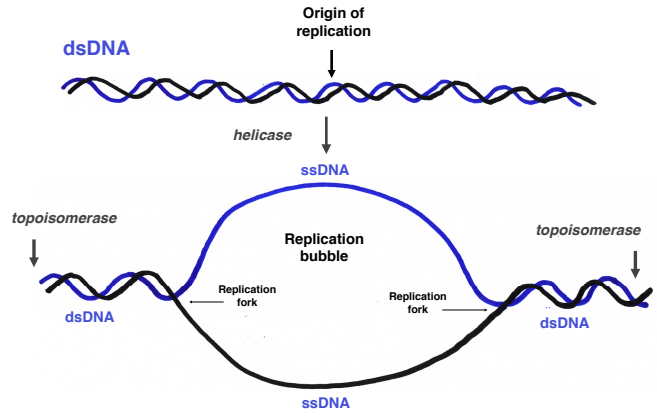
Helicases and the replication bubble



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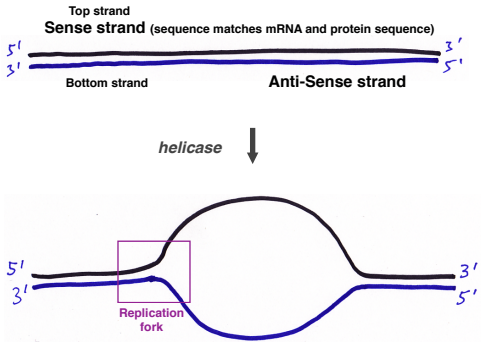
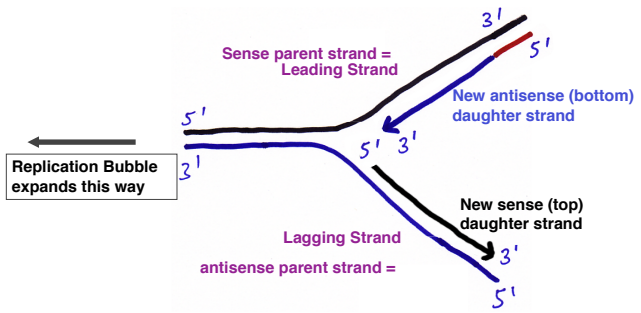


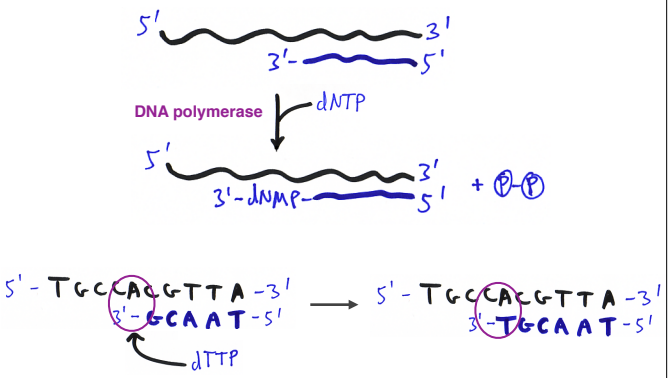
2

At the Replication Fork:



3

DNA Extension



dNTP = deoxy-nucleotide triphosphate
dNMP = deoxy-nucleotide monophosphate

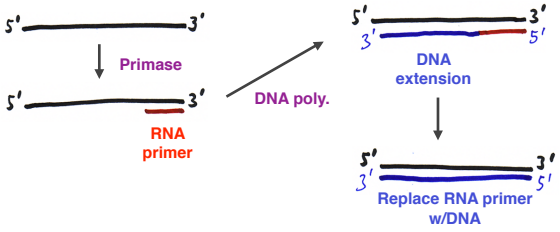
4

Priming

DNA polymerase can't just jump on a parent ssDNA strand and start making a new daughter strand.

DNA polymerase can only extend a short double-stranded segment.

So, parent strand needs to be primed with a short piece of RNA by a primase enzyme.



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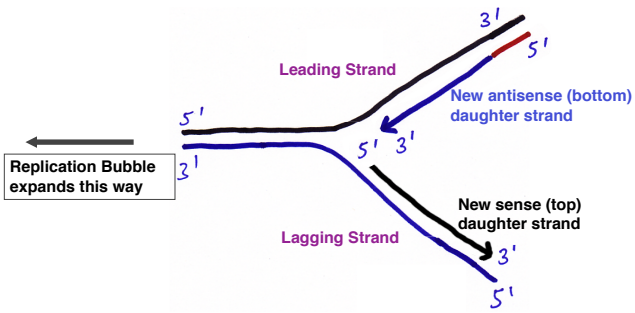
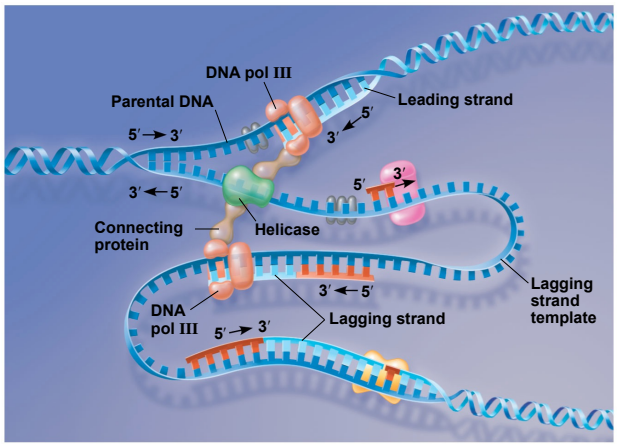
At the Replication Fork:



6

Figure 16.18



7

DNA repair enzymes

1. Mismatch: 1 error every 10,000 base-pairs (e.g. C:T instead of A:T)

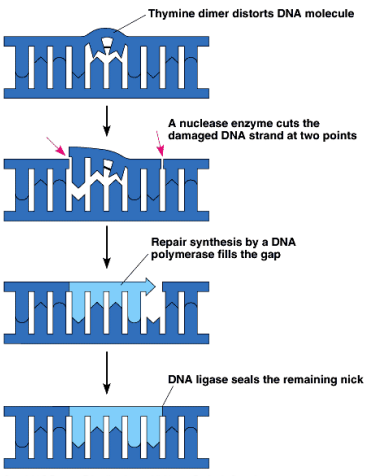
   DNA polymerase has proofreading activity.

2. Excision repair: damage to non-replicating DNA that needs to be cut out and replaced. (e.g. ultraviolet radiation causes two adjacent thymines to be covalently cross-linked.)

   Nuclease excises damaged DNA; polymerase and ligase fill in the gap.

8

DNA repair



9

Summary of DNA Replication

10

1. Beginning at origin, dsDNA is unwound by helicase to make replication bubble.
2. Replication proceeds away from origin in both directions at the 2 replication forks.
3. New DNA strands are primed by a short piece of RNA primer constructed by primase.
4. Leading strand is synthesized continuously by DNA polymerase, growing 5' -> 3'.
5. Lagging strand is synthesized discontinuously by DNA polymerase as Okazaki fragments, which are stitched together by DNA ligase.
6. Errors in the DNA is corrected by proof-reading by DNA polymerase, and other repair enzymes.

DNA replication

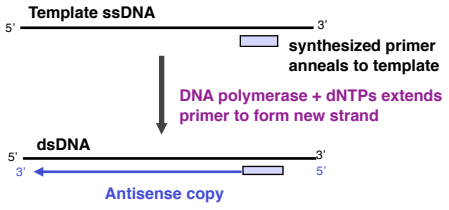
11

All organisms (prokaryotes and eukaryotes) use essentially the same enzymes and reactions to replicate their DNA.

DNA replication can be carried out in a test-tube just by adding DNA, primers, and DNA polymerase.

DNA Replication in a test-tube: The Polymerase Reaction

12



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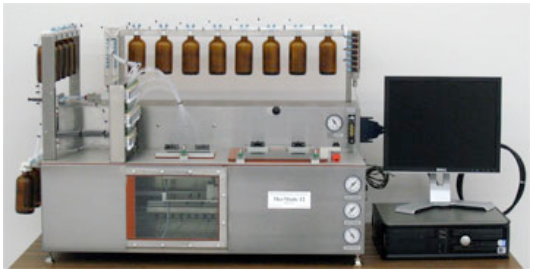
DNA Synthesis machine



The MerMade-6 Oligonucleotide synthesizer is designed and priced for low throughput synthesis at a low cost. Based on the proven MM12 design the MM6 is rugged, reliable, and easy to service. This 6 column machine offers a wide scale range (50nmole to 200micromole) and On Line Trityl Monitoring (up to 6 columns) at about the same price as competing 4 column machines on the market. Offering much more flexibility and speed this DNA/RNA synthesizer allows the operator to add and remove columns at any point during a run and to synthesize different scales and chemistries on each column, while not wasting reagents or time.

http://www.bioautomation.com

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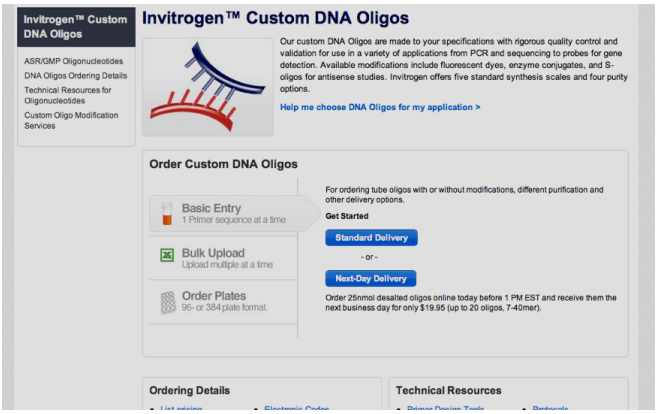

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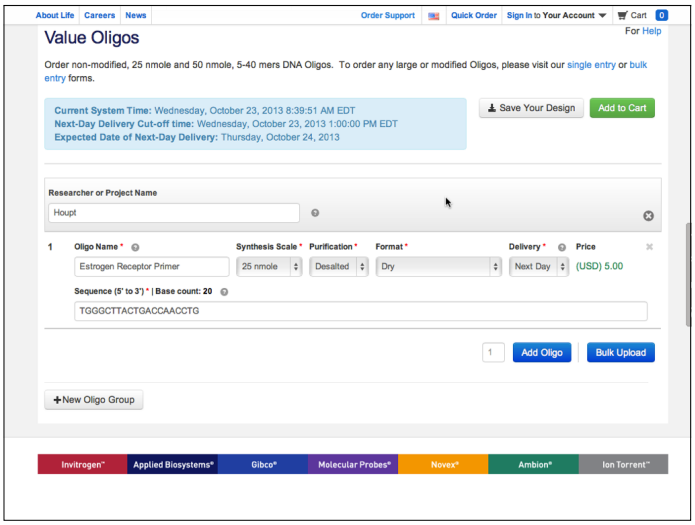
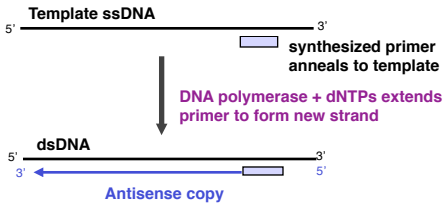


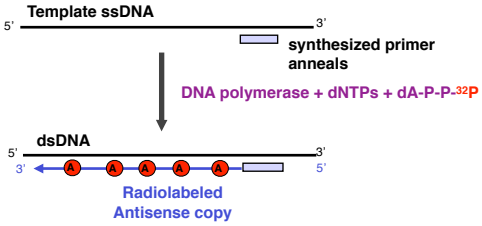
DNA Replication in a test-tube: The Polymerase Reaction

16



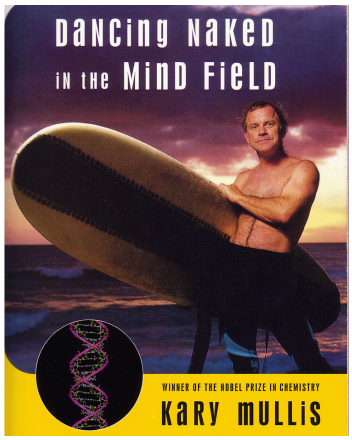
Radioactive DNA Labeling: The Polymerase Reaction

17

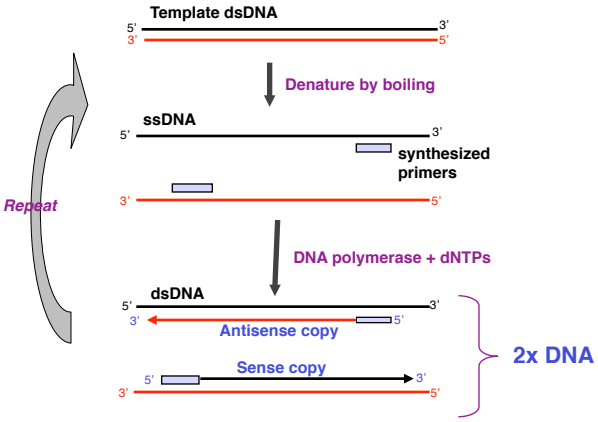


Inventor of PCR

18



Polymerase Reaction



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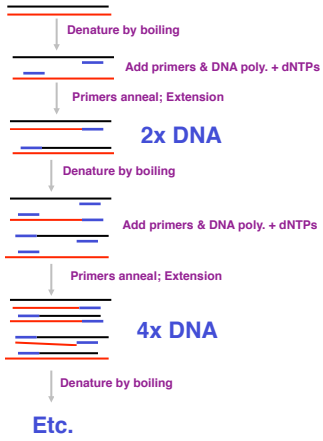
Polymerase Chain Reaction



20

Polymerase Chain Reaction (PCR)

One Round of PCR:

Denature Template

Anneal Primers

Extend New Strand

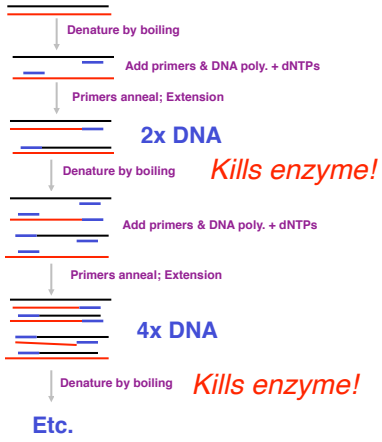
24 rounds = 2^{24} = 16.7 million copies

30 rounds = 2^{30} = 1 billion copies

1. Denature Template, Anneal Primers, Extend New Strand — 2x
2. Denature Template, Anneal Primers, Extend New Strand — 4x
3. Denature Template, Anneal Primers, Extend New Strand — 8x
4. Denature Template, Anneal Primers, Extend New Strand — 16x
5. Denature Template, Anneal Primers, Extend New Strand — 32x
6. Denature Template, Anneal Primers, Extend New Strand — 64x

21

Polymerase Chain Reaction (PCR)



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Thermus aquaticus (Taq) polymerase

Bacteria from a hot spring near Great Fountain Geyser, Yellowstone Park

Thermal range is 50-80° C (122-176° F), and its optimum is around 70° C (158° F). So it will survive repeated boiling at 100° C.

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PCR Terminology

Template
DNA to be amplified, eg. extracted from tissue, or RT reaction product

Primers

T_m
temperature at which 1/2 of complementary ssDNA hybridizes to form dsDNA

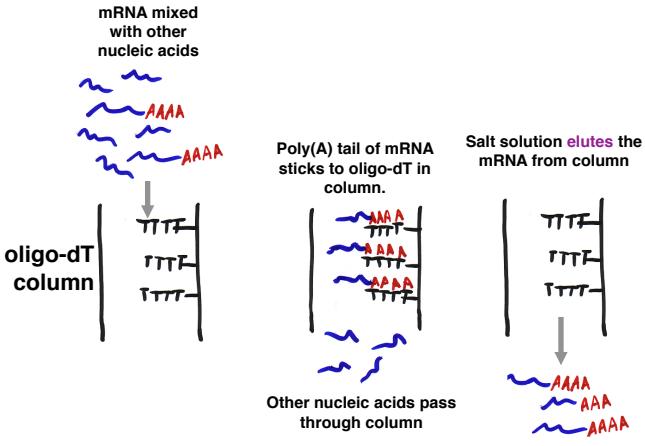
Taq Polymerase
but can be different varieties

Reaction Mix
source of dNTPs, salt conditions, Mg++ (Sybr Green if for qPCR)

Product DNA

24

oligo-dT mRNA Extraction and Reverse Transcriptase



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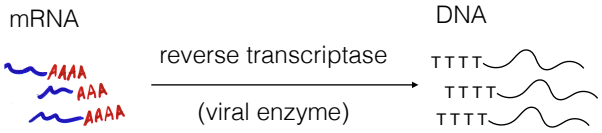
oligo-dT mRNA Extraction and Reverse Transcriptase



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PCR Cycling Parameters:

Annealing
at T_m + 2°

Extension
72° C, 1 min for every 1kb of template length

Denaturing
95°

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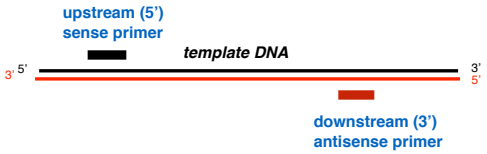
PCR Amplifies between Primers



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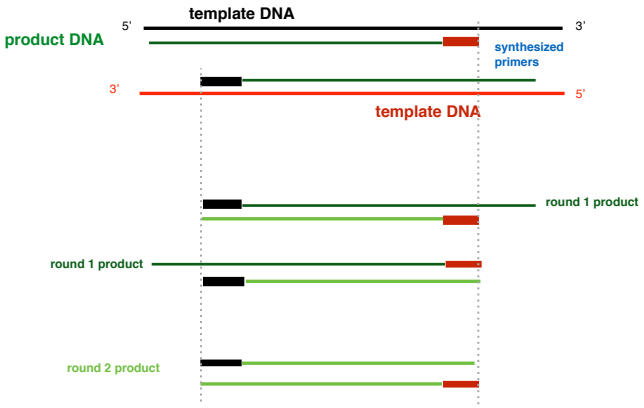
PCR Amplifies between Primers



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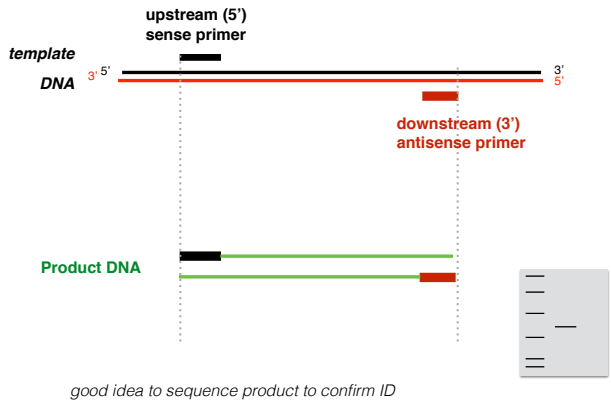
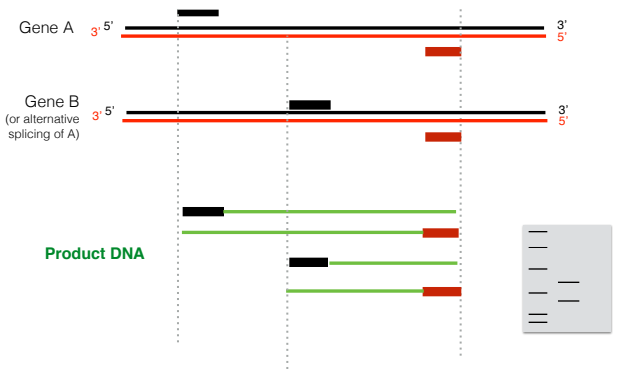
PCR Amplifies between Primers



good idea to sequence product to confirm ID

30

PCR Amplifies between Primers



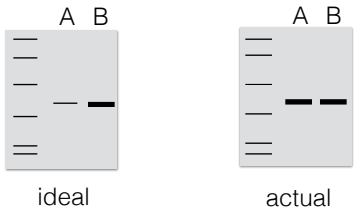
31

qPCR : Quantitative (Real-Time) PCR

- Amplification should be log-2 linear (but isn't)
- Reaction saturates at high level

Tube A: 2 copies of Gene → 32 million copies

Tube B: 4 copies of Gene → 64 million copies



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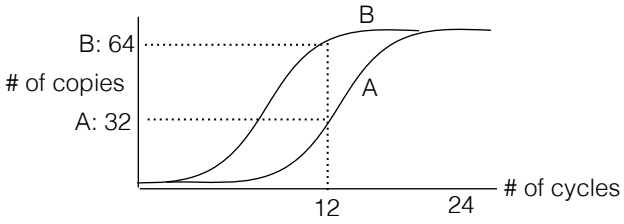

33

qPCR : Quantitative (Real-Time) PCR

34

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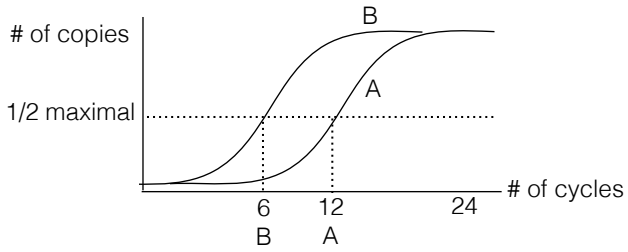

qPCR : Quantitative (Real-Time) PCR

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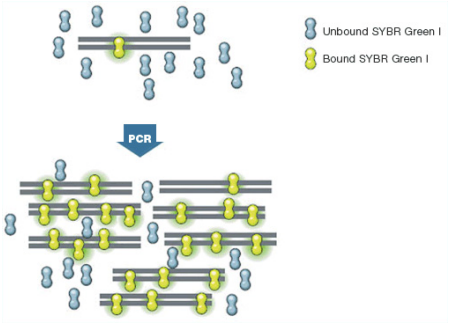
SYBR Green - Fluorescence increases as total double-stranded DNA increases



http://www.di.uq.edu.au/proj19proc

36

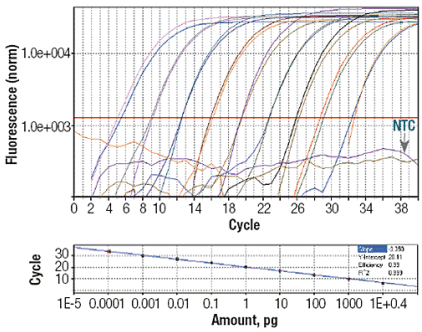
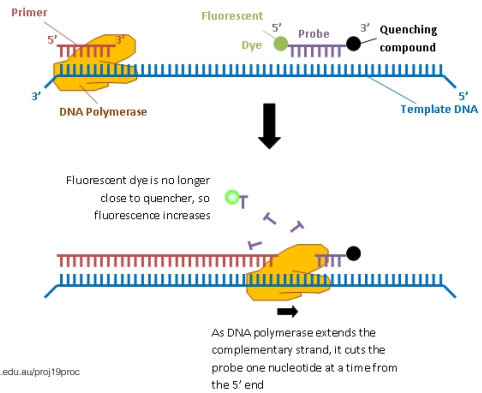


Figure 1. Uniform and reproducible results.
Amplification of 10-fold dilutions of supercoiled plasmid DNA, starting from 10 ng down to 0.1 fg, using the Maxima® SYBR Green/ROX qPCR Master Mix (2X) in duplicate reactions. Reactions were performed on the Eppendorf Mastercycler® ep *realplex instrument. The amplification plot and standard curve show the linearity across 9 orders of magnitude. NTC is the non-template control.*

http://www.seoulin.co.kr/shop/products/product_01.php?category=11154a100000000

qPCR : Quantitative (Real-Time) PCR

Taqman - Fluorescence increases as specific product increases



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