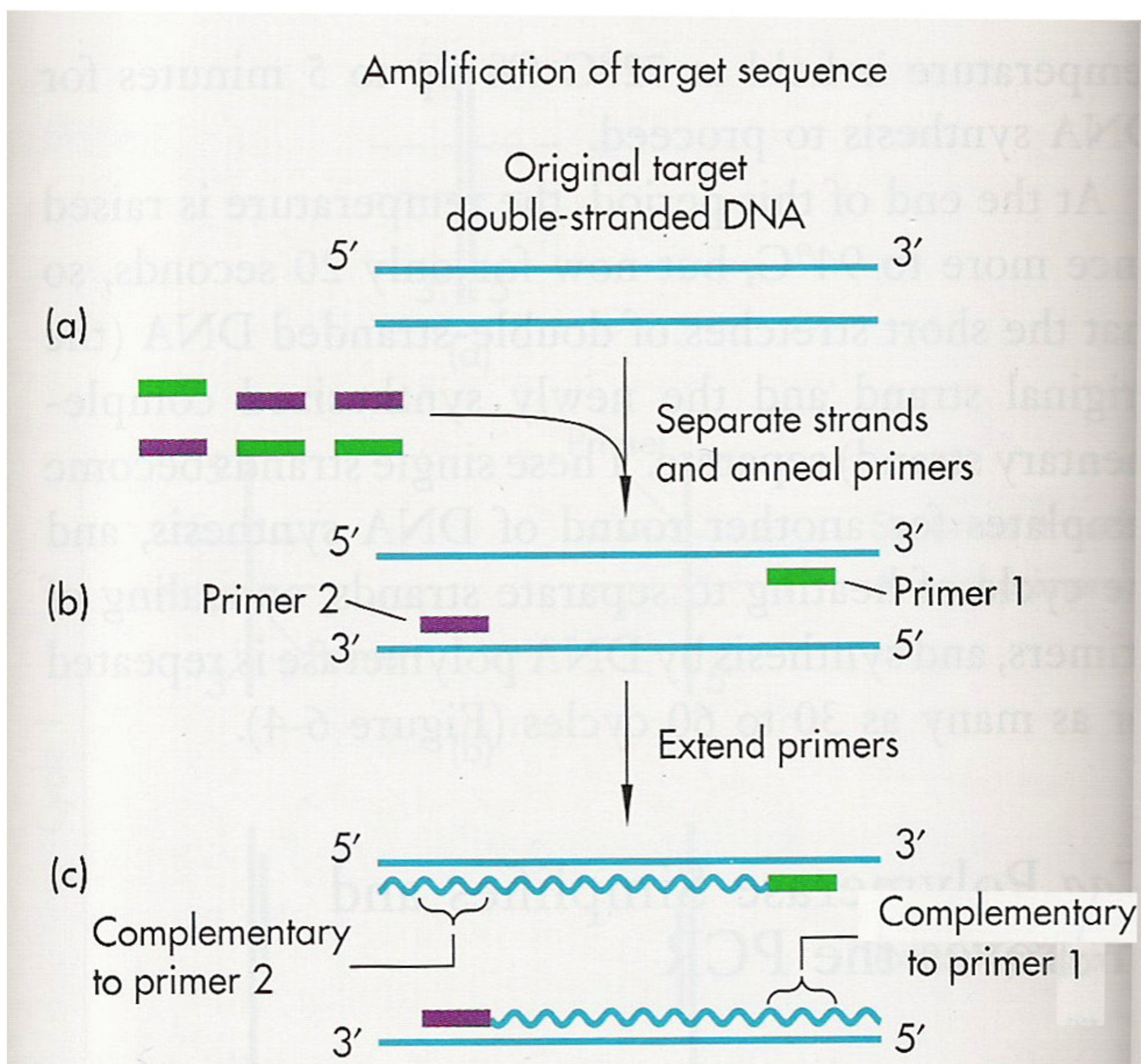
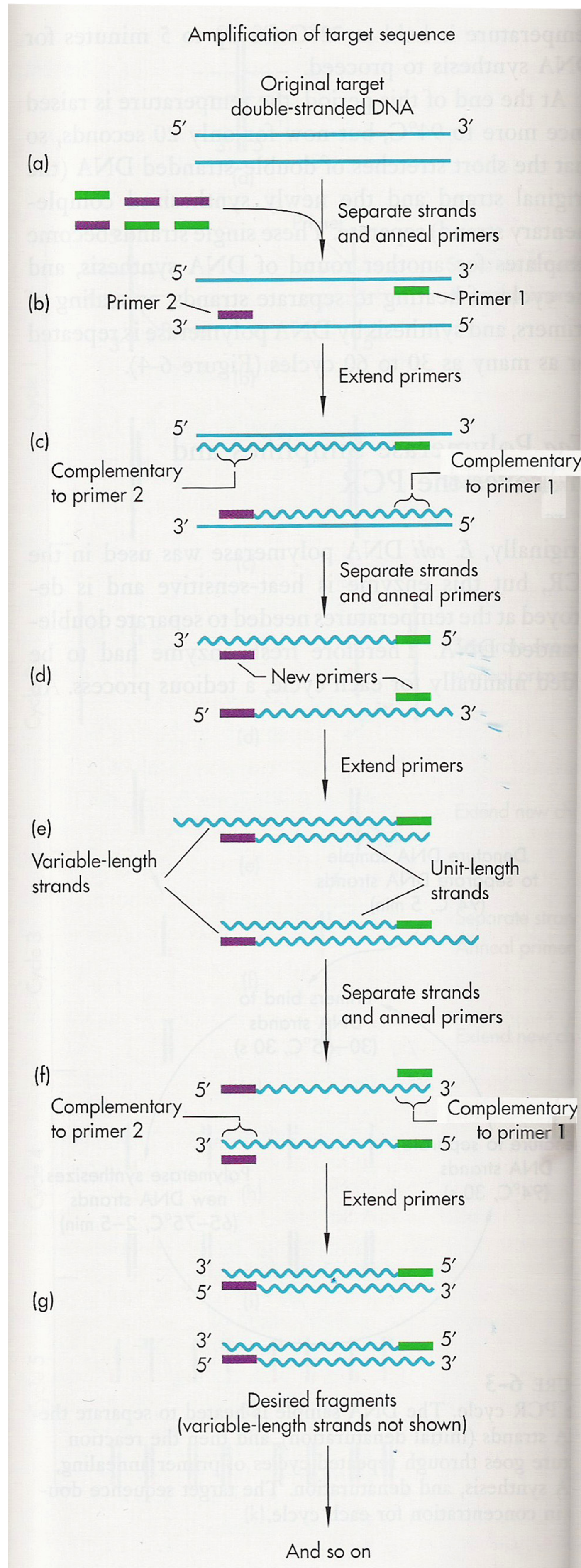
Polymerase Chain Reaction

• Relies on repeated cycles of MELT-ANNEAL-POLYMERISE
• The MELT separates the strands of dsDNA (temp ~95˚C)
• The ANNEAL uses PRIMERS that are designed to match the 3' ends of each of the two strands that result from the melt. ie one primer for the coding strand and a second for the template strand. We add access primers because one equivalent of each used in each cycle. (temp ~54˚C)
• The POLYMERISation step is catalyzed by a polymerase enzyme (temp ~72˚C). The high temperature means that the enzyme needs to be thermostable. We normally use one called *Taq* polymerase from a bug that was isolated from a thermal vent in Yellowstone called *Thermus aquaticus*. The substrates as for any polymerase are dNTPs for all 4 bases.
• Developed by Kary Mullis in 1984.
• Up to 100,000 cycles are possible.



• These first dsDNA products have overhanging lengths left over from the original DNA but as we make more copies in each cycle these are no longer at a significant concentration because primers don't template for these regions. They can therefore be ignored as an impurity at later stages.



Amplification of target sequence
Original target
double-stranded DNA
5′
3′
(a)
Separate strands
and anneal primers
(b)
Primer 2
Primer 1
Extend primers
(c)
Complementary
to primer 2
Complementary
to primer 1
Separate strands
and anneal primers
(d)
New primers
Extend primers
(e)
Variable-length
strands
Unit-length
strands
Separate strands
and anneal primers
(f)
Complementary
to primer 2
Complementary
to primer 1
Extend primers
(g)
Desired fragments
(variable-length strands not shown)
And so on