An Introduction to Polymerase Chain Reaction (PCR)

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Introduction

- The technique was invented by Dr. Kary Mullis, 1983,
- for which he received the Nobel Prize in Chemistry in 1993.
- *in vitro* method (test tube system) for DNA replication.

Published papers with 'PCR'

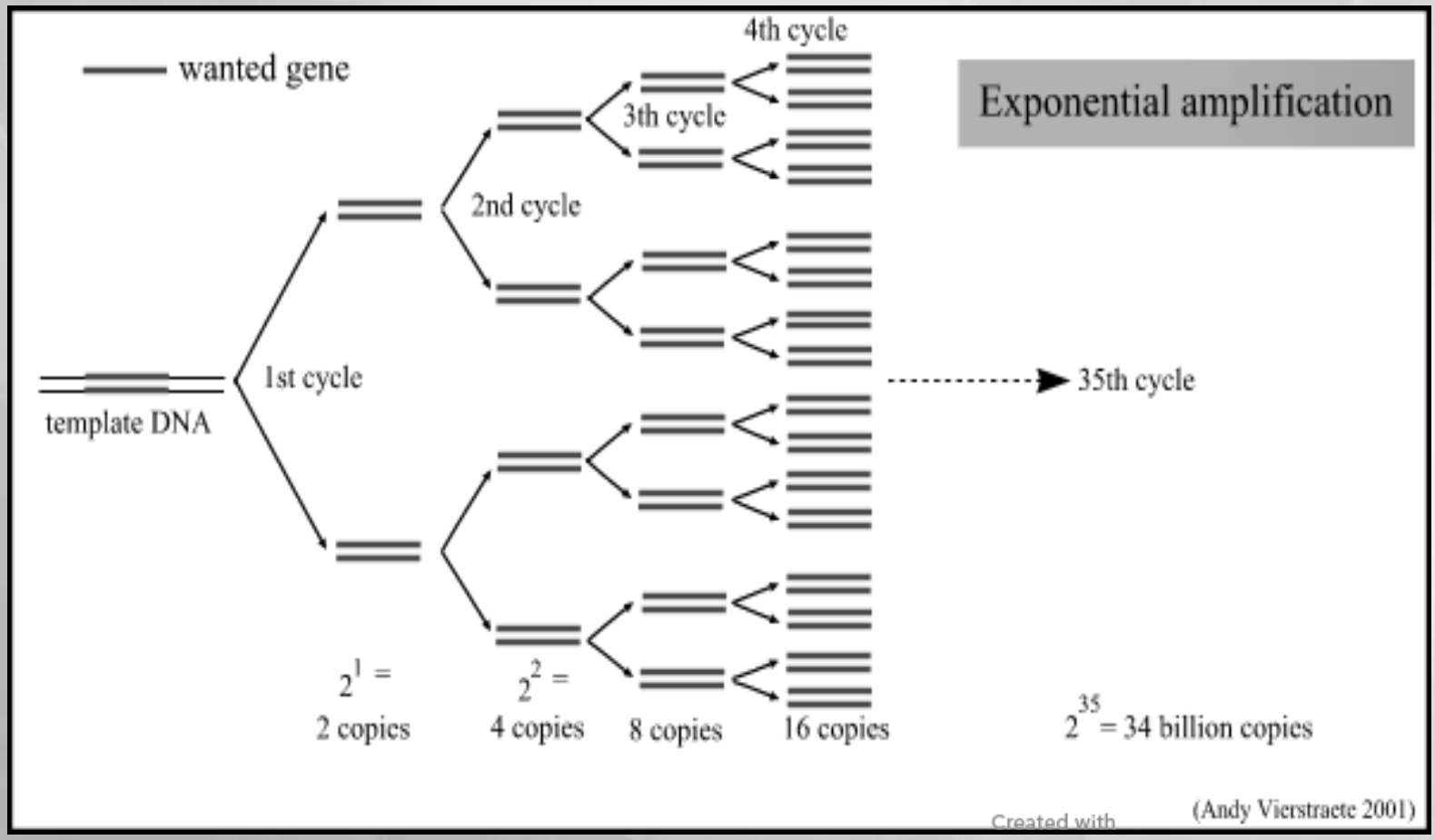
- 1989 - 219
- 1990 – 496
- 1991 – 711
- 1992 – 906
- 1993 –1030
- 1994 – 857 (>4000)
- 1995 – 823
- 1996 – 796
- 1997 – 732
- 1998,10 - >73,000
- 1999,4 - >81,000
- 2000,10 – 121,305
- 2001,2 – 125,563
- 2002,3 – 149,572
- 2003,2 – 170,841
- 2004,2,23-195,193
- 2004,2,26-195,265
- 2006,3,22 - 255,788
- 2006/4/18 – 257,737
- 2007/3/9 – 283,607
- 2007/4/11 - 286,486

Introduction

- PCR will allow a short stretch (specific sequence) of DNA (usually fewer than 3000 base pairs) to be amplified to more than million fold.
- Millions of copies of a segment of DNA can be made within a few hours.

Introduction

- **DNA amplification by PCR (overview)**



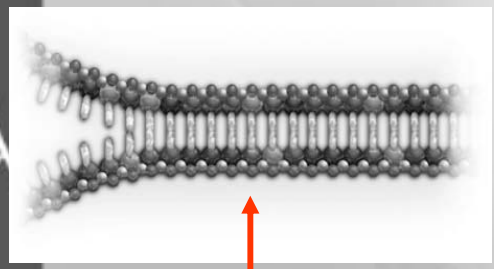
What do we need for PCR?

- Target DNA (Template).
- Two primers: (forward and reverse)
- Nucleotides: (the 4 dNTP'S: A, T, C, G)
- Heat-stable DNA polymerase:
  (like Taq DNA Polymerase)
- Buffer and Cofactor $MgCl_2$ (Mg^{++}, K^{+}).
- Thermal cycler.

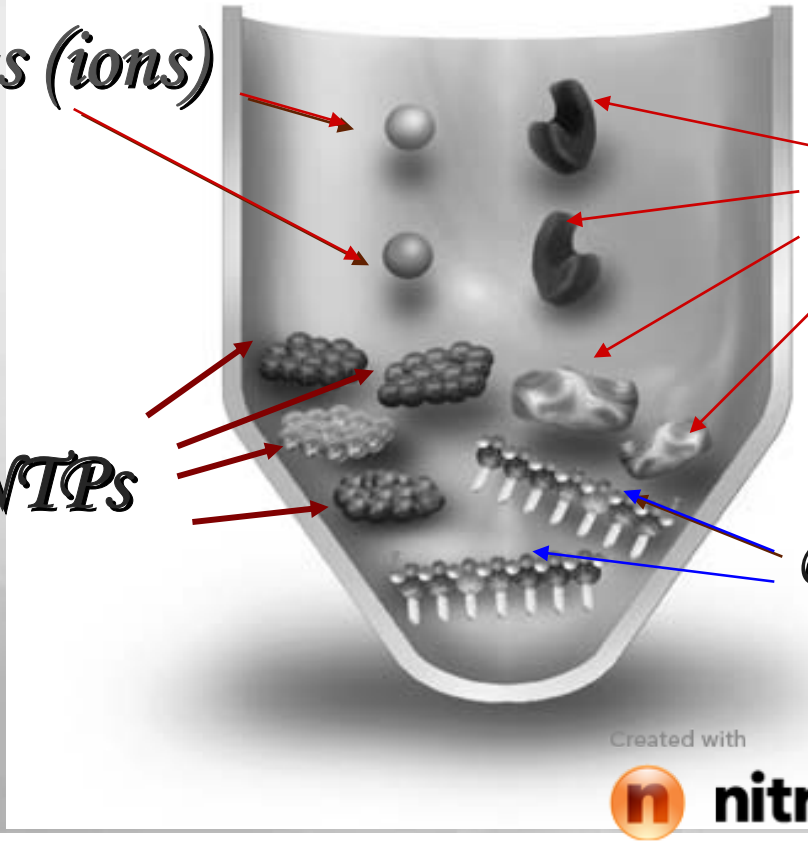
PCR Procedure

Essential Components of PCR Reaction

All the required components are inserted into an Eppendorf tube



Template DNA



The Reaction

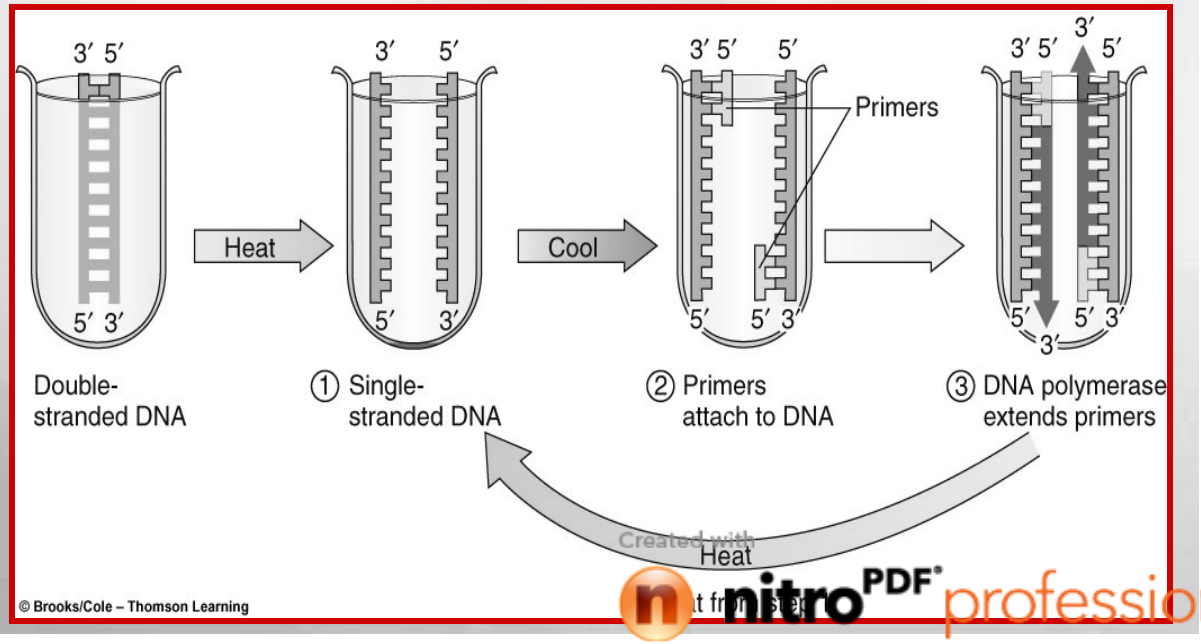
PCR Eppendorf tube containing reaction mix. placed in a thermocycler.

PCR tube

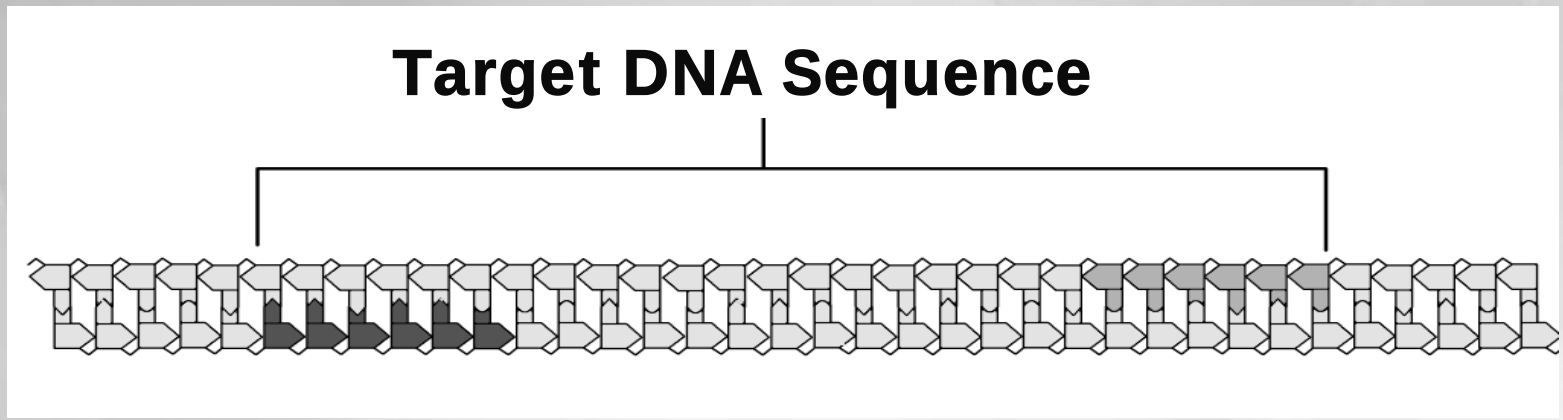
THERMOCYCLER

PCR Cycle

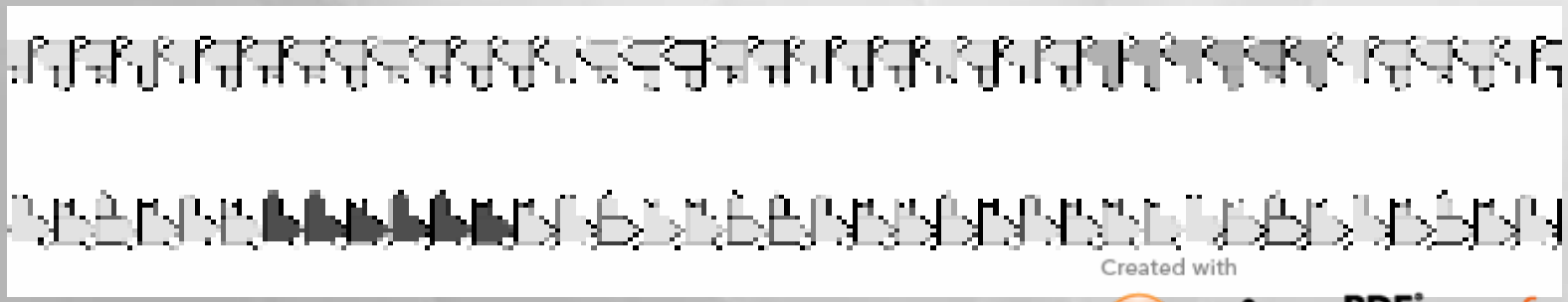
- **Each cycle (Round) of PCR contains 3 steps:**
  - **1- Denaturation**
  - **2- Primer annealing**
  - **3- Primer extension**
- **The cycle usually repeated for 25 – 40 times.**



PCR cycle (round)

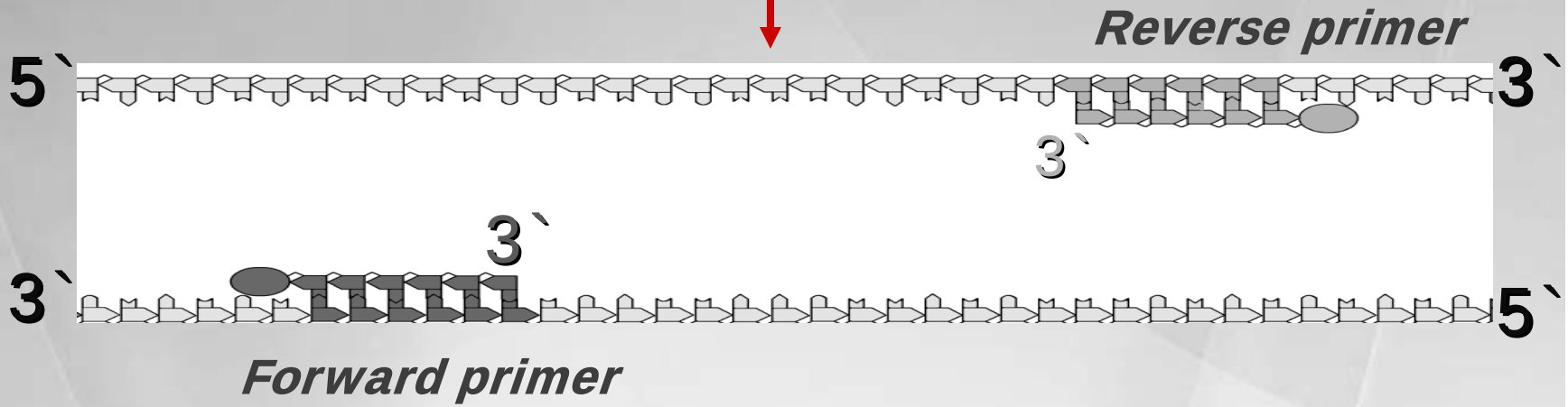


Denaturation (95°C) ~ 1 min.



Primer Annealing (55°C)

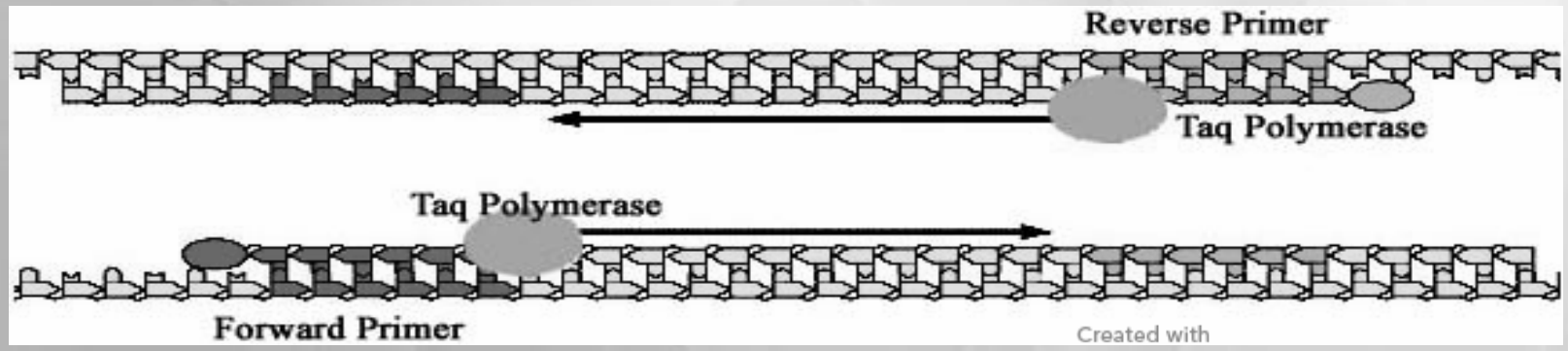
~1 min



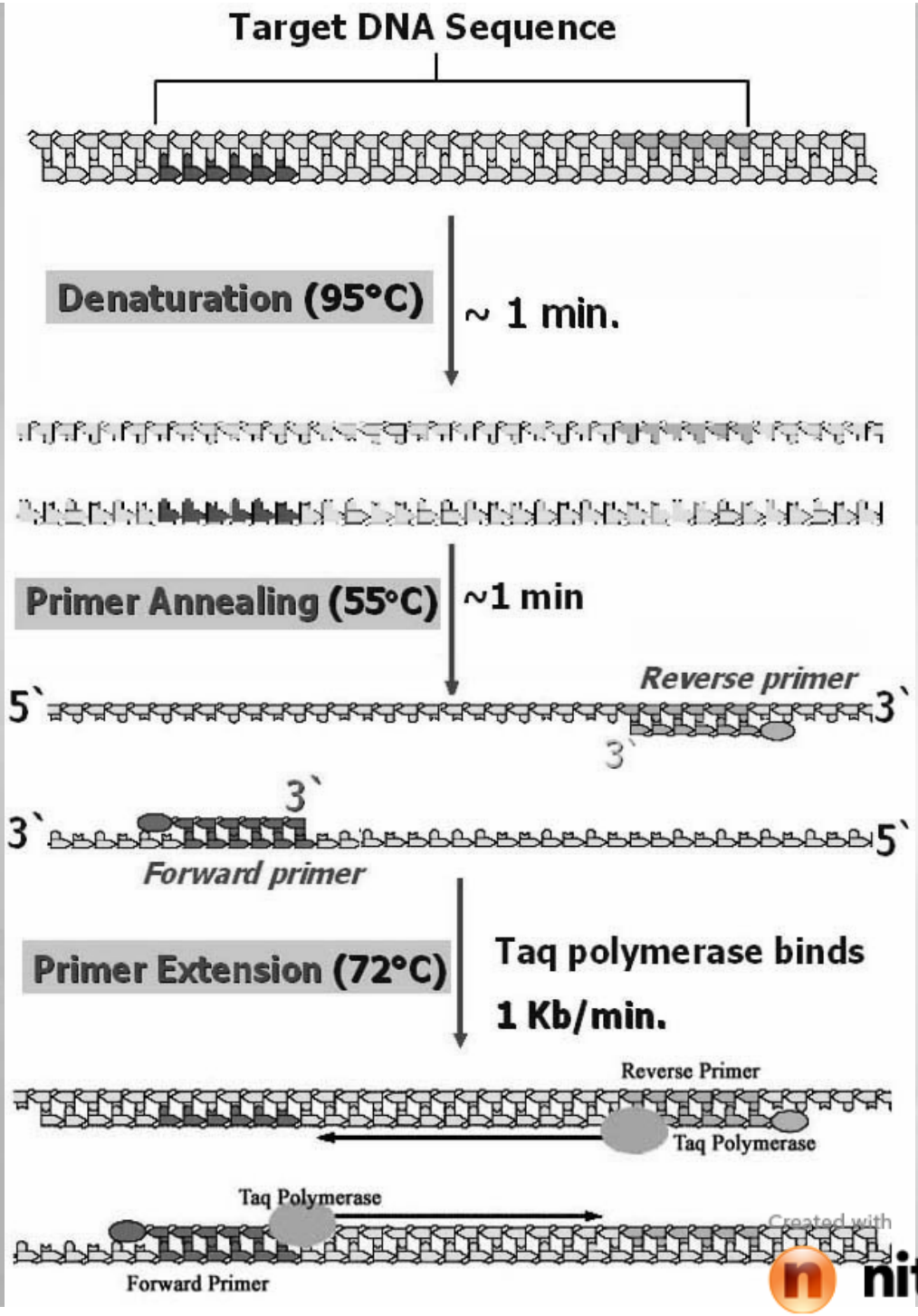
Primer Extension (72°C)

Taq polymerase binds

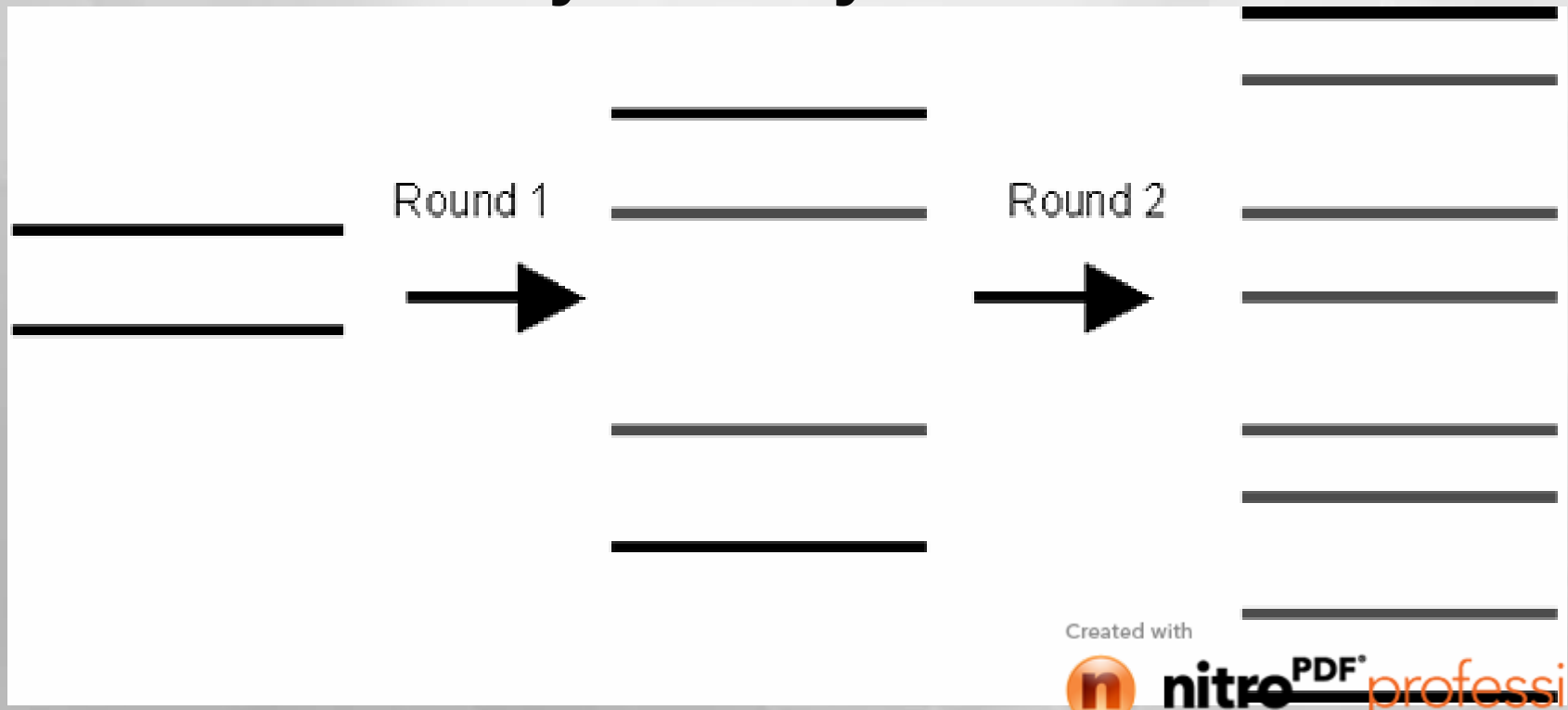
1 Kb/min.

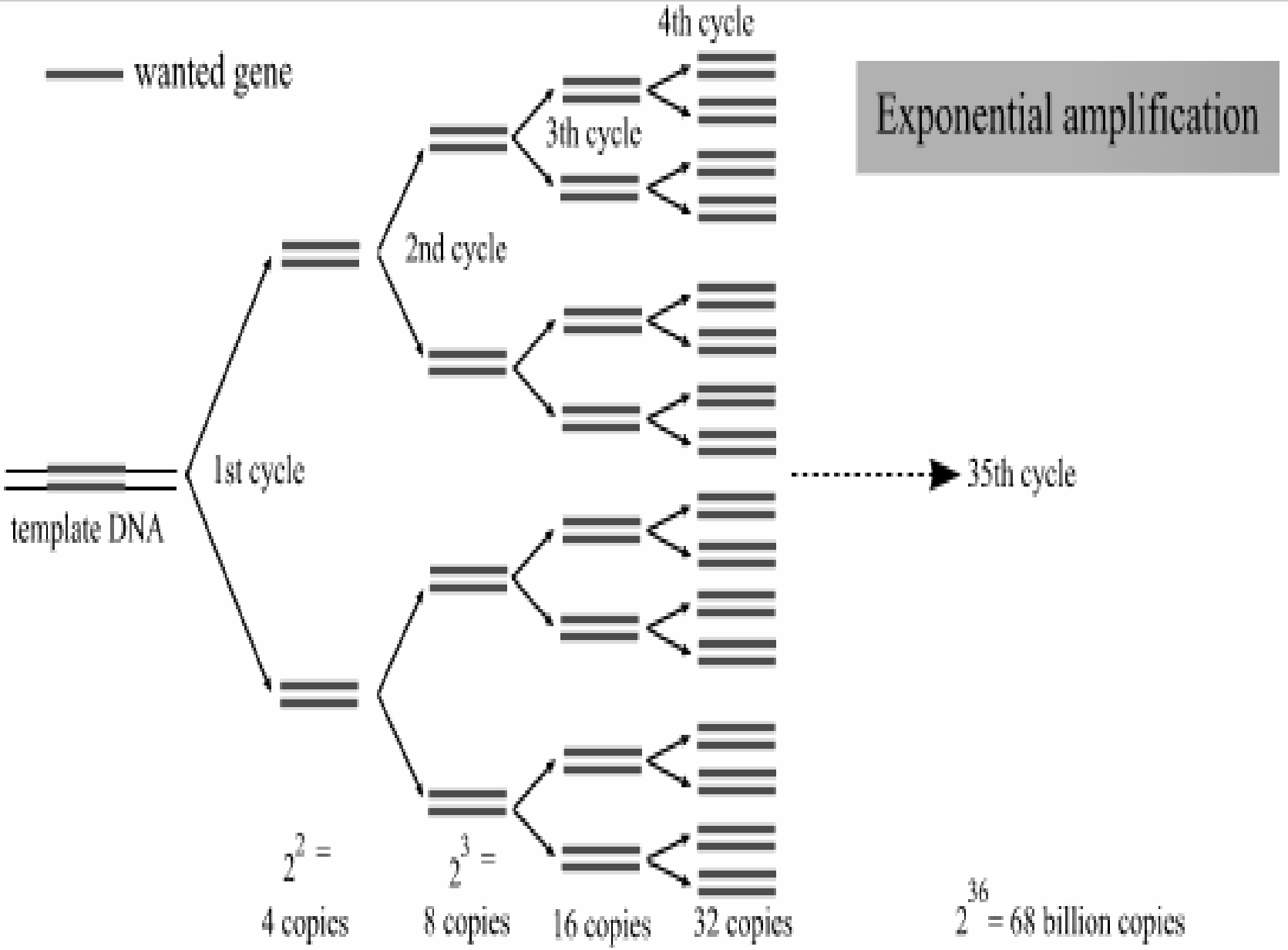


PCR Cycle (round)



- **The mixture is heated again at 90-95°C to denature the molecules and separate the strands and the cycle is repeated.**
- **Each new strand then acts as a template for the next cycle of synthesis.**





Exponential amplification

(Andy Vierstraete 1999)

- **A thermal cycler automatically changes the temperature at the correct time for each of the stages.**

How much amplification do you get?

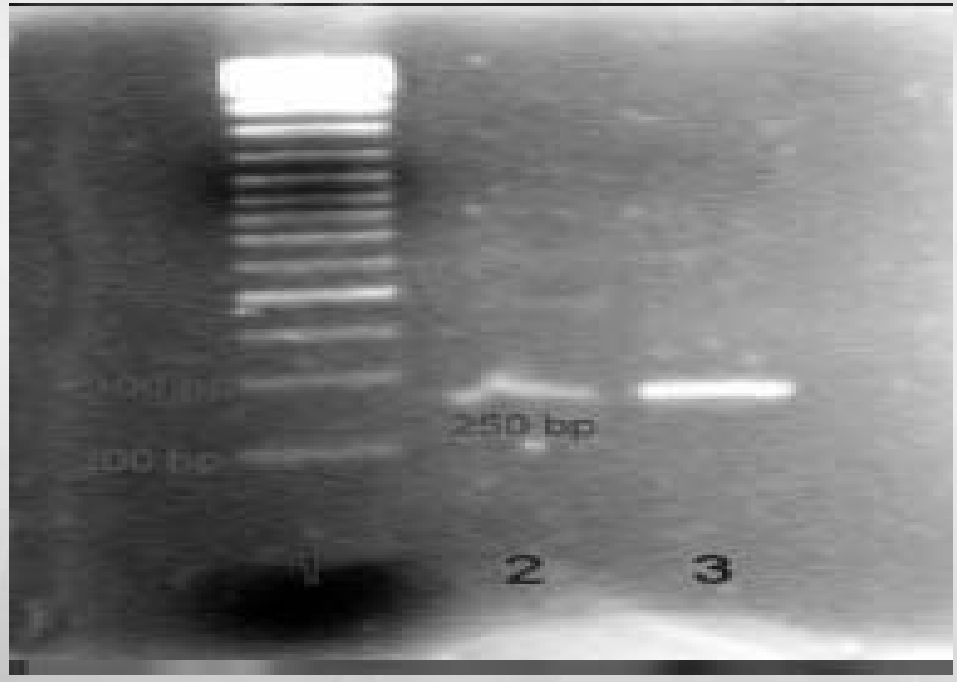
- **Amplicons = A X 2^{n-2}**
- **n = number of cycles**
- **A = starting target copy number**

- **Ex.**
- **6 cycles = 26^{6-2} (or 16) DNA copies.**
- **26 cycles = 2^{24} (or 16.77 million) DNA copies.**

- **PCR animation and Video**

- **The amplified product can be detected using gel electrophoresis to view the band containing DNA fragments.**

- Animation.



Primer features:

- **single stranded DNA molecules.**

- Types of primers
  - Random (short 10 – 15 b) [e.g. RAPD-PCR]
  - Specific (long 17 – 24 b)

***as the primer increases in size, the chances of matching the target size increase.**

***Longer the primer, the higher the annealing temperature.**

- **They are manufactured commercially**

Taq DNA Polymerase features :

- **A heat-stable DNA polymerase must be used in the reaction.**

- **Heat stable <u>Taq DNA polymerase</u> was purified from the hot springs (hydrothermal vents) bacterium *Thermus aquaticus* in 1976.**

- **Taq polymerase has optimal enzymatic activity at 72°C .**

Taq DNA Polymerase features :

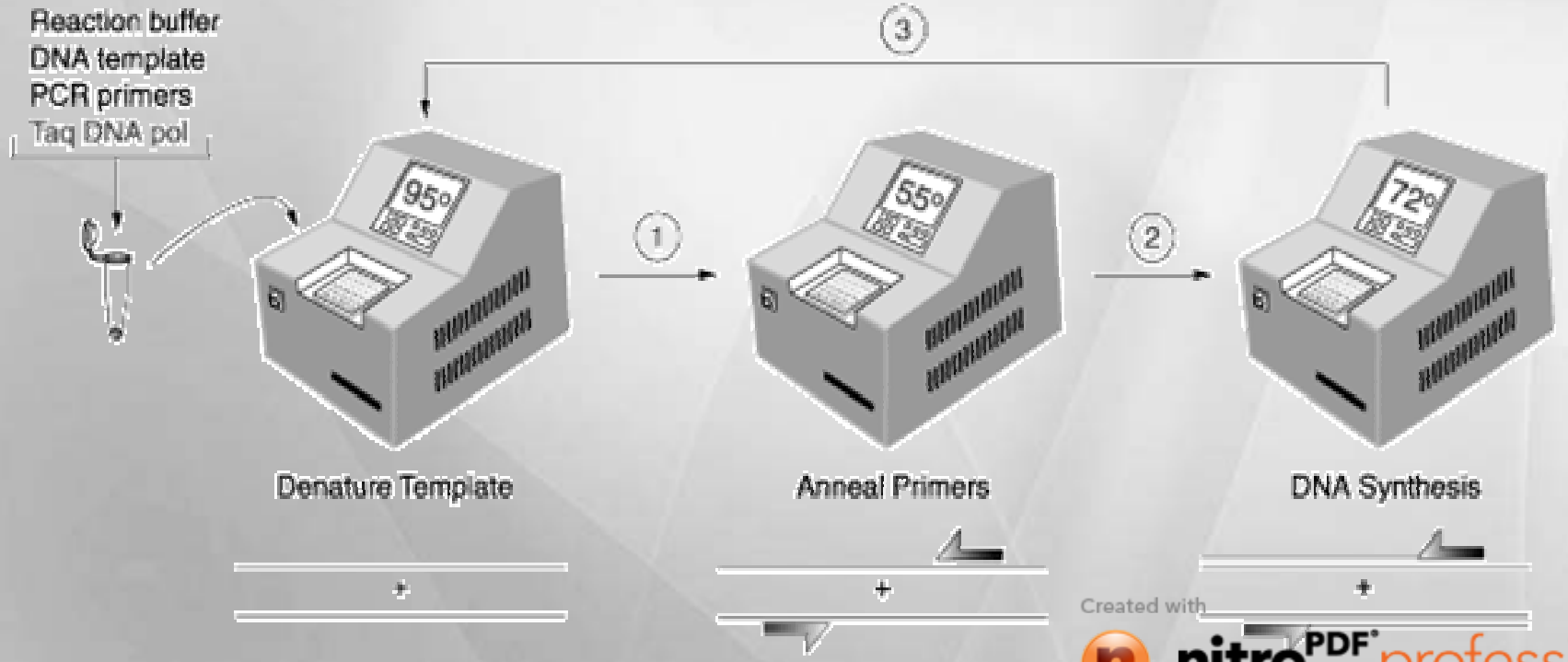
- Its enzymatic halflife (at 95°C) is 40 min
- Taq Polymerase extends the DNA chain by adding ~1.0 Kb per min.

Taq DNA Polymerase features :

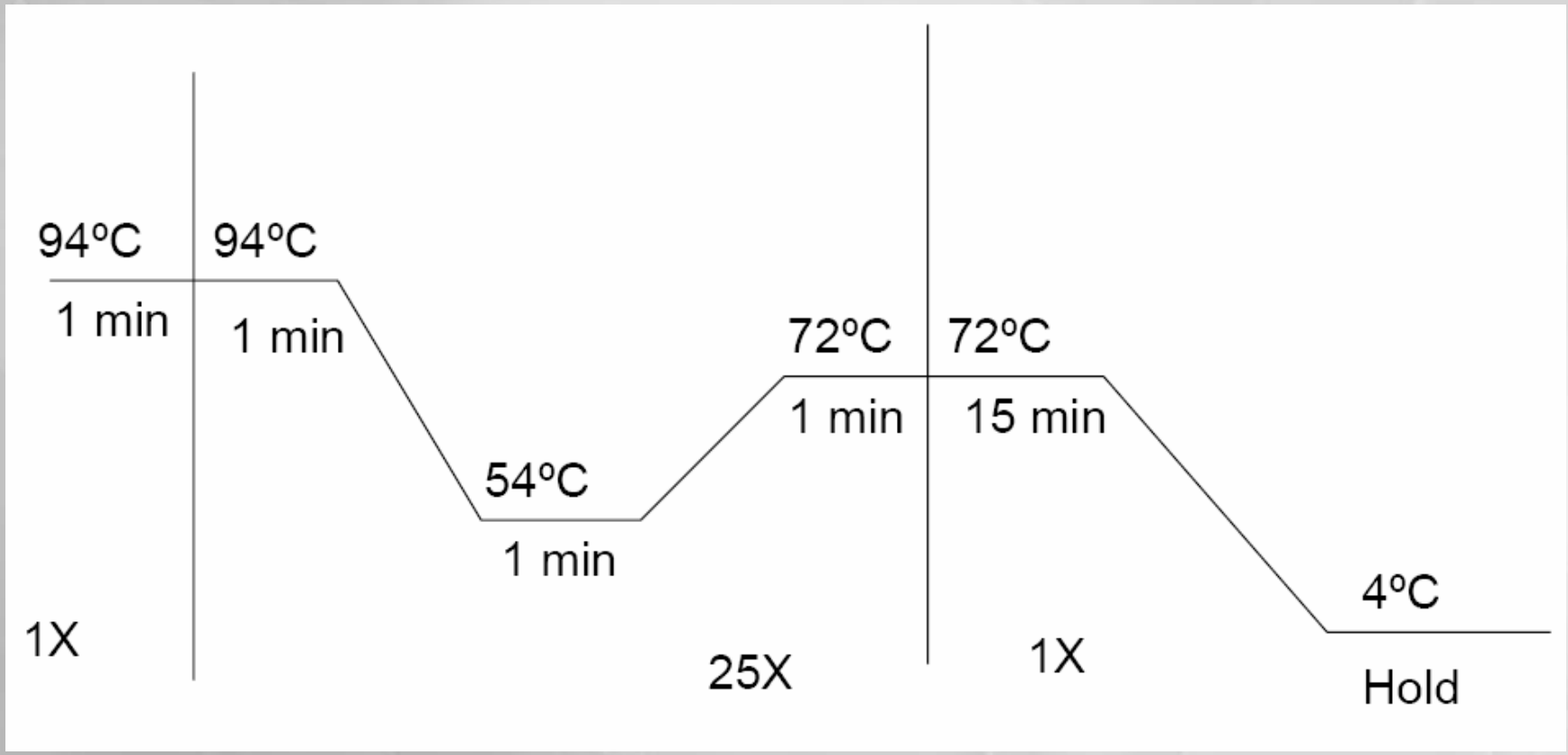
Polymerase	T ½, 95°C	Extension Rate (nt/sec)	Source
Taq pol	40 min	75	*T. aquaticus*
Amplitaq	80 min	>50	*T. aquaticus*
Vent	400 min	>80	*Thermococcus litoralis*
Deep Vent	1380 min	?	*Pyrococcus GB-D*
Pfu	>120 min	60	*Pyrococcus furiosus*
Tth	20 min	>33	*T. thermophilus*

Thermal Cycler:

- It heats and cools the reaction tubes within it to the precise temperature required for each step of the reaction.
- It is available in different specificity and models.



Programming the Thermocycler



Advantages of PCR

- PCR is fast (2–5 hours).
- DNA or RNA can be amplified.
- High-yield amplification can be achieved (10^6 to 10^9 amplification).
- DNA from one cell equivalent can be amplified.
- Works on damaged DNA.
- PCR products can be directly sequenced.
- Flexible.

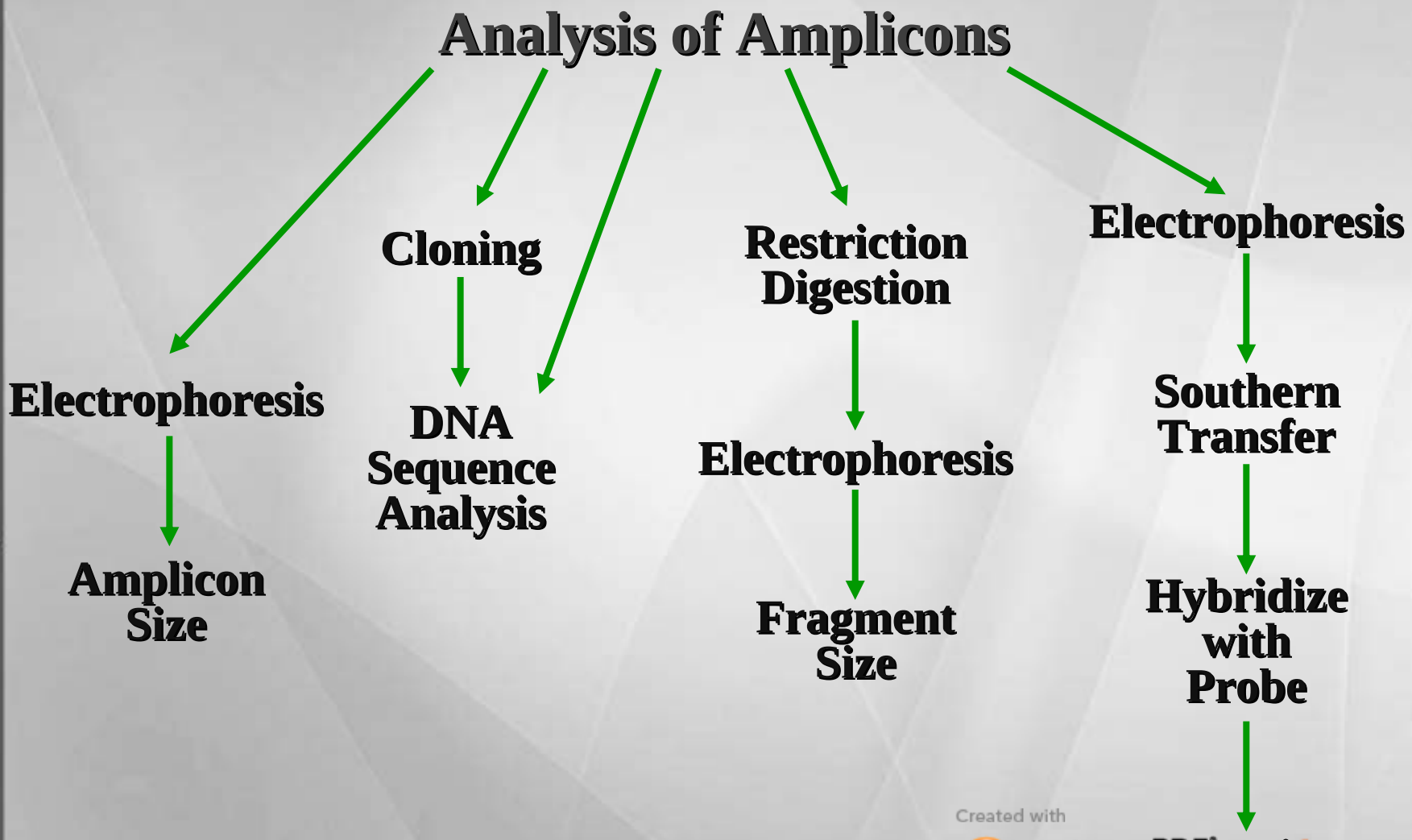
Disadvantages of PCR

- Need information about Target DNA sequence.
- Highly susceptible to contamination or false amplification.
- Amplification may not be 100% specific.
- Specificity of amplification is dependent on temperature and Mg++ concentration.

Disadvantages of PCR

- **Analysis and product detection usually takes longer than the PCR reaction itself.**
- **There is an upper limit to the size of DNA synthesized by PCR.**

Analysis of PCR Products



References

- **Micklos, David, Greg Freyer and David Crotty. DNA Science a First Course. New York:Cold Spring Habor Laboratory Press, 2003.**
- **Purves, Sadava, Orians, Heller. "Life." 6th ed. Sinauer Associates, 2001.**
- **Demidov.V, Broude. N(2004). DNA Amplification: Current Technologies and Applications**
- **Websites:**
- http://faculty.plattsburgh.edu/donald.slish/PCRmov.html (Animation)
- http://www.accessexcellence.org/RC/AB/IE/PCR_Xeroxing_DNA.html
- http://www.people.virginia.edu/~rjh9u/pcranim.html (PCR Animation)
- http://www.escience.ws/b572/L3/L3.htm (PCR Animation)
- http://homepages.strath.ac.uk/~dfs99109/BB211/RecombDNAtechlect4.html
- http://en.wikipedia.org/wiki/Polymerase_chain_reaction
- http://www.escience.ws/b572/L3/L3.htm
- http://allserv.rug.ac.be/~avierstr/principles/pcrani.html