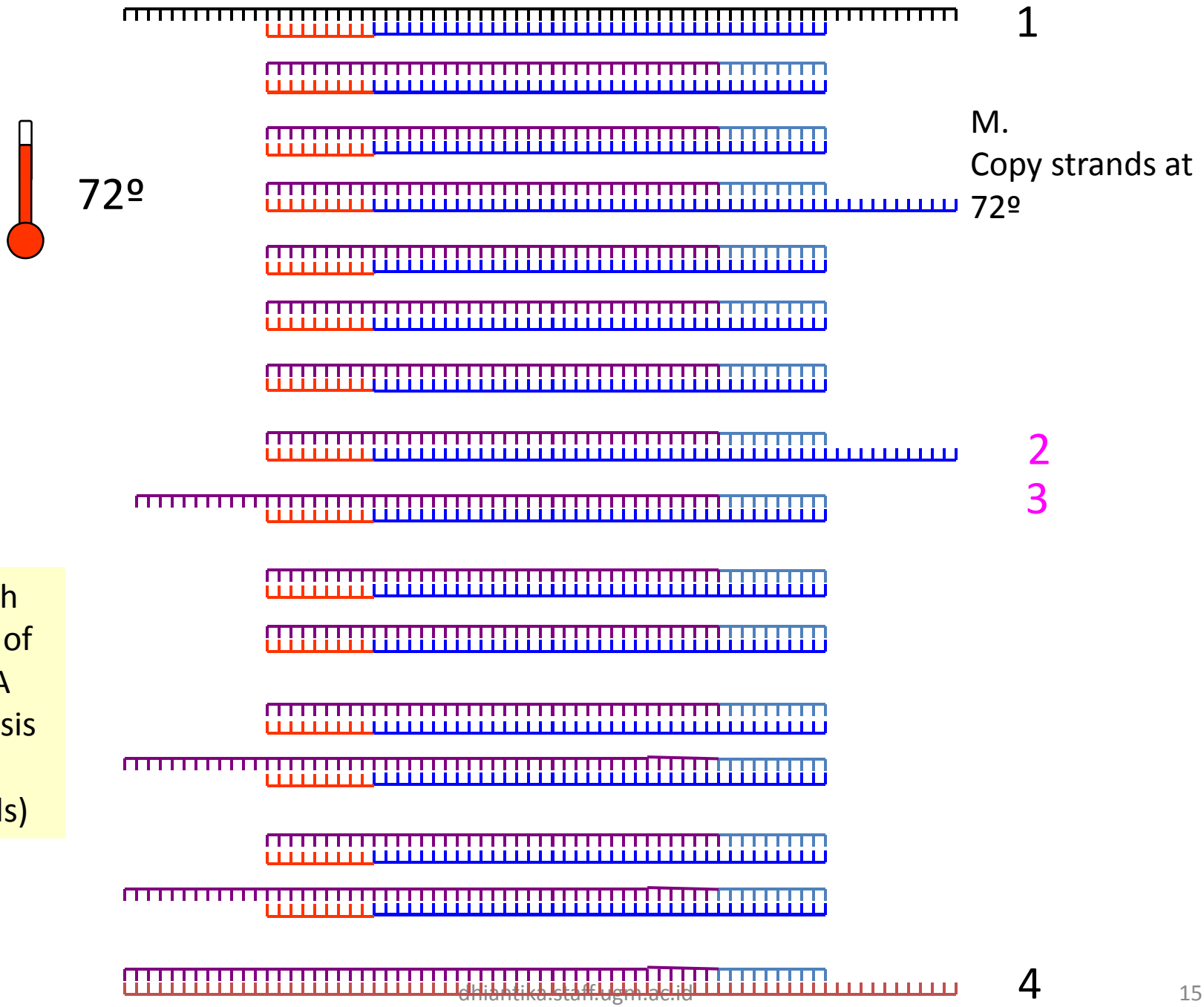


Third round of cDNA synthesis (16 strands)



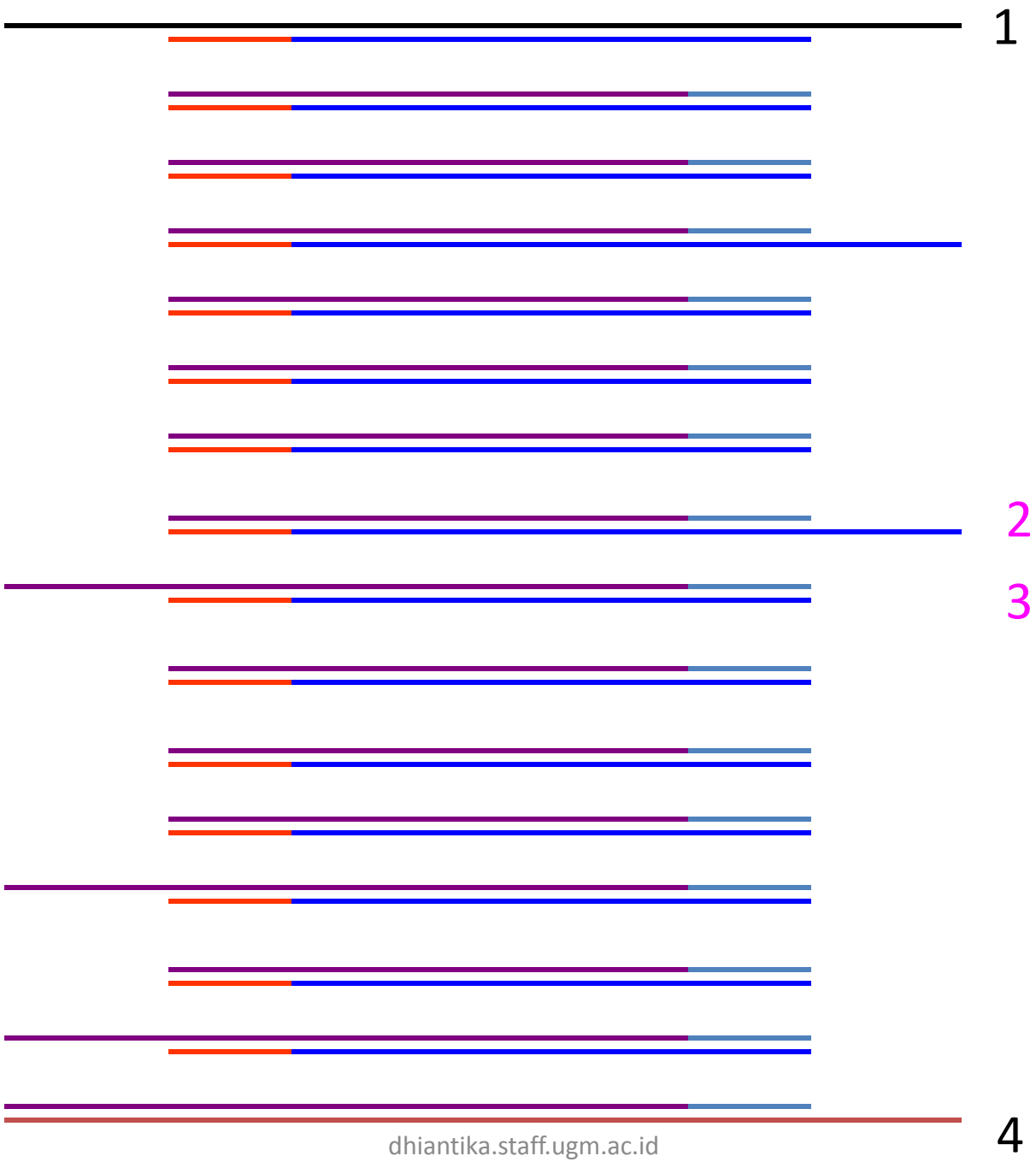


L.
Denature at 96º
Anneal primers at 50º

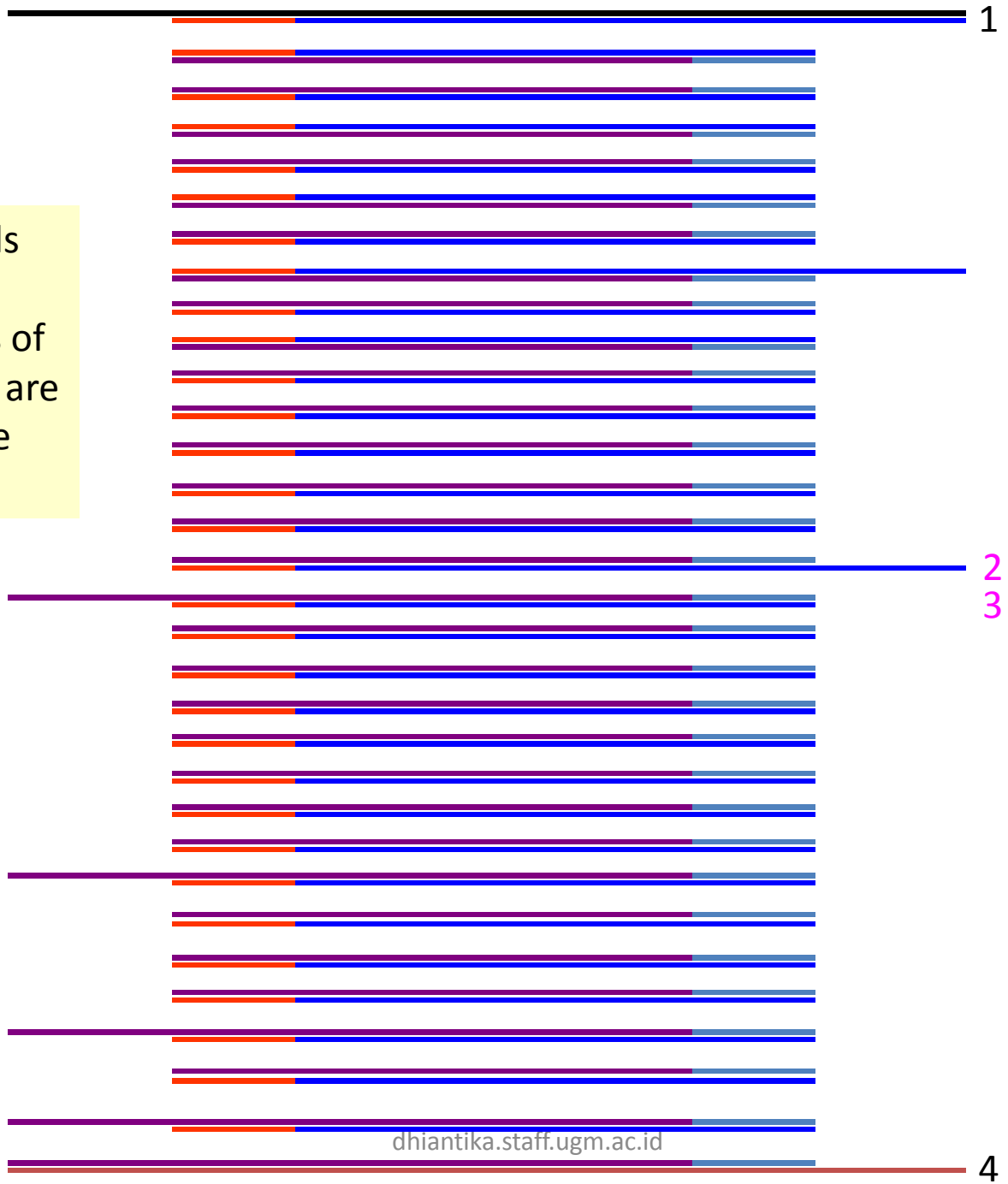


Fourth round of cDNA synthesis (32 strands)

cDNA strands (32) are now shown as lines

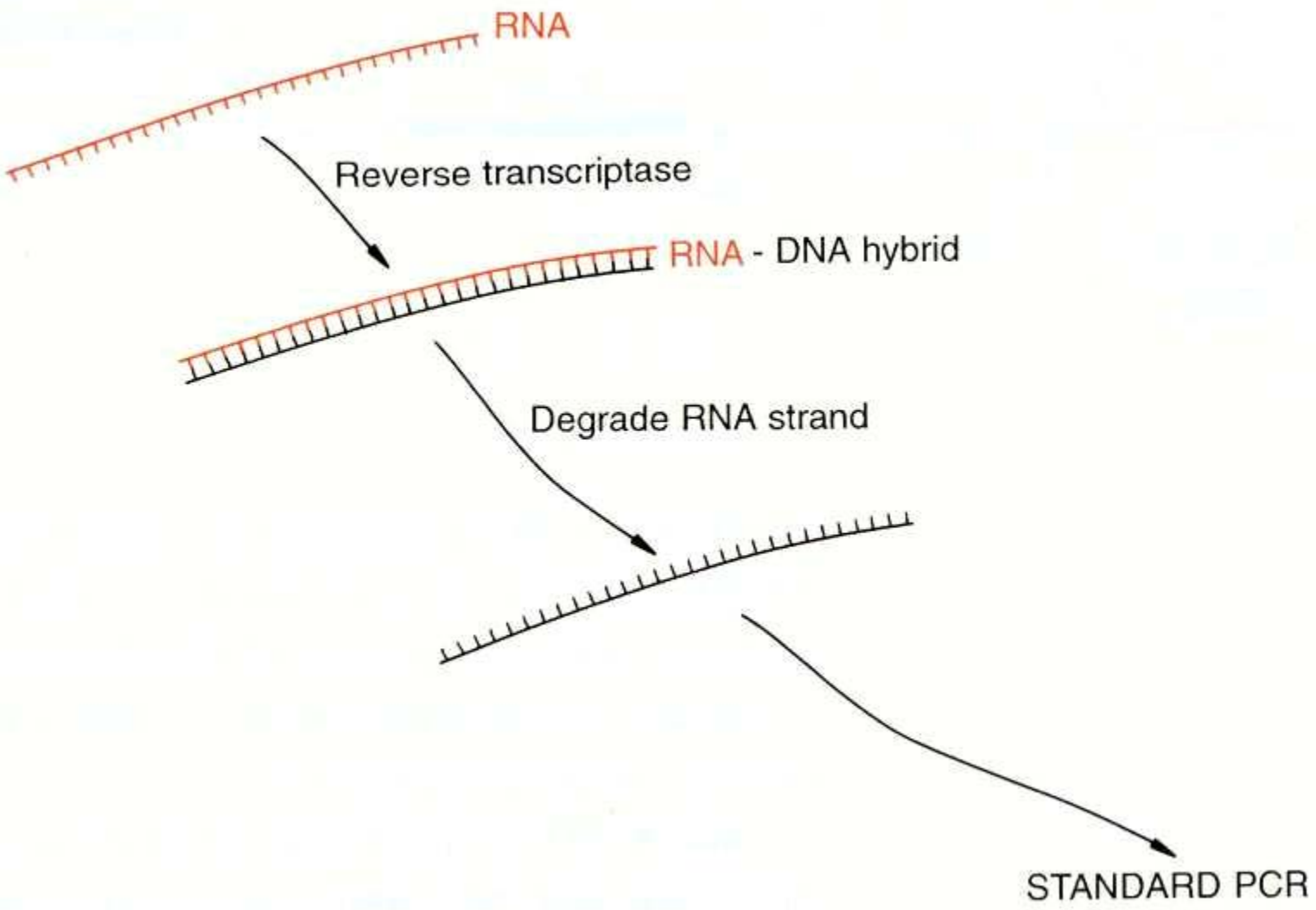


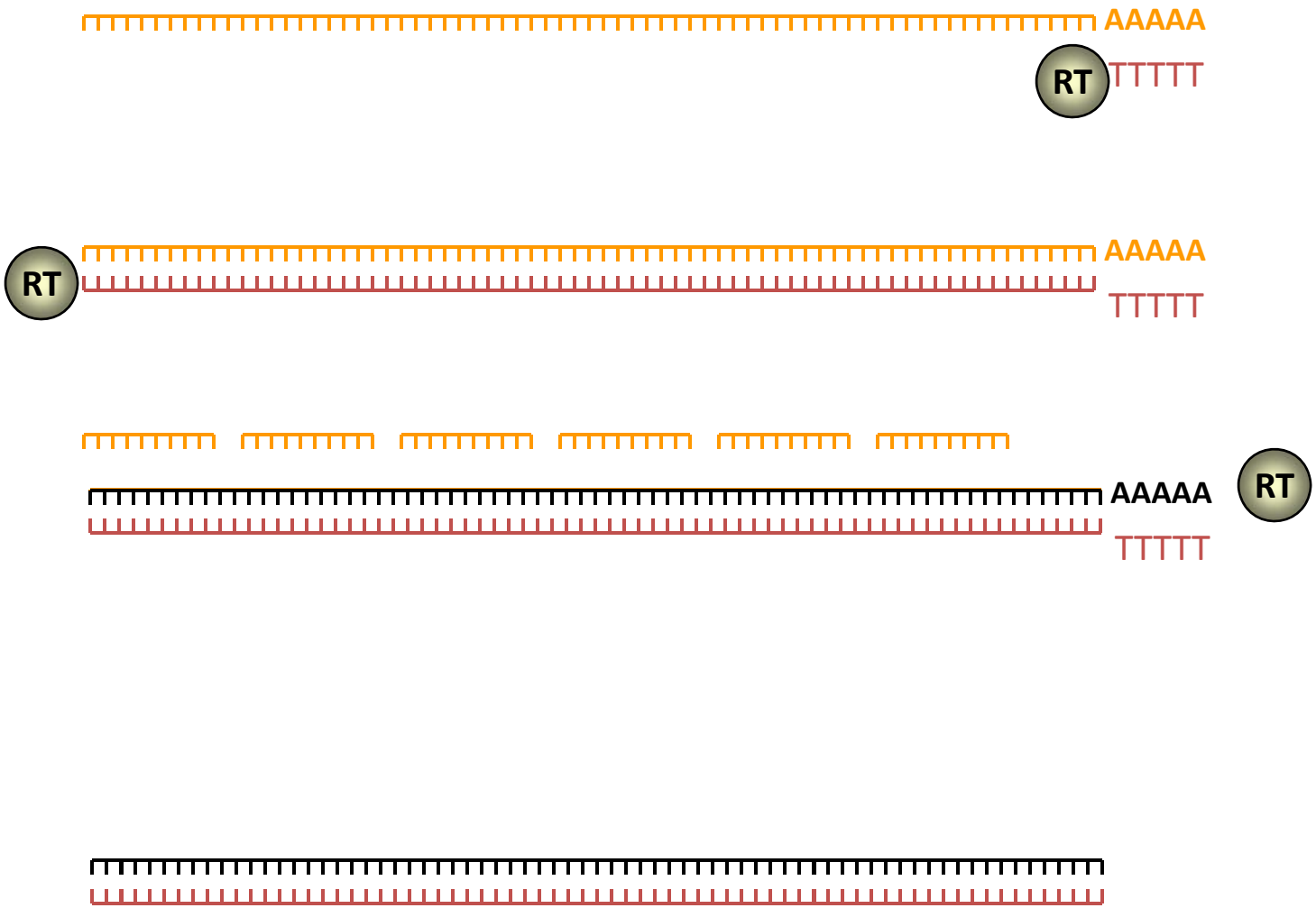
After 5 rounds there are 32 double strands of which 24 (75%) are are same size



RT-PCR

Reverse transcriptase (RT)-PCR adalah amplifikasi fragmen DNA yang diperoleh dari fragmen mRNA. Produk yang dihasilkan adalah cDNA.





Oligo dT primer is bound to mRNA

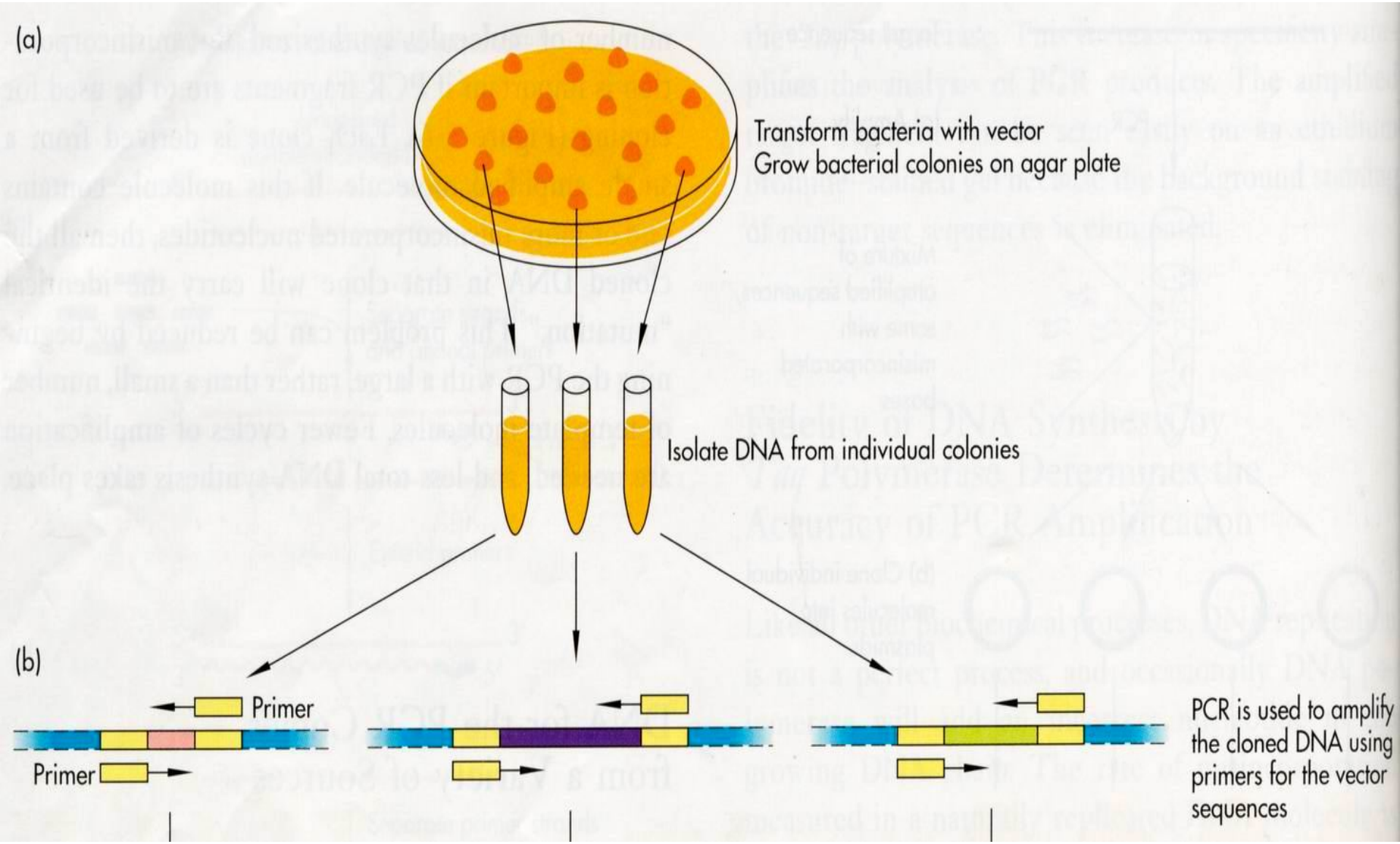
Reverse transcriptase (RT) copies first cDNA strand

Reverse transcriptase digests and displaces mRNA and copies second strand of cDNA

Double strand cDNA

Conversion of mRNA to cDNA by Reverse Transcription

Amplifikasi frgamen DNA yang sudah diklonkan kedalam vektor dengan menggunakan primer yang mengenali urutan nukleotida pada vektor.



Kloning produk PCR yang menghasilkan fragmen DNA dengang beberapa basa yang tidak berpasangan.