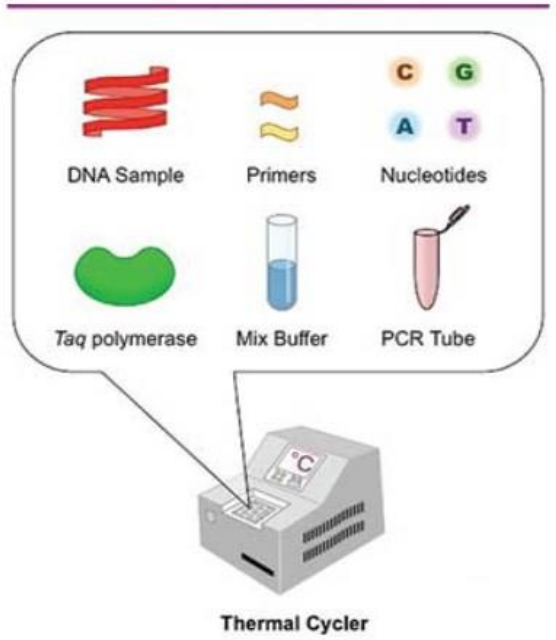
انواع PCR



Gradient PCR

Long PCR

Nested PCR

Colony PCR

RFLP PCR

Rapid PCR

RT-PCR

Quantitative PCR=Real time PCR

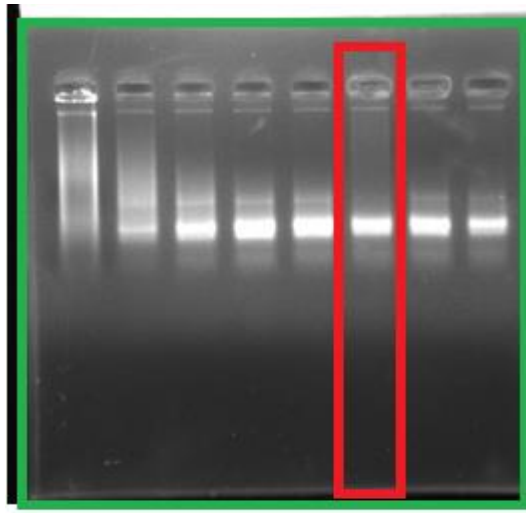
Hot start PCR

Touch down PCR

Gradient PCR

Gradient PCR is a technique that allows the empirical determination of an optimal annealing temperature using the least number of steps. This optimization can often be achieved in one experiment. The Eppendorf Mastercycler Gradient provides a gradient function that in one single run evaluates up to 12 different annealing, elongation, or denaturation temperatures

The PCR is normally started at 5°C below the calculated temperature of the primer melting point (T_m)

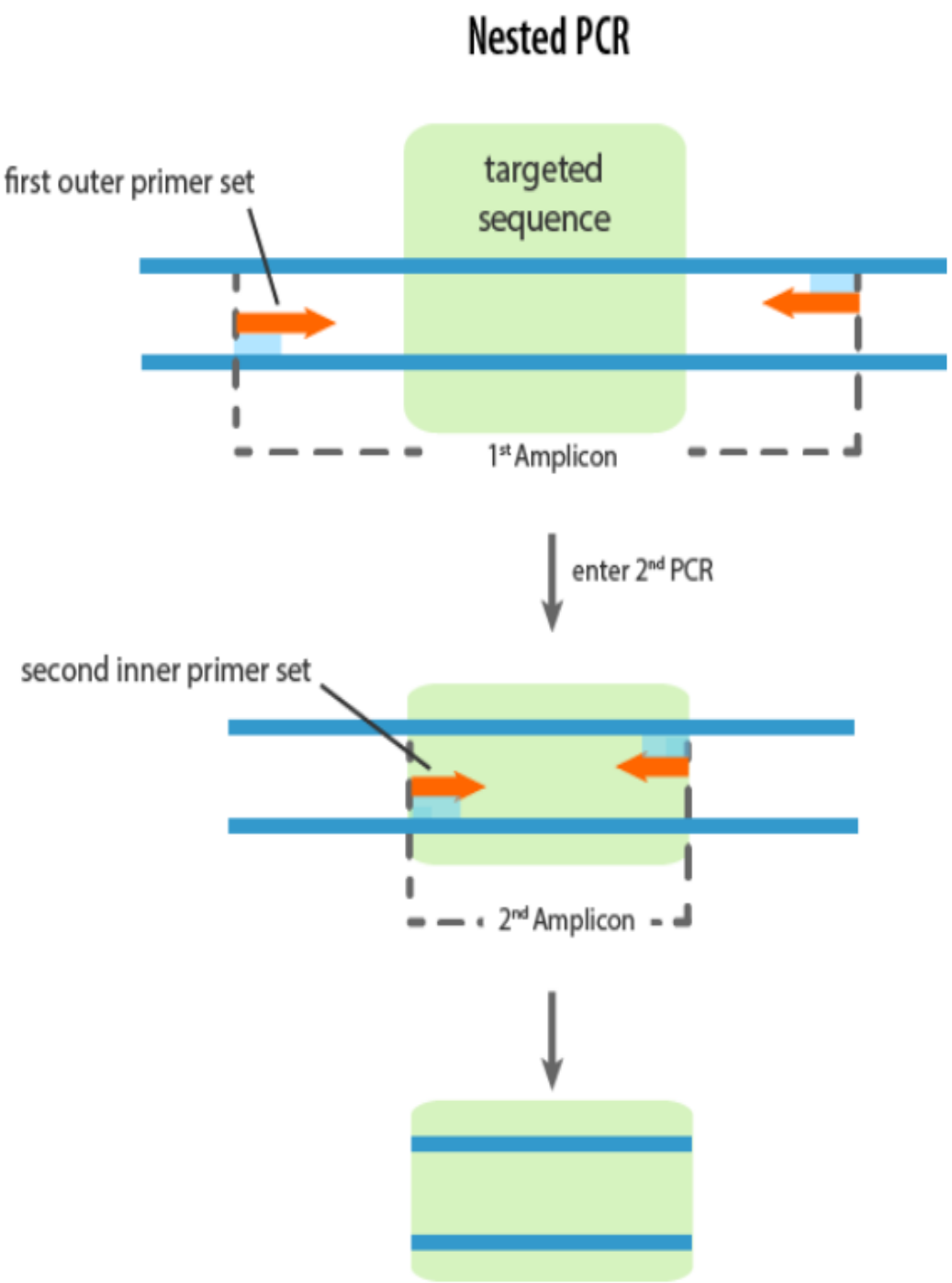


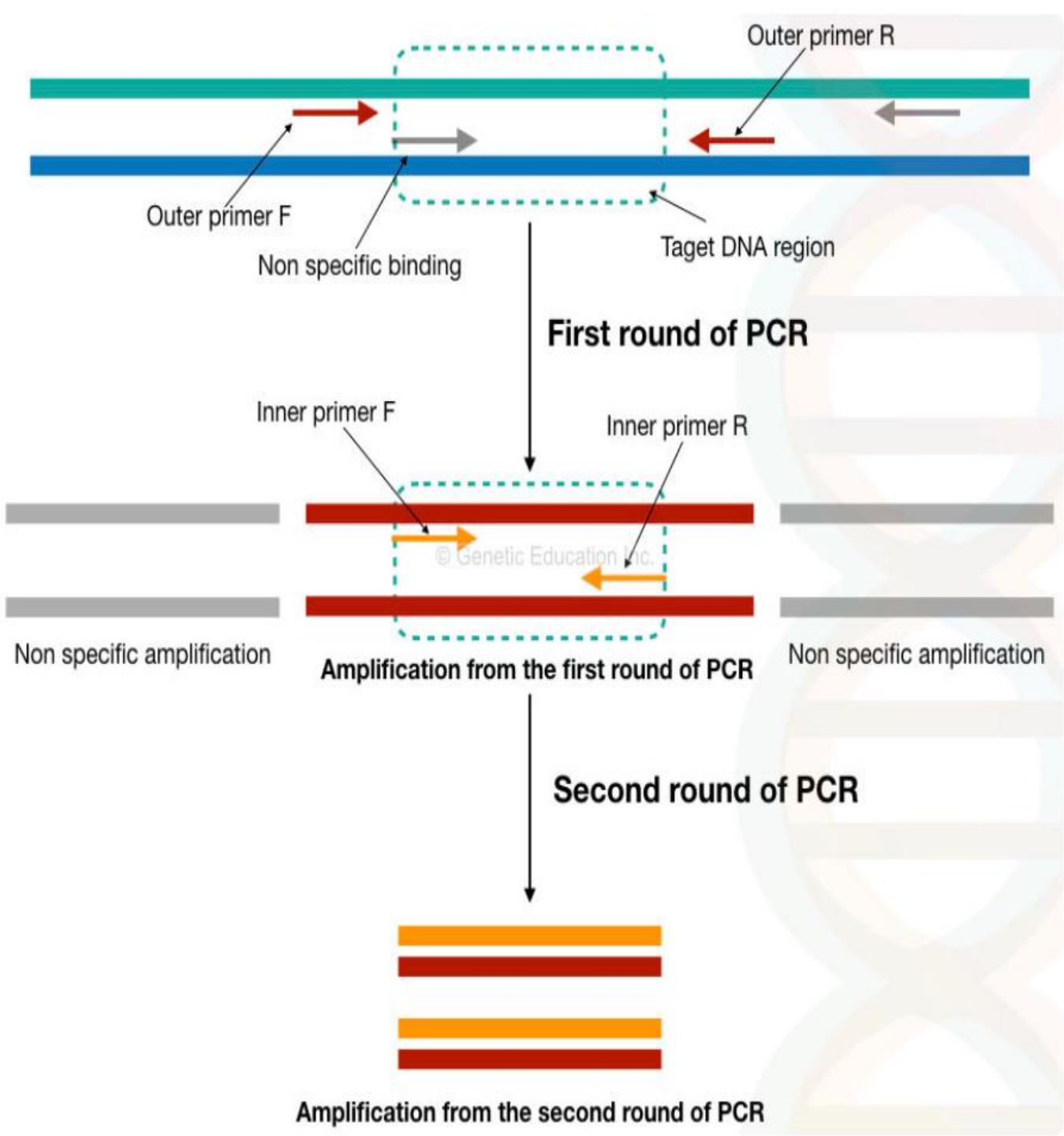
Nested PCR

In the year 1993, *Kamolvarin* and coworkers described the method for use of two sets of primers for increasing sensitivity and specificity of the PCR.

Nested PCR is used to increase the **specificity** of DNA amplification by reducing the non-specific amplification of DNA. A nested PCR assay has **2 sets of primers (outer pair and inner pair)** for a single locus and two successive PCRs. In the first PCR run, the outer pair primers are used to generate DNA products as the regular PCR does and thus their DNA products may contain non-specifically amplified DNA fragments. These products then enter a second run of PCR using the second set "inner" primers whose binding sites are completely or partially different from and located after the 3'end of each of the outer pair primer used in the first PCR reaction. Therefore, a second PCR product was produced and shorter than the first one.

PCR Steps (first PCR: 25 cycles and second PCR: 35 cycles)


Nested PCR
first outer primer set
targeted sequence
1st Amplicon
enter 2nd PCR
second inner primer set
2nd Amplicon


Outer primer R
Outer primer F
Non specific binding
Taget DNA region
First round of PCR
Inner primer F
Inner primer R
© Genetic Education Inc.
Non specific amplification
Amplification from the first round of PCR
Non specific amplification
Second round of PCR
Amplification from the second round of PCR

Multiplex-PCR

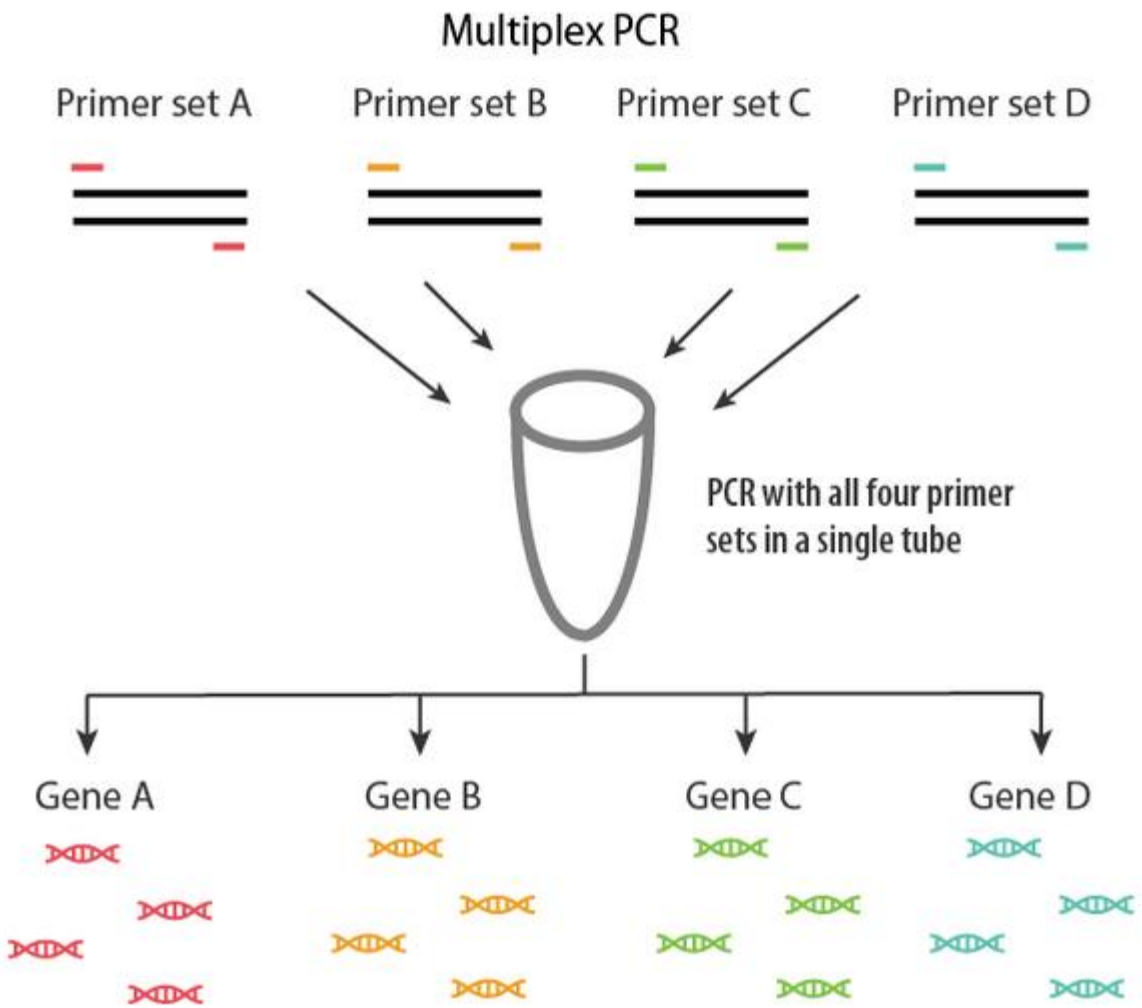
Multiplex PCR is a widely used molecular biology technique for amplification of multiple targets in a single PCR experiment. In a multiplex-PCR assay, different target DNA sequences can be amplified simultaneously by using multiple primer pairs in a reaction mixture. Annealing temperature and primer sets should be optimized so that all primer pairs can work correctly within a single reaction. Amplicon sizes of different genes such as their base pair length should be different so that distinct bands can be visualized by gel electrophoresis.

pathogen identification

mutation analysis

gene deletion analysis

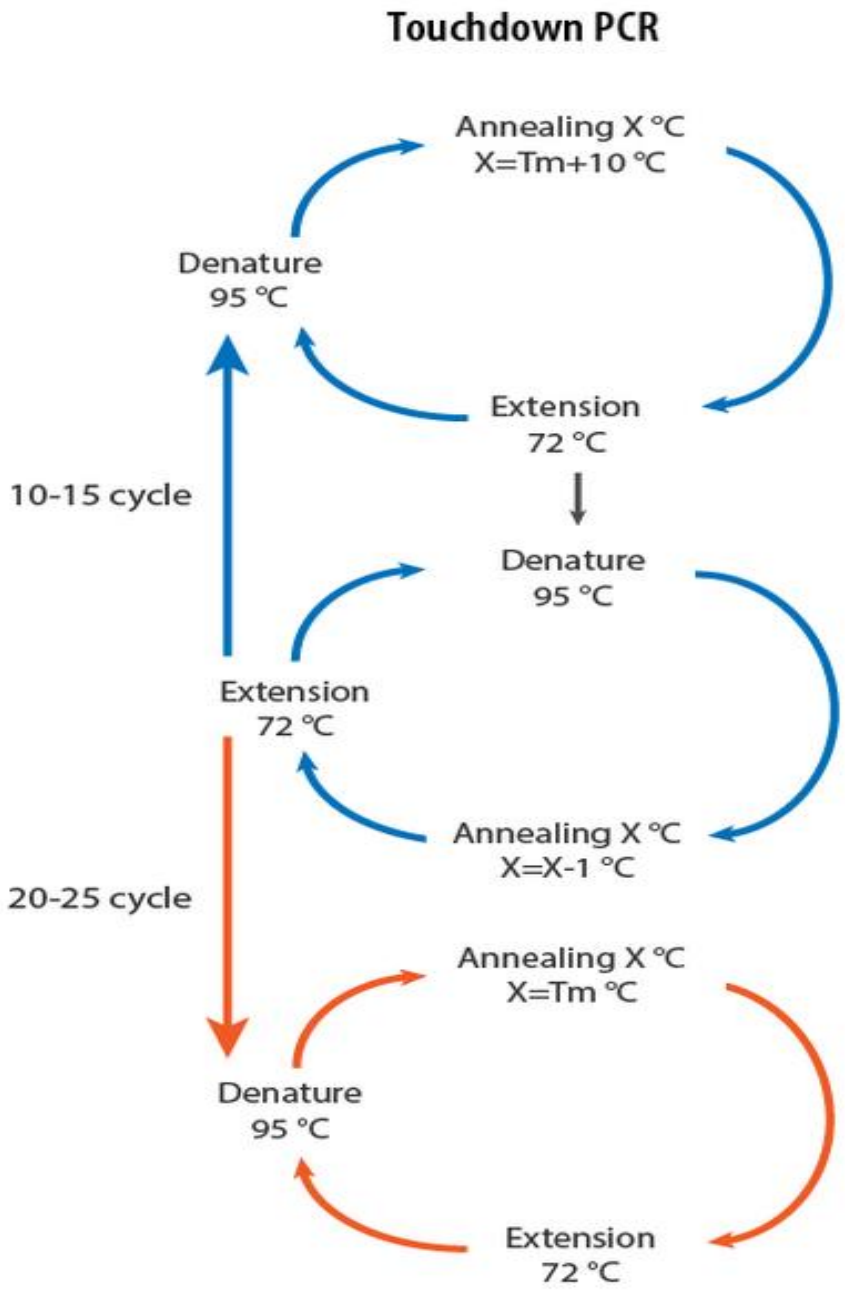
forensic studies


Multiplex PCR
Primer set A
Primer set B
Primer set C
Primer set D
PCR with all four primer sets in a single tube
Gene A
Gene B
Gene C
Gene D

Touch Down PCR

A typical **Touch Down PCR** cycling condition has two phases. The first phase of touchdown programming uses an annealing temperature that is approximately **10°C above the calculated Tm (melting temperature).** The temperature is reduced by 1°C every successive cycle until the calculated Tm range is reached. This is done for a total of 10-15 cycles. Phase 2 follows generic PCR amplification of up to 20-25 cycles using the final annealing temperature reached in the touchdown phase. The cycles and temperature drop during touchdown phase can be adjusted from 1-3 cycles per 1-3°C drop in temperature if non-specific products are still observed or if the yield is low

Touch Down PCR **increases the specificity** of PCR by using higher annealing temperatures at the earlier cycles and **increases the efficiency** by lowering the annealing temperatures gradually toward the end of cycles. This method dramatically increases the **quality of outcome of PCR**


Touchdown PCR
Annealing X °C
X=Tm+10 °C
Denature
95 °C
Extension
72 °C
10-15 cycle
Denature
95 °C
Extension
72 °C
Annealing X °C
X=X-1 °C
20-25 cycle
Annealing X °C
X=Tm °C
Denature
95 °C
Extension
72 °C

ARMS-PCR

The allele-specific PCR is also called as the (amplification refractory mutation system) ARMS-PCR because of the use of two different primers for two different alleles. Refractory= resistant to something.

ARMS primer (10 pM)
Add strong mismatch near the 3' end of the primer (at -2 position ideally)

internal control primers (1-3 pM)

Importantly, the PCR cycles for ARMS-PCR are lower than the normal PCR reaction. Set a PCR cycle between 22 to 25 but not 35. As the PCR cycles increase the chance of false-positive results increases.

point mutation

genetic disease diagnosis

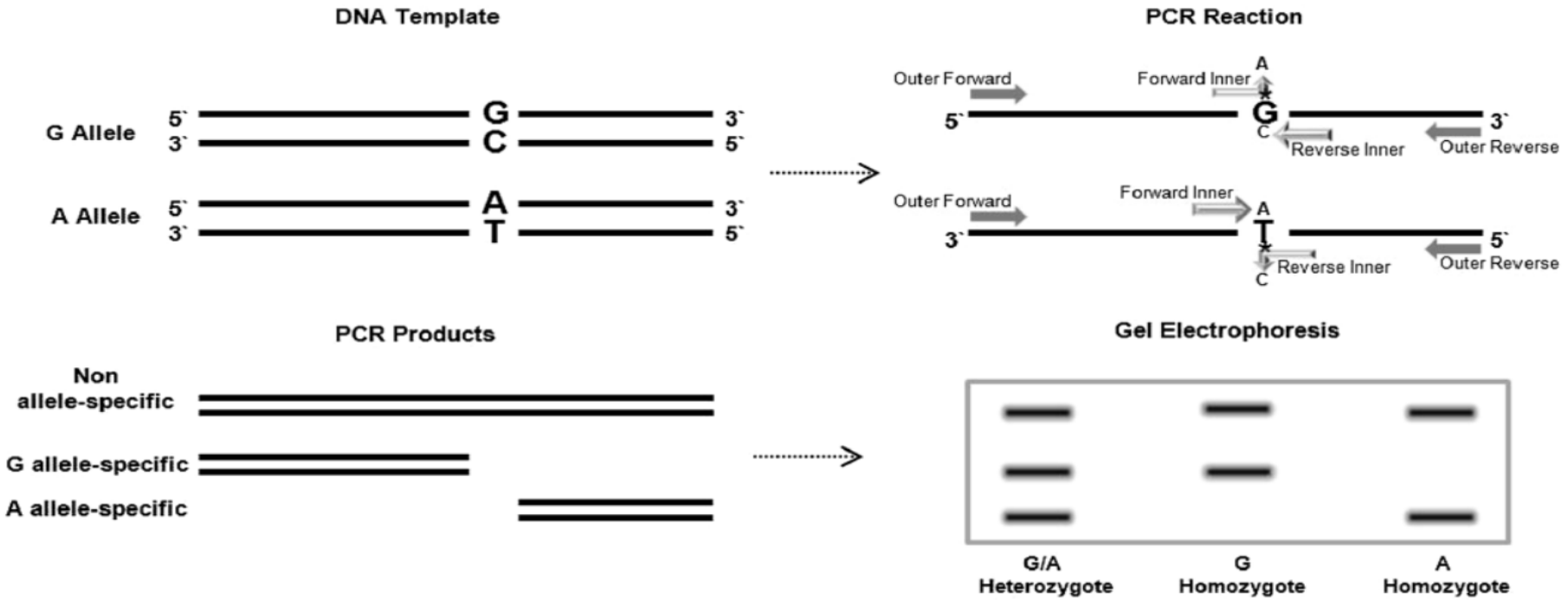
Sickle cell anaemia

Thalassemia

cystic fibrosis

Suppose our DNA sequence has **G-A point mutation** viz, G in normal allele and A in place of G in the mutant allele

We have to design a forward primer in such a manner that for normal allele the primer contains C (complementary to G) at 3' end and the mutant primer contains T in place of C



Schematic illustration of the tetra-primer ARMS–PCR assay for SNP genotyping

در صورتی که یک جهش تنها سبب تغییر در یک نوکلئوتید شود به آن SNP می‌گویند

PCR-RFLP

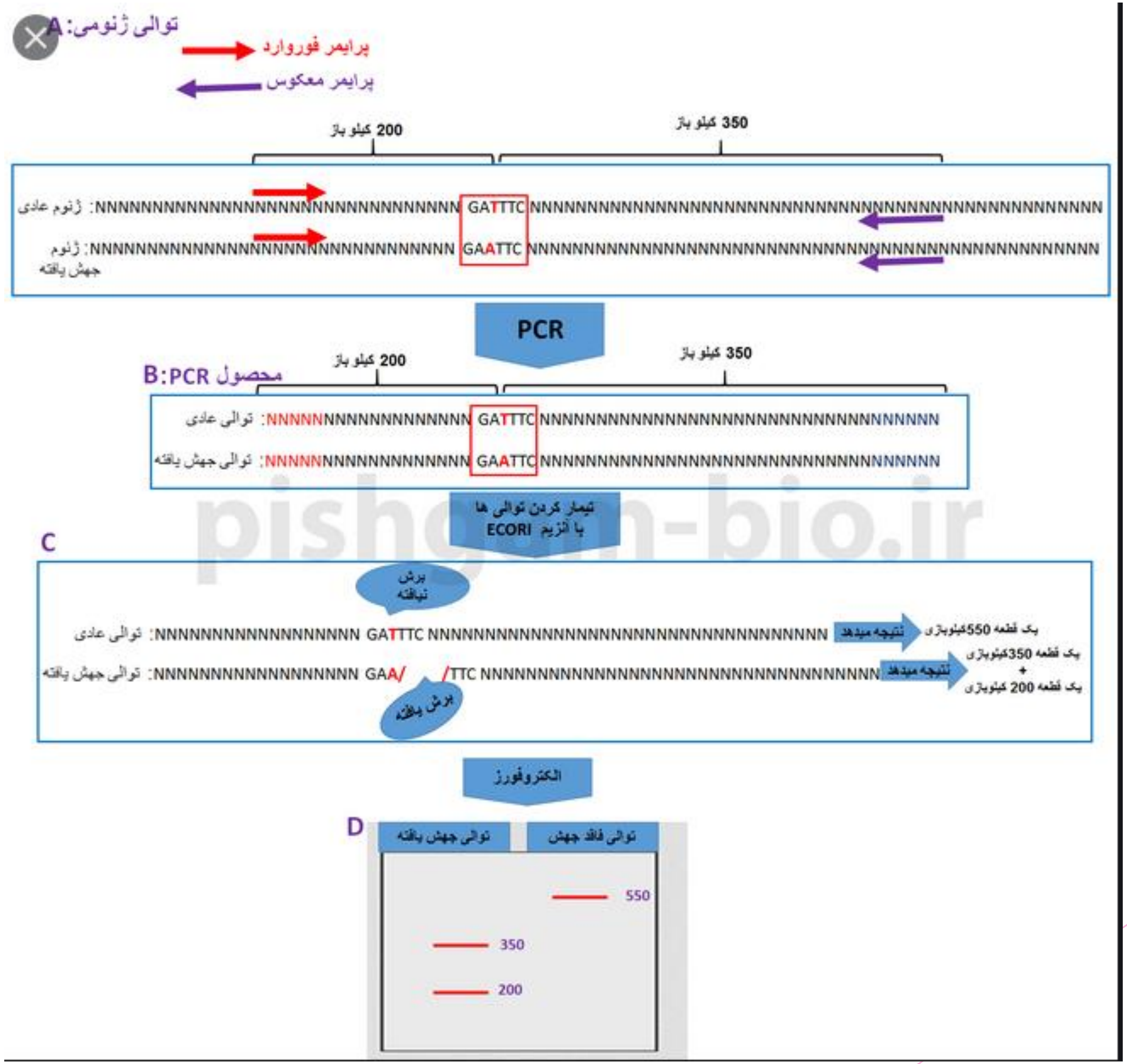
means

PCR-restriction fragment length polymorphism

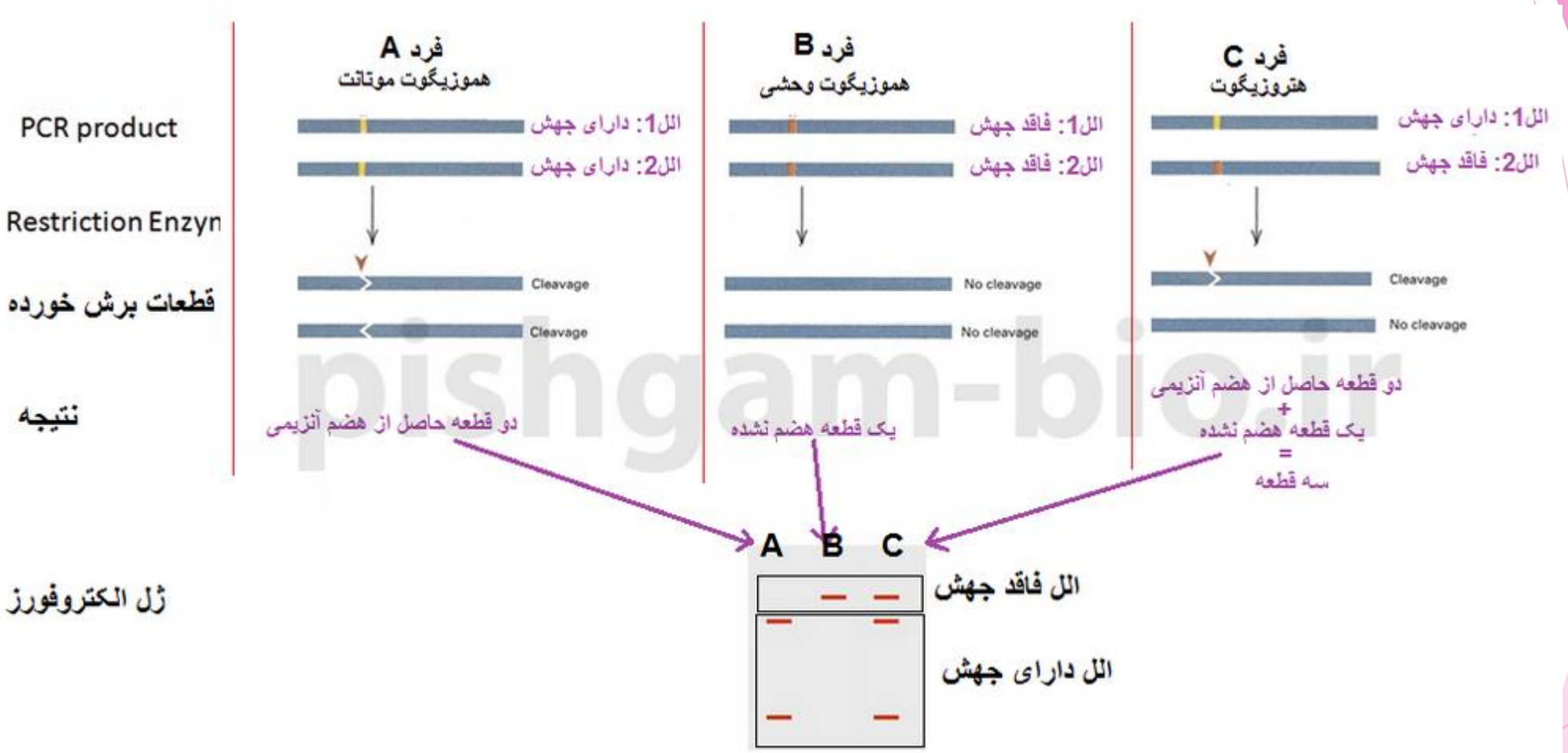
جهش‌ها (تغییرات تک نوکلئوتیدی ((SNPو پلی مورفیسم ها در ژنوم)

DNA-finger printing

پلی مورفیسم: در صورتی که فراوانی یک جهش در جامعه بیشتر از 1٪ باشد با آن جهش پلی مورفیسم می‌گویند.



A: توالی ژنومی
پرایمر فوروارد
پرایمر معکوس
200 کیلو باز
350 کیلو باز
ژنوم عادی
GATTTC
ژنوم جهش یافته
GAATTC
PCR
B: PCR محصول
200 کیلو باز
350 کیلو باز
توالی عادی
GATTTC
توالی جهش یافته
GAATTC
تیمار کردن توالی ها
با آنزیم ECORI
C
برش نیافته
توالی عادی
GATTTC
نتیجه میدهد
یک قطعه 550کیلوبازی
توالی جهش یافته
GAA/
/TTC
برش یافته
نتیجه میدهد
یک قطعه 350کیلوبازی
+
یک قطعه 200 کیلوبازی
الکتروفورز
D
توالی جهش یافته
توالی فاقد جهش
550
350
200



Colony PCR

استفاده از کلنی باکتری (سلول کامل بدون نیاز به مراحل لیز کردن و استخراج)

روش انجام Colony PCR: مقداری بسیار کمی از کلنی را با استفاده از خلال دندان استریل برداشته و پس از همژن کردن در آب به عنوان الگو در واکنش PCR استفاده می شود.

هدف از colony PCR: غربال گری سریع کلنی های ترانسفورم شده

متدهای غربال گری کلنی های ترانسفورم شده پس از انجام کلونینگ:

1- Colony PCR
2- Nested PCR
3- Mini-prep
4- White-blue complementation
5- Run on gel: Size