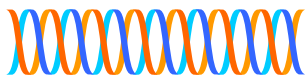


PCR: creare il DNA in provetta



DNA stampo



Buffer =
soluzione di
reazione

G C
A T
dNTP= nucleotidi

Primers



Taq Polimerasi

Termociclatore



1 Una molecola di DNA con una specifica sequenza da copiare viene riscaldata a 90 °C per denaturalarla.

2 Quando la miscela si è raffreddata, primer sintetizzati artificialmente si legano al DNA a singolo filamento.

3 Vengono aggiunti dNTP e DNA polimerasi resistenti alle alte temperature per sintetizzare due nuovi filamenti di DNA. Il processo viene ripetuto, raddoppiando la quantità di DNA sintetizzata.

4 Il processo si ripete, raddoppiando la quantità di DNA.

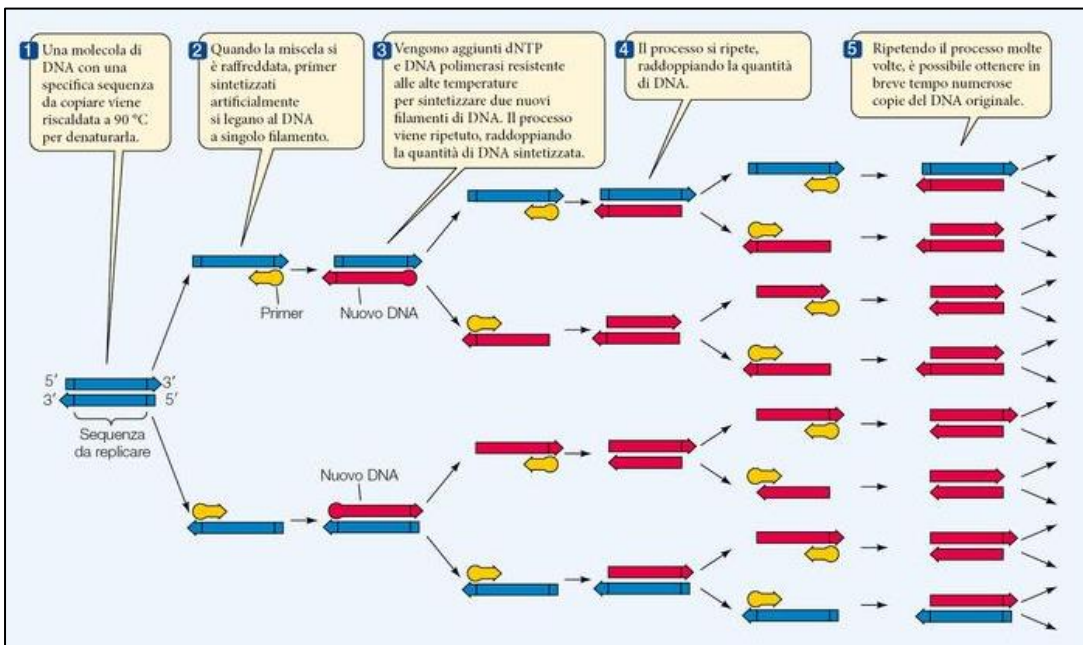
5 Ripetendo il processo molte volte, è possibile ottenere in breve tempo numerose copie del DNA originale.

5' 3'
3' 5'
Sequenza da replicare

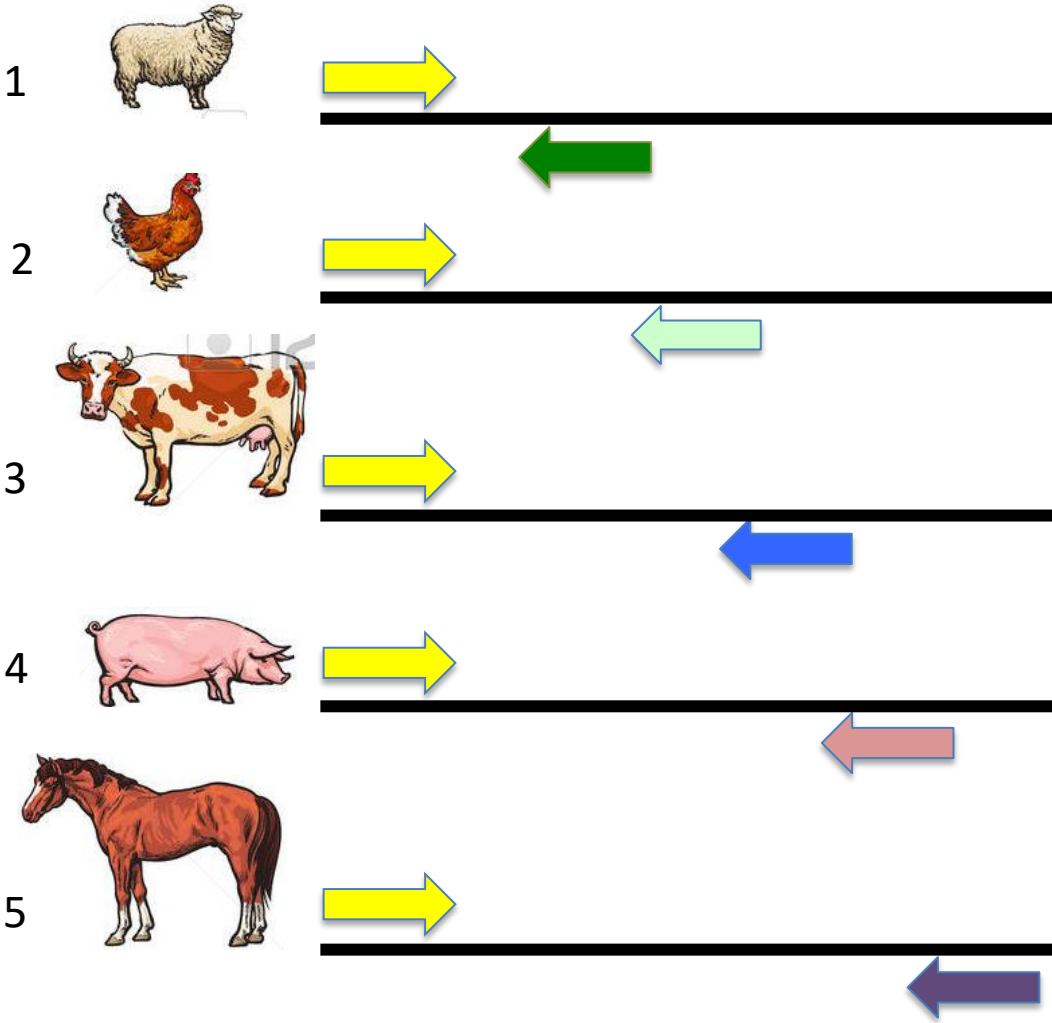
Primer

Nuovo DNA

Nuovo DNA



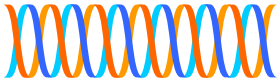
IL GENE DEL CITROCROMO b



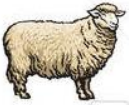
Per l'amplificazione si utilizza un *Primer* che è efficace sul DNA genomico di tutte le specie indicate ed uno specie-specifico che si consente l'amplificazione solo in una specie

PCR specie specifica

DNA stampo
(Il DNA di una sola
specie per tubo)



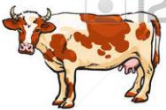
1



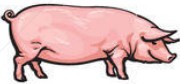
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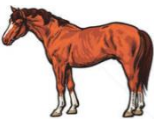
3



4

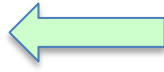


5



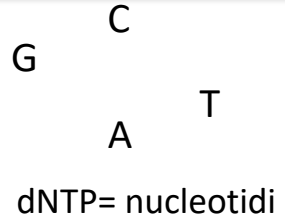
Primers (tutti)

efficace su tutti
i DNA genomici



specie-specifico

altri componenti
(tutti)

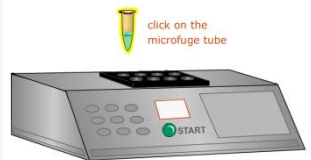


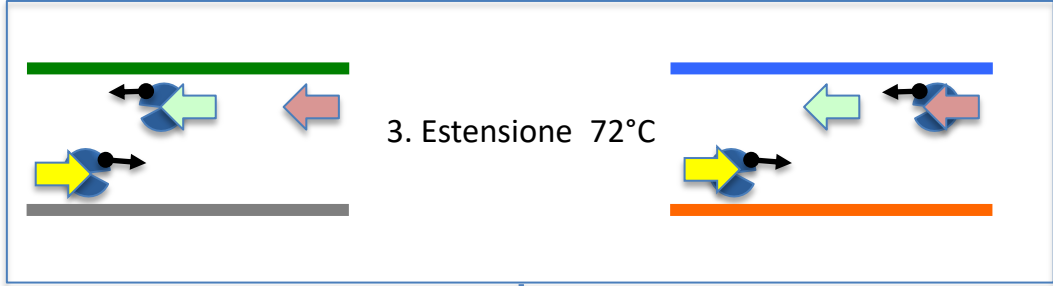
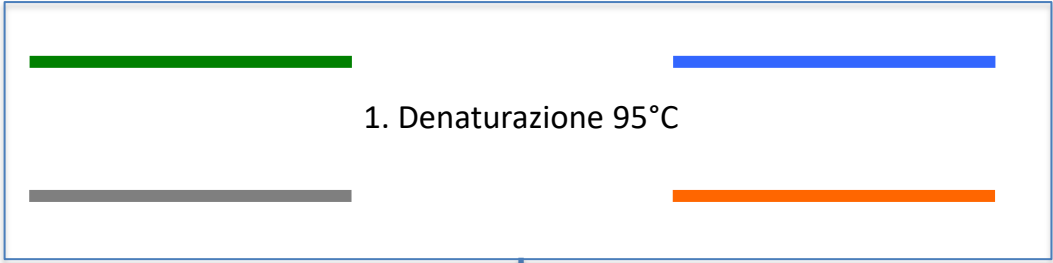
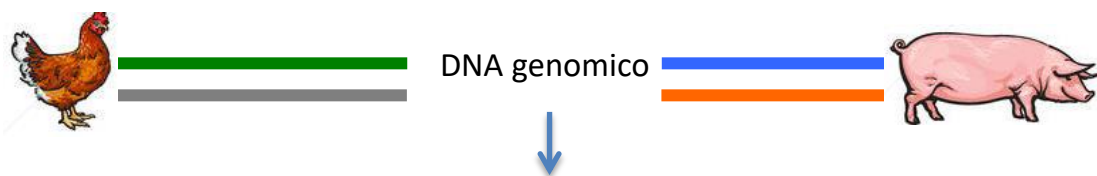
Taq Polimerasi



Buffer =
soluzione di
reazione

Termociclatore





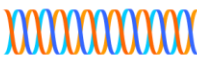
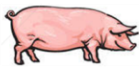
Analisi su gel d'agarosio



DNA amplificato =
227 paia di basi



DNA amplificato =
398 paia di basi



DNA genomico

Primers



.. CCTCCAGCCCCCTCCAACATCTCATCATGATGAAA..... AGCATCCATATTCTTTATCTCGCTCTT... TACGGTCATCACAAATCTACTATCAGC..
 .. GGAGGGTCGGGGGAGGTTGTAGAGTAGTACTACAAA..... TCGTAGGTATAAGAAATAGACGCAGAA... ATGCCAGTAGTGTTTAGATGATAGTCG

1. Denaturazione 95°C

.. CCTCCAGCCCCCTCCAACATCTCATCATGATGAAA..... AGCATCCATATTCTTTATCTCGCTCTT... TACGGTCATCACAAATCTACTATCAGC..
 .. GGAGGGTCGGGGGAGGTTGTAGAGTAGTACTACAAA..... TCGTAGGTATAAGAAATAGACGCAGAA... ATGCCAGTAGTGTTTAGATGATAGTCG

2. Appaiamento 55°C

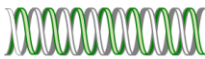
AGCATCCATATTCTTTATCTCGCTCTT... TACGGTCATCACAAATCTACTATCAGC..
 GCGGAGTAAGAAGAAGTAGACATAGAA ATGCCAGTAGTGTTTAGATGATAGTCG..

3. Estensione 72°C

CCTCCAGCTCCATCAAAATCTCATCTTGATGAAA
 .. GGAGGGTCGGGGGAGGTTGTAGAGTAGTACTACAAA

AGCATCCATATTCTTTATCTCGCTCTT... TACGGTCATCACAAATCTACTATCAGC..
 GCGGAGTAAGAAGAAGTAGACATAGAA ATGCCAGTAGTGTTTAGATGATAGTCG..

CCTCCAGCTCCATCAAAATCTCATCTTGATGAAA
 .. GGAGGGTCGGGGGAGGTTGTAGAGTAGTACTACAAA



DNA genomico

Primers



.. CCTCCAGCCCCCATCAACATCTCTGCTTGATGAAA..... CGCCTCATTCTTCTTCATCTGTATCTT... CACCGTACTCACAAACCTATTATCAGC..
 .. GGAGGGTCGGGGGAGGTTGTAGAGACGTACTACTAAA..... GCGGAGTAAGAAGAAGTAGACATAGAA... GCTCATAGTAGATTTGTGATGACCCT..

1. Denaturazione 95°C

.. CCTCCAGCCCCCATCAACATCTCTGCTTGATGAAA..... CGCCTCATTCTTCTTCATCTGTATCTT... CACCGTACTCACAAACCTATTATCAGC..
 .. GGAGGGTCGGGGGAGGTTGTAGAGACGTACTACTAAA..... GCGGAGTAAGAAGAAGTAGACATAGAA... GCTCATAGTAGATTTGTGATGACCCT..

2. Appaiamento 55°C

CGCCTCATTCTTCTTCATCTGTATCTT... CACCGTACTCACAAACCTATTATCAGC..
 GCGGAGTAAGAAGAAGTAGACATAGAA ATGCCAGTAGTGTTTAGATGATAGTCG..

3. Estensione 72°C

CCTCCAGCTCCATCAAAATCTCATCTTGATGAAA
 .. GGAGGGTCGGGGGAGGTTGTAGAGACGTACTACTAAA

CGCCTCATTCTTCTTCATCTGTATCTT... CACCGTACTCACAAACCTATTATCAGC..
 GCGGAGTAAGAAGAAGTAGACATAGAA ATGCCAGTAGTGTTTAGATGATAGTCG..

CCTCCAGCTCCATCAAAATCTCATCTTGATGAAA
 .. GGAGGGTCGGGGGAGGTTGTAGAGACGTACTACTAAA

