Nested PCR – Long PCR

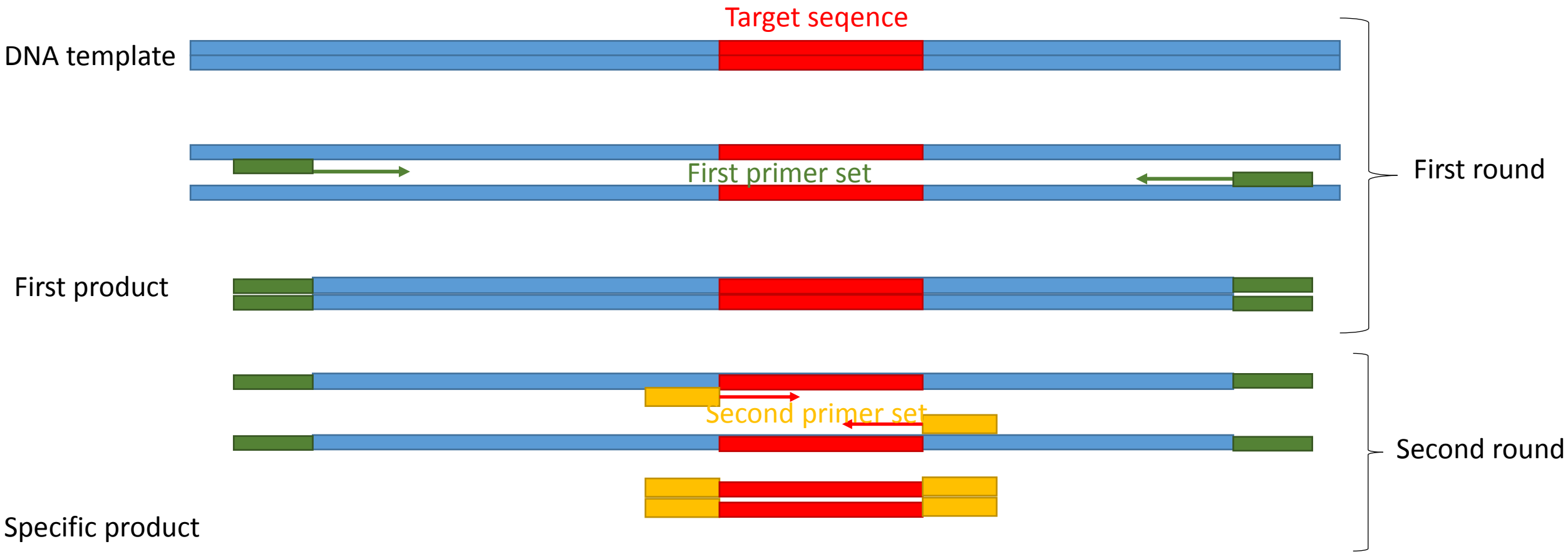
Katalin Dobos

NRIRR

PCR Training course – 13-19/06/2016

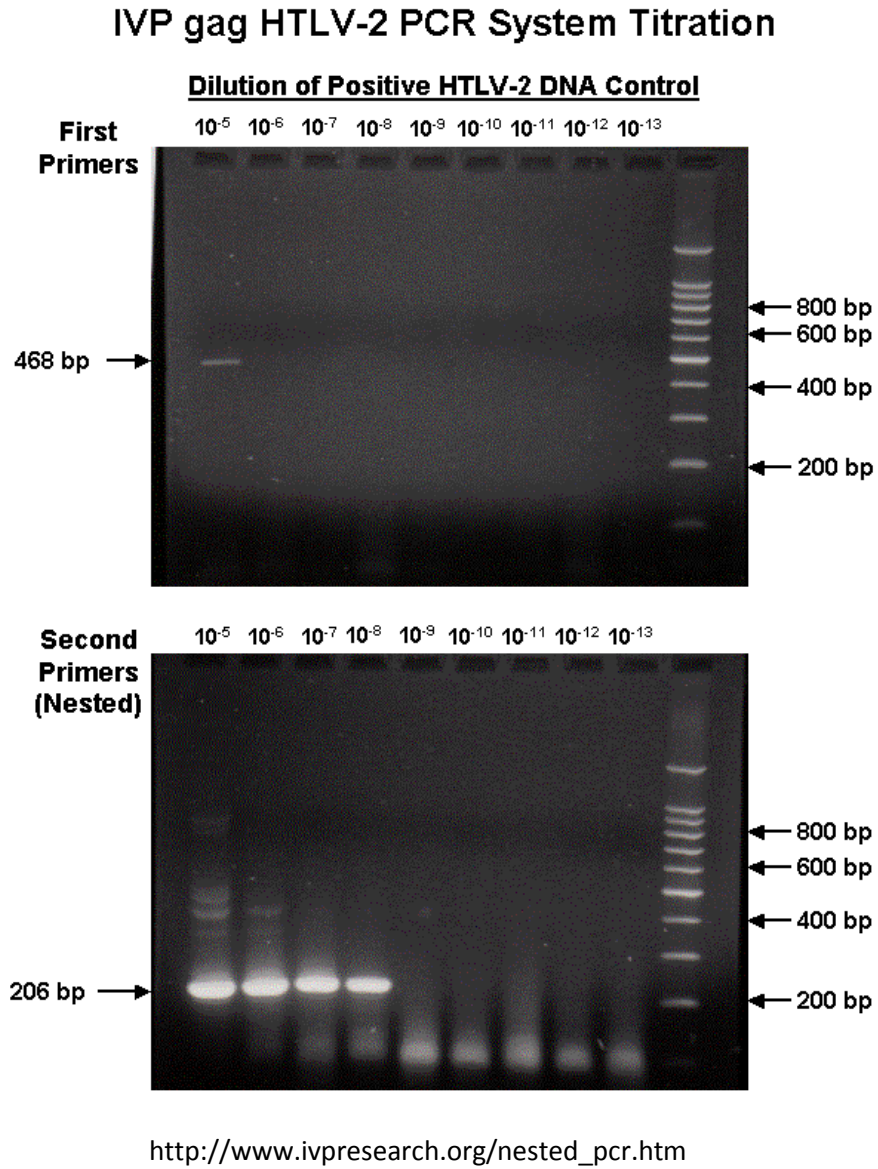
Nested PCR

- Modification of polymerase chain reaction
- Reduce the non-specific product
- 2 round of PCR
- First round: outer primer
  - Shorter primer
  - possible non-specific product
- Second round: inner primer
  - Longer primer within the outer primer
  - The template is the product of first round
  - Very improbably non-specific product


Target seqence
DNA template
First primer set
First round
First product
Second primer set
Second round
Specific product

Advantages

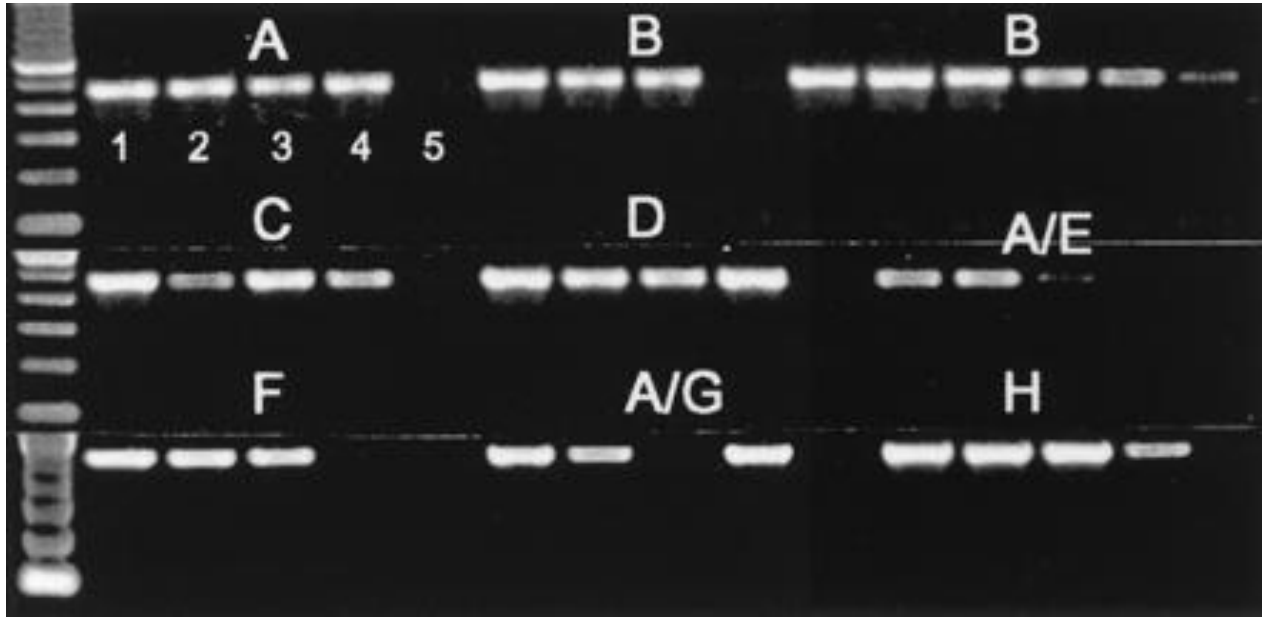
- Specific
- Sensitive



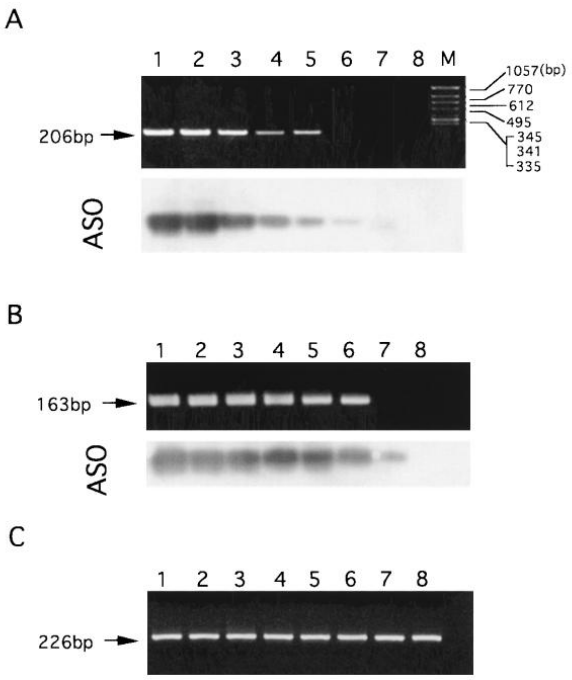
http://www.ivpresearch.org/nested_pcr.htm

Applications

- Infection diagnosis: 1 HIV-1 genome/PCR reaction
- Tumor monitoring: 1 tumor cell/10^5 lymphocyte



http://jcm.asm.org/content/37/8/2581.full



https://www.jstage.jst.go.jp/article/tjem/193/2/193_2_127/_pdf

Special technics

- Semi-nested PCR
- Nested-RT-PCR
- Nested PCR-RFLP
- Quantitative nested PCR

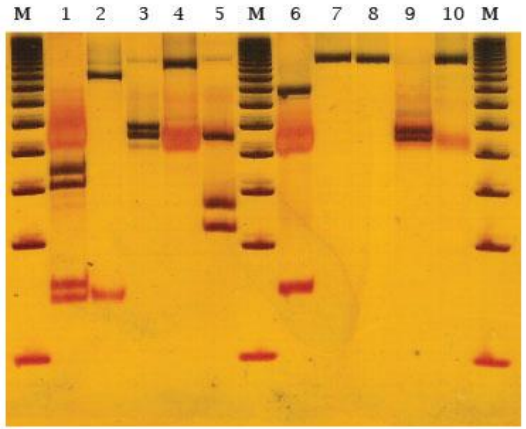
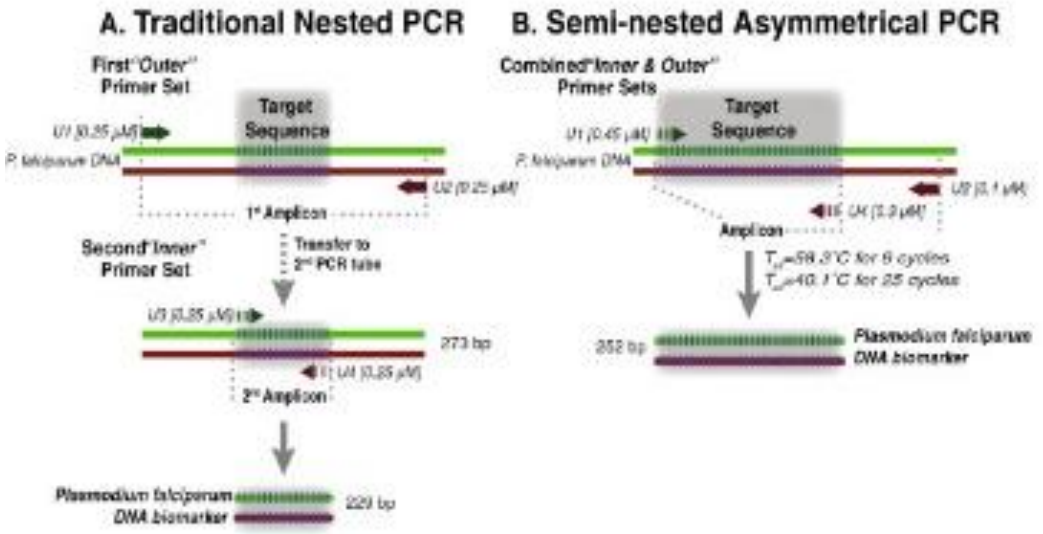


Figure 1: Restriction enzyme patterns of MY09/11 amplicon from HPV types 6 and 16. Lanes M, 50 bp ladder size marker; lanes 1 to 5, HPV 6 digested with *Rsa* I, *Dde* I, *Hinf* I, *Pst* I and *Hae* III; lanes 1 to 5, HPV 6 digested with *Rsa* I, *Dde* I, *Hinf* I, *Pst* I and *Hae* III.

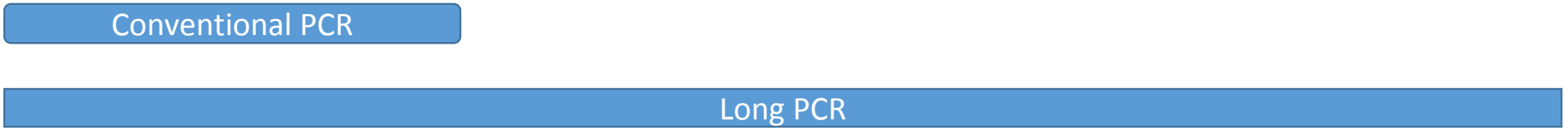
http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-86702011000500009



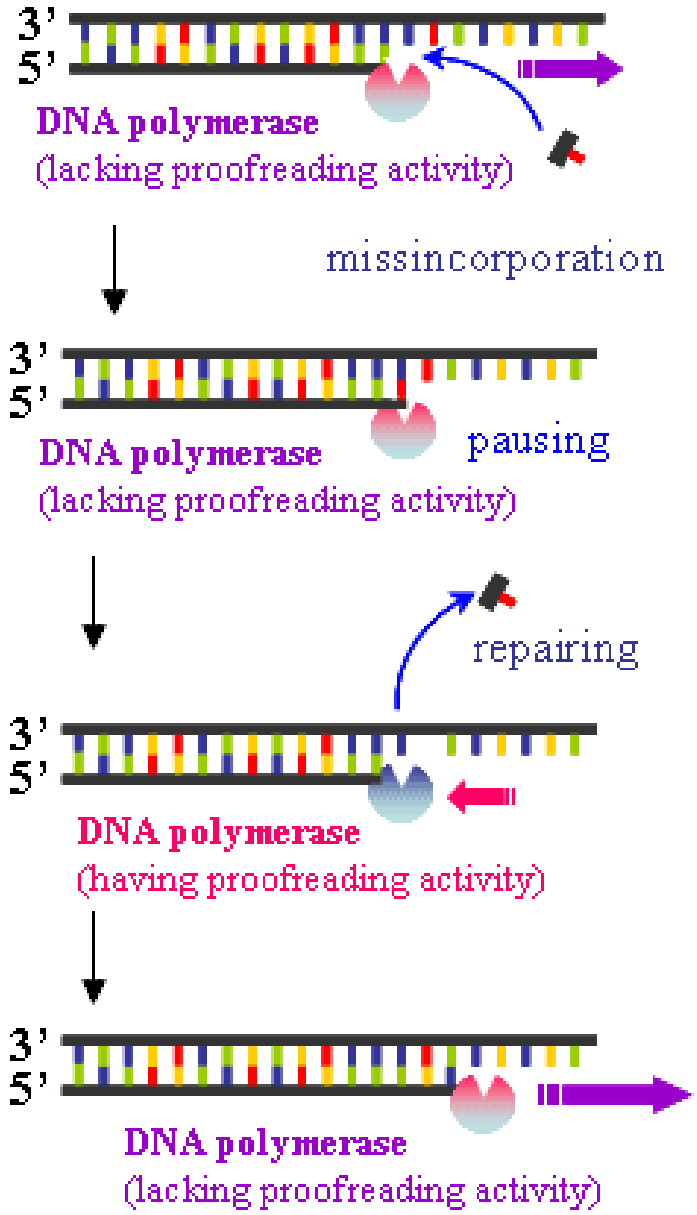
Schematic of nested PCR (A) and semi-nested asymmetric PCR (B). Note... - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/237201285_fig1_Schematic-of-nested-PCR-A-and-semi-nested-asymmetric-PCR-B-Note-that-the-semi-nested [accessed Jun 9, 2016]

Long PCR

- Traditional polymerase (Taq) enzymes can amplify ~5kb
- Some thermostable polymerase has proofreading activity, but alone not efficient for amplifying long templates ~ 20 kb in chromosomal genome, ~ 40 kb in viral genome



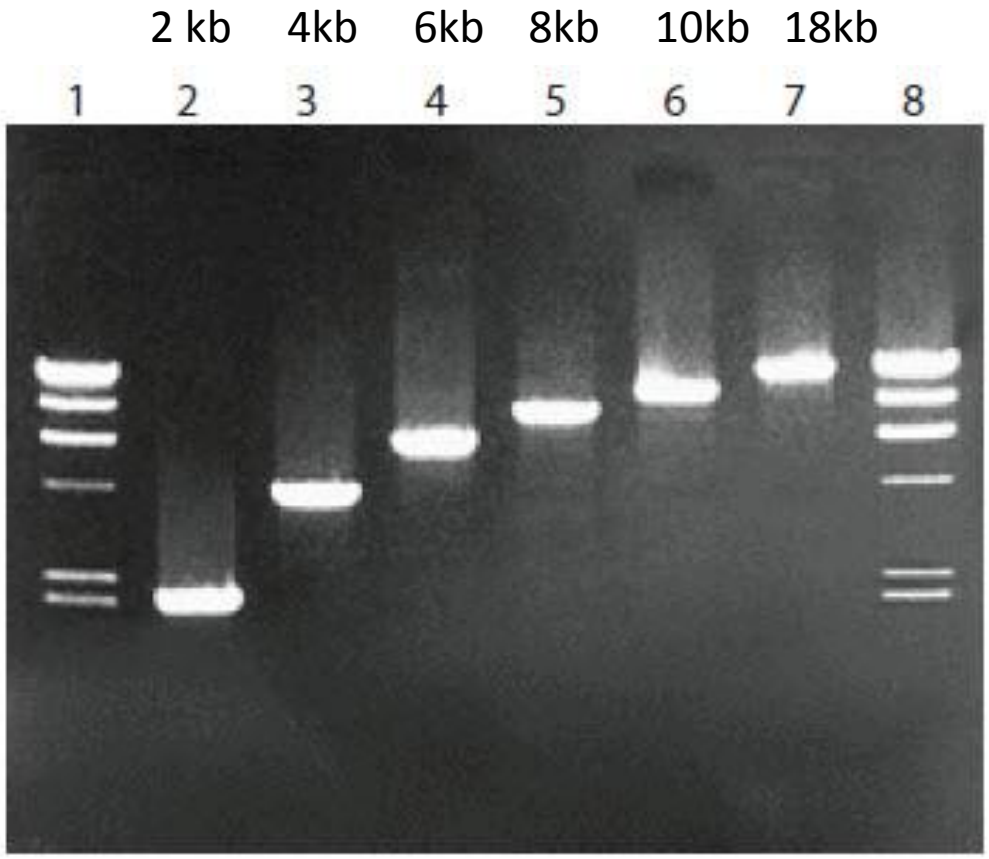
- For long DNA amplification necessary two thermostable polymerase
  - 1. nonproofreading (Taq)
  - 2. proofreading with 3’ -> 5’ exonuclease activity (Pfu, Tli, Pwo) - > lower concentration
    - When nonproofreading polymerase misincorporates dNTP, a polymerization stops or becomes very slowly, the proofreading polymerase remove the misincorporated dNTP



http://www.toyobo-global.com/seihin/xr/lifescience/technology/002.html

- Longer extension time: typically 10-20 min
- Higher temperature
- Lower pH

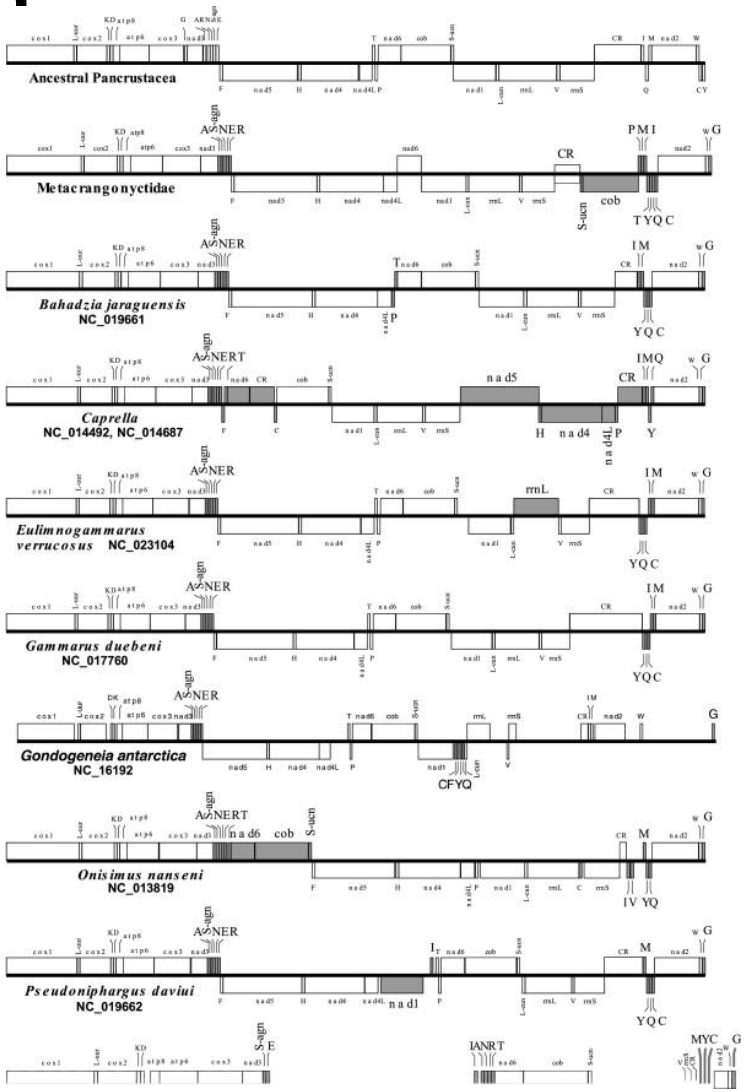
94°C	1 min	
↓		
98°C	20 sec	⎫
68°C	3 min (for 2 and 4 kb products)	⎪
	5 min (for 6 and 8 kb products)	⎪ 30 cycles
	15 min (for 10 and 18 kb products)	⎭
↓		
72°C	10 min	



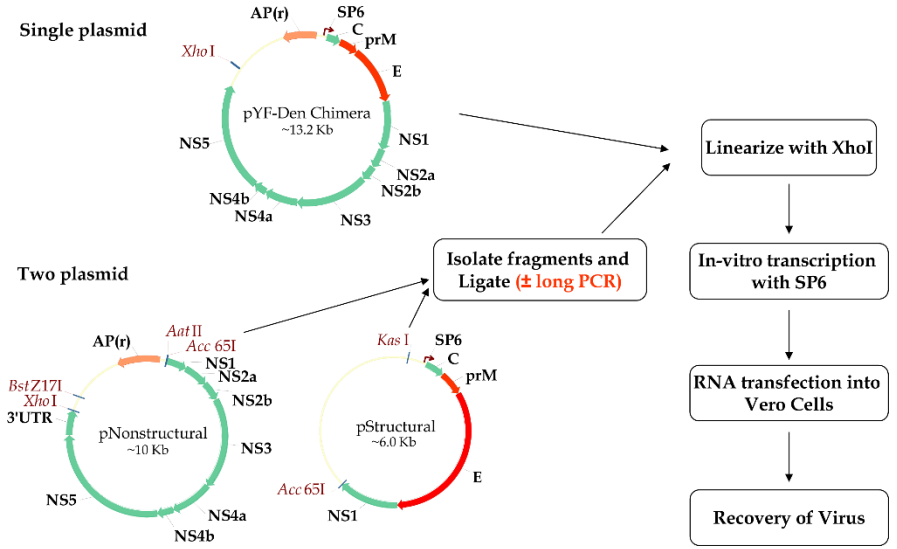
http://www.clontech.com/LK/Products/PCR/Resources/Application_Notes/Long_PCR

Applicatior

- Vaccine
- Genetic polymorphisms
- Phylogenetic
- Cloning



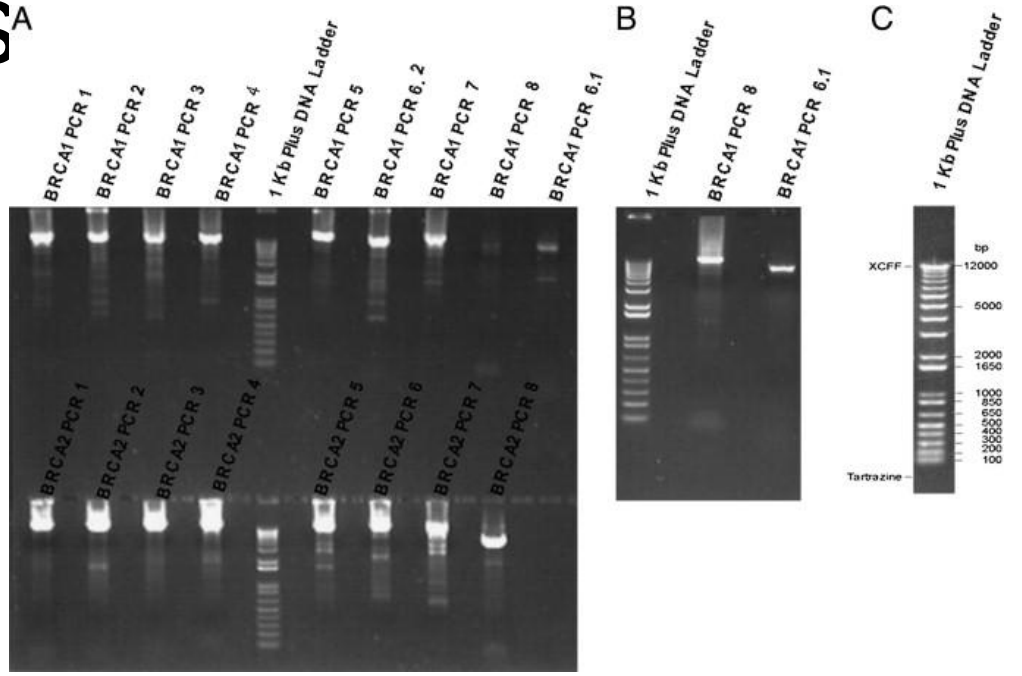
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4112215/



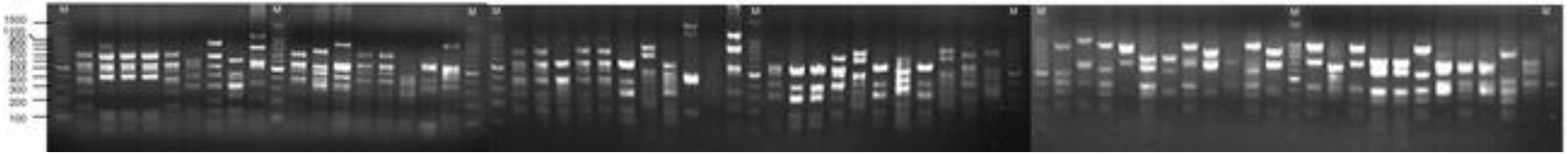
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0152209

Advanced technics

- Long-PCR + Next generation sequencing
- Long-PCR-RFLP
- RT-Long-PCR



http://www.sciencedirect.com/science/article/pii/S1525157812001407



http://onlinelibrary.wiley.com/doi/10.1111/jam.12057/full