

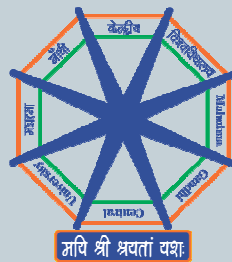
Course: M.Sc. Biotechnology

Paper: BIOT4009: Genetic Engineering and Gene Therapy

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UNIT – III

POLYMERASE CHAIN REACTION-3



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Types and Applications of PCR

Important types (component dependent) and features

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Important PCR Types (DNA as Template)

Conventional PCR: a DNA template and a pair of primers

Multiplex-PCR: a DNA and many pair of primers

AP-PCR: many DNA and single primer (arbitrary) in separate reactions

Anchored-PCR: one primer is fixed and one primer is designed

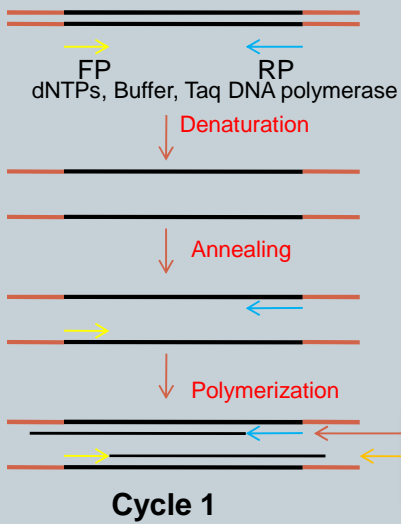
Asymmetric PCR: One primer (forward or reverse) is exhausted after few cycles

Inverse PCR: primers oriented in reverse direction (in close vicinity)

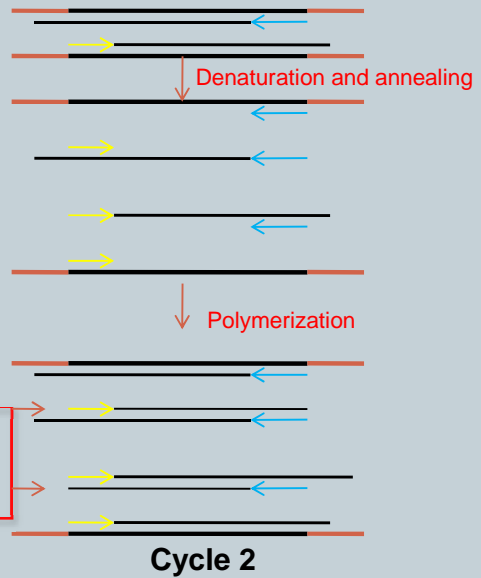
Conventional PCR

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ds DNA as initial template and a pair of primers



Product of Cycle 1



Products of indefinite length

Targeted products of defined length

Targeted products of defined length are exponentially amplified from cycle 3 onwards and soon outnumber the template and product of indefinite length

Conventional PCR contd.

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In case, native *Taq* DNA polymerase is used in PCR
It adds additional template independent A residue at
3' end

Cetus corporation has synthesized a recombinant *Taq*
DNA polymerase which adds additional T at 3' end


This activity is called terminal extendase activity

Taq DNA polymerase has no proof reading activity and
higher error rate

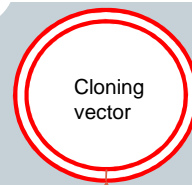
These properties are used in PCR based gene cloning

PCR based T/A cloning

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 Gene raised by PCR with
Taq DNA Polymerase
 (Additional A at 3' end)



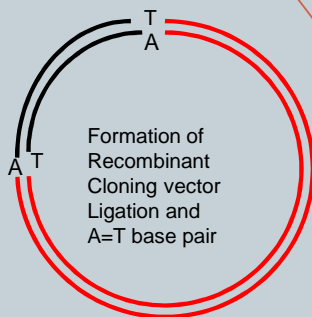
URS for Hae III
 at MCS
 ↓
 Cut with Hae III



↓
 Treatment with Engineered *Taq* DNA
 Polymerase of Cetus corporation in
 presence of dTTP

T

Linear vector with additional T at 3' end



Transformation of host bacteria

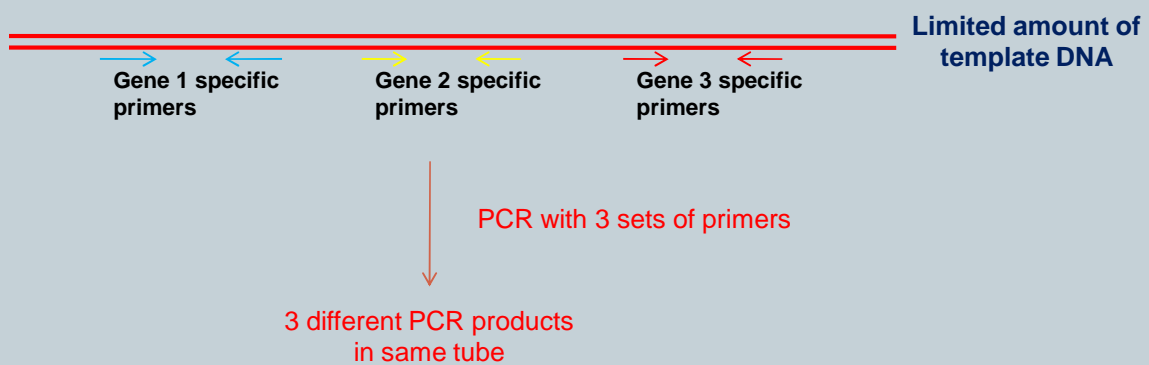
Selection of transformants and
 multiplication

Clones of gene
 inserted in the vector

Multiplex PCR

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- DNA template in limited amount
- PCR optimized with many pair of primers

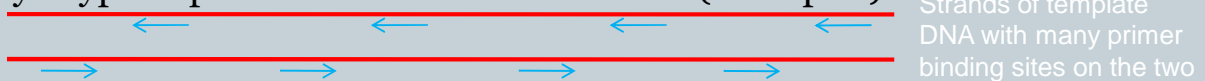


Arbitrary Primed-PCR (AP-PCR)

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Small arbitrary primers (6-12 nts) are used (not gene specific)

Only 1 type of primer is used in one reaction (not 1 pair)

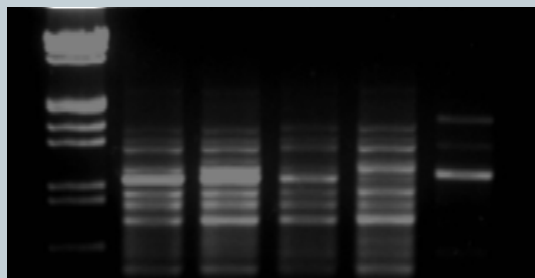


PCR
↓ Taq DNA Polymerase,
dNTPs, Buffer

Multiple PCR products of
different size and sequence

↓ Agarose gel (1-2% agarose) electrophoresis

Markers 1 2 Samples 3 4 5

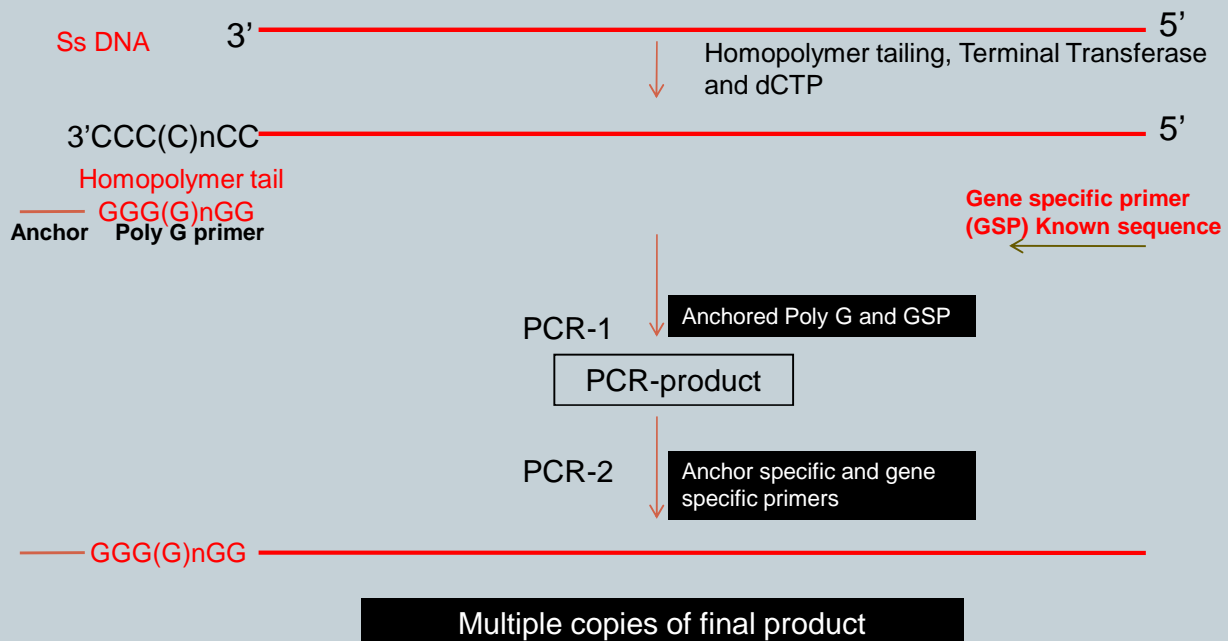


→ Calculation of
similarity index and
assessment of genetic
relationship/ mutation

Anchored-PCR

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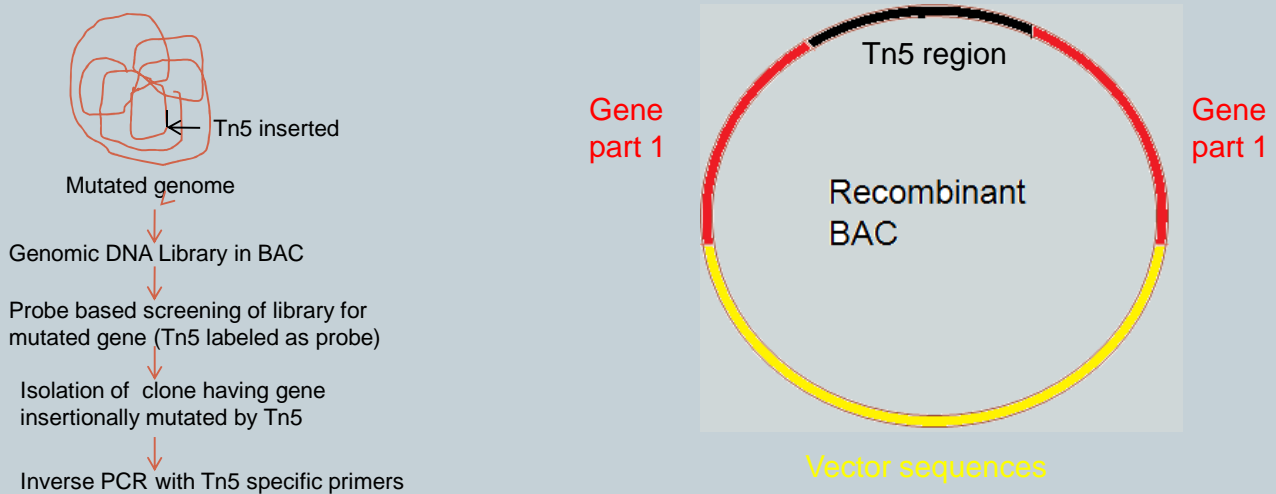
- When only one primer sequence (5') is known/ available



Inverse-PCR

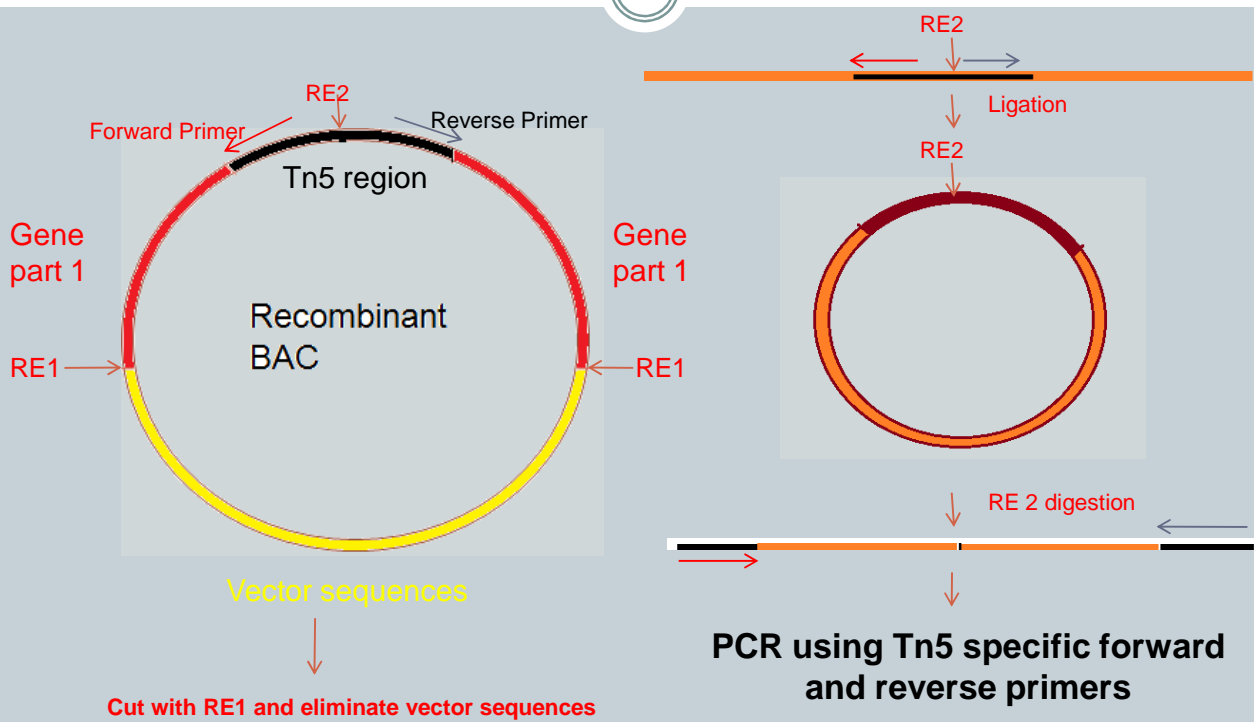
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Specially used when structure and function of an unknown gene is to be known through insertional mutagenesis. E.g. Tn5 mediated mutagenesis



Inverse-PCR

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Thanks



TO BE CONTINUED