PCR in the Classroom

UC Davis - PCR Workshop
Friday, September 26, 2003

A little history…

In 1983, Kary B. Mullis conceived the procedure.

He went on to Cetus Corp in Emeryville, CA where it was developed further.

In 1993, he was awarded the Nobel Prize in Chemistry.

Now, this procedure that few in the general public know about has completely revolutionized virtually every aspect of biotechnology research.

Current Uses…

PCR mimics cellular DNA replication.

Because of this, it is very useful in the laboratory for generating large quantities of DNA for research.

However, this technique is also helpful for:

- ✓Making cDNA and genomic DNA libraries.
- ✓Analyzing trace amounts of DNA from crime scenes.
- ✓Creating specific or random mutations in DNA for research purposes.
- ✓Analyzing the induction of specific genes in an organism.
- ✓Rapid detection of specific diseases when there is a suspected outbreak.
- ✓Identifying specific organisms or clones.

How it works…

Initially, the DNA to be replicated, or amplified, needs to be sequenced so researchers can make primers that are complimentary to the sequence.

Then the DNA sample is added to a small tube that contains the primers, nucleotides, buffer, and Taq Polymerase. Taq Polymerase is a DNA polymerase from *Thermus aquaticus*, a hot-spring bacterium. Taq polymerase is heat-stable because of this.

The mixture is placed in a thermal cycler. The mixture is heated up to about 95°C. This causes the two strands of the DNA to separate as in the beginning of DNA replication.

The mixture is then cooled to about 50-65°C to allow the primers to attach or "anneal" to the DNA.

How it works…

Once the primers have had a chance to anneal, the thermal cycler heats up the mixture to about 72°C. This allows Taq Polymerase to extend the DNA strands and "zip" up the DNA.

At this point, there are two *almost* identical DNA molecules that were created from just one.

This process is repeated for up to 40 cycles. Theoretically, the DNA is doubled in every reaction, but this doesn't actually happen. What really happens is that there is a huge shortage of the template DNA in the early cycles which leads to inefficient replication.

Similarly, last few cycles are inefficient because primers and dNTP's are in short supply and the Taq Polymerase begins to loose activity.

What the components do…

Primers allow the process to be specific. They bind to the 3′ end of the template DNA and start the copying of the DNA in a 3′ to 5′ direction.

The longer the primer, the more base pairing, the more specific.

Because G's and C's form three hydrogen bonds between base pairs, they are held more tightly together. So, a primer with more G-C content can have a higher annealing temperature. This makes it more specific.

There are also common primers that are used for amplifying genes in commercially available vectors (plasmids). These allow almost any gene to be amplified regardless of the gene's actual sequence.

What the components do…

Polymerase is the enzyme that copies the DNA. Taq Polymerase is the most common polymerase used in PCR, but there are other options.

Some polymerases are genetically modified versions of Taq Polymerase that have been altered to increase their proofreading capabilities, binding strength to the DNA, or heat sensitivity. In addition to these features, Taq Polymerase also leaves a 5'-A overhang on the ends of its products. This has actually become a benefit to using Taq as it allows easier cloning in some commercial vectors.

Pfu Polymerase is a polymerase that lacks the proofreading function of Taq polymerase. By altering other conditions, you can induce random mutations in your genes if you wish. Pfu Polymerase also creates a "blunt-end" product.

What the components do…

dNTP's are deoxyNucleotide TriPhosphates, or the building blocks of DNA. They are usually composed of equal concentrations of each nucleotide.

However, for sequencing purposes, you can use de-deoxynucleotide triphosphates. These do not allow any further nucleotides to be added once they are used in a chain. So, the reaction stops. This is why the sequencing method is called the chain-termination method.

What the components do…

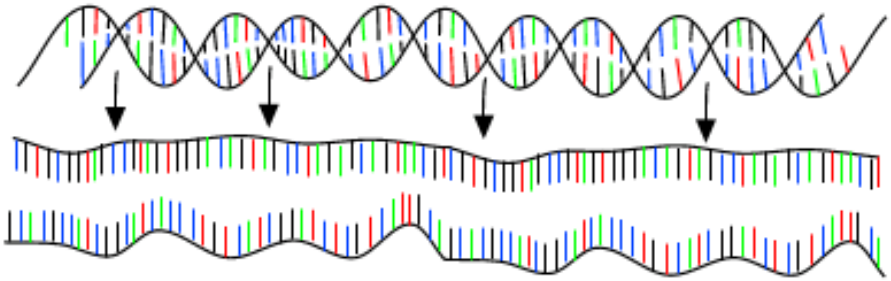
$MgCl_2$ is Magnesium Chloride. All polymerases require some concentration of magnesium chloride to function.

Altering the concentration of magnesium chloride allows you to tailor the efficiency of your polymerase. This could be useful if you wanted to introduce mutations.

These are the three steps to every cycle of a PCR reaction.

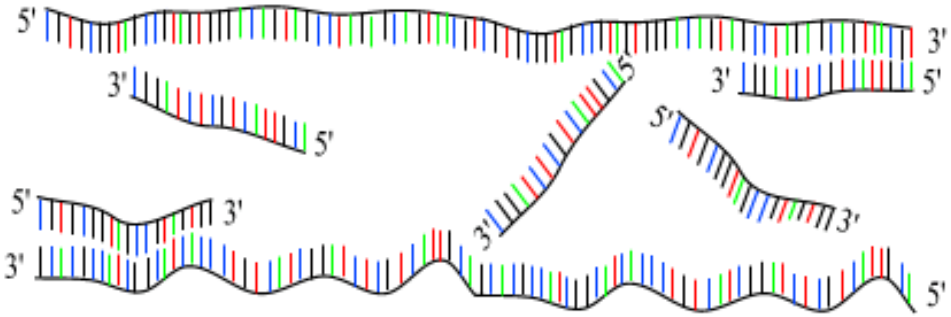
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

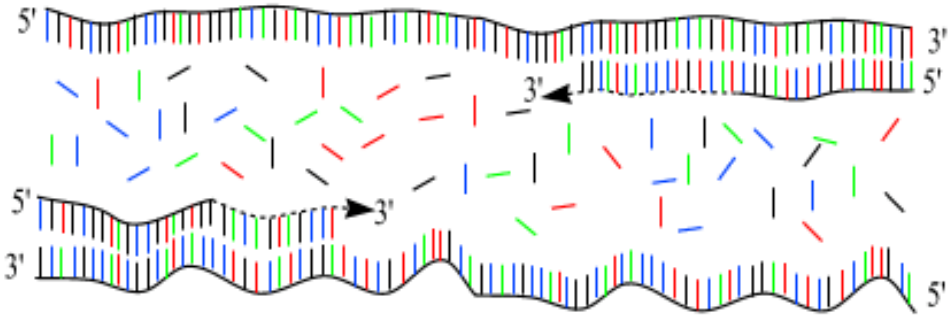
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



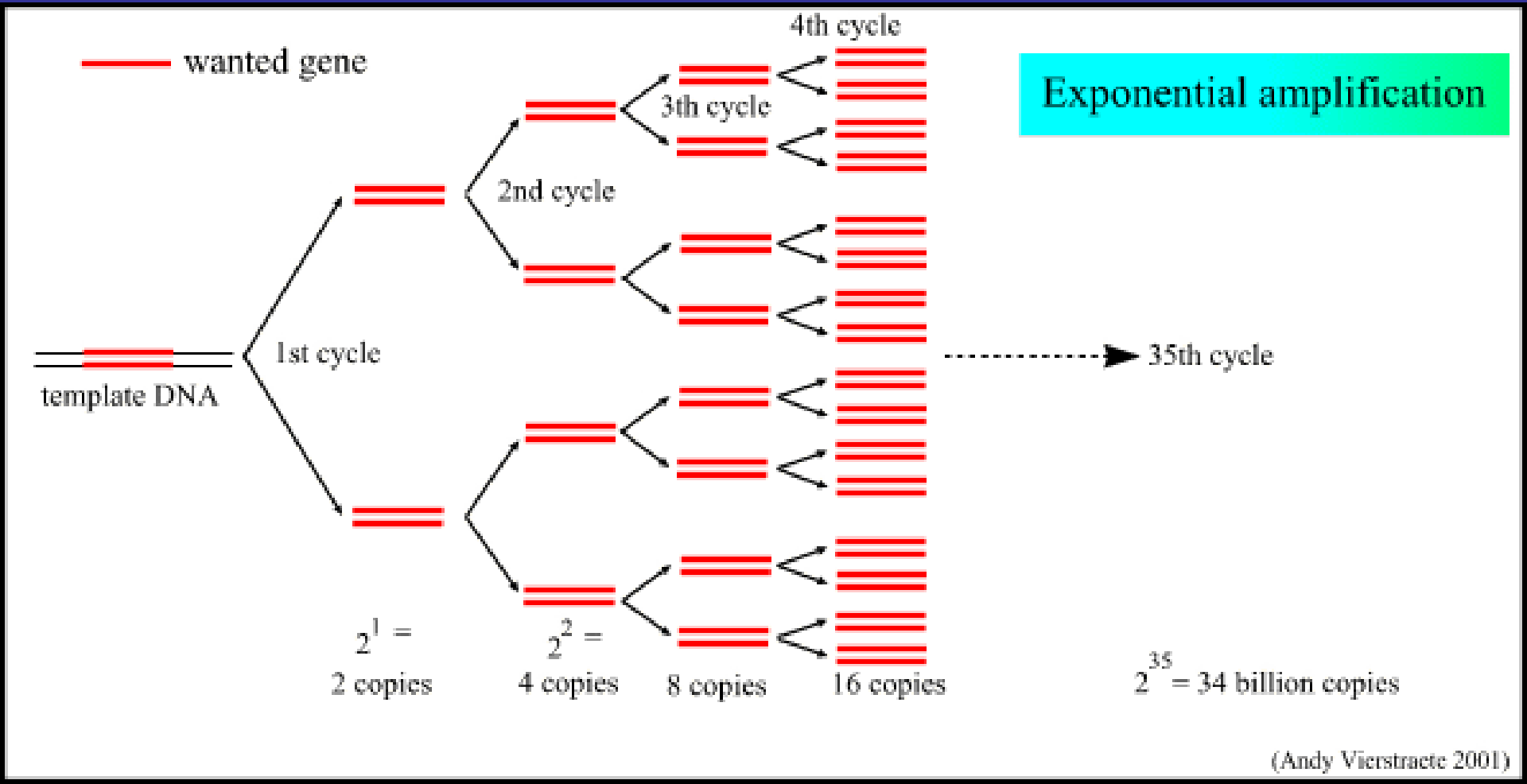
Step 3 : extension

2 minutes 72 °C

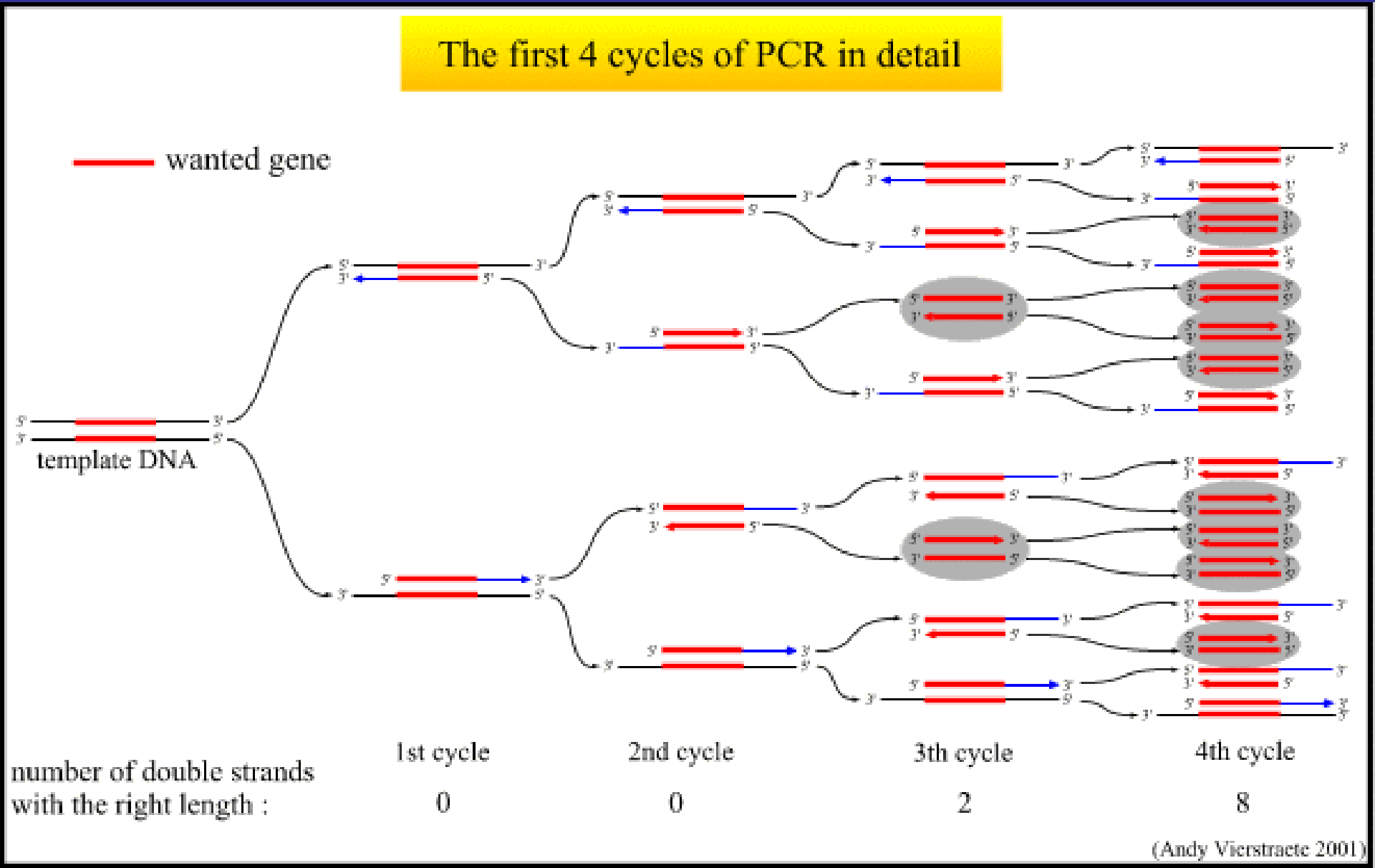
only dNTP's

(Andy Vierstraete 1999)

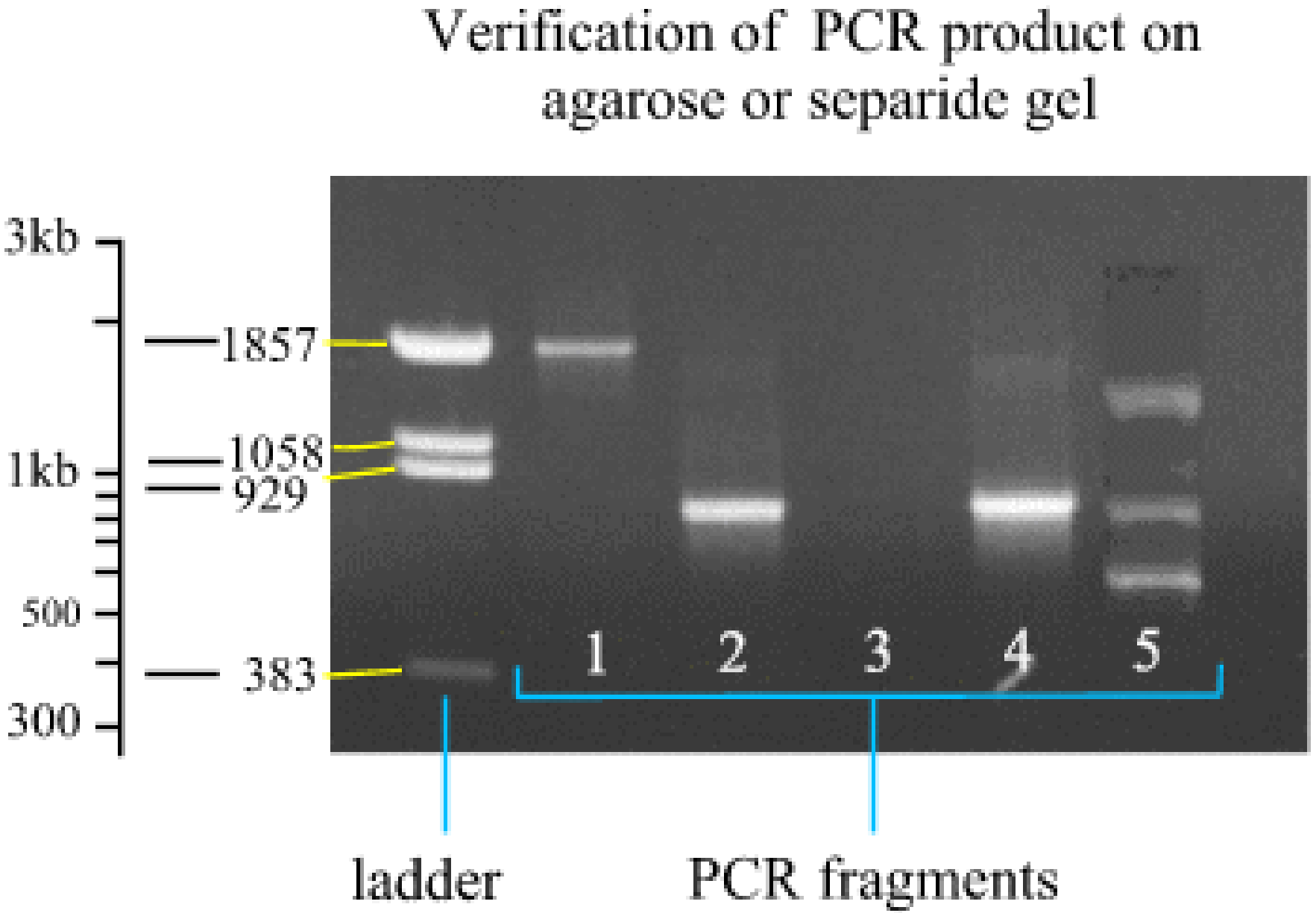
This is the beauty of PCR. DNA is exponentially amplified.



Not all of the products are initially the same.



After amplification, the reactions are run on a gel.



How our experiment should work…

Alu is a 300 bp repeat of "junk" DNA. Only a small portion of the DNA in our cells is ever used for transcription. PV 92 is the locus (or location) of one possible place where an Alu repeat may be found.

✓Primers have been made that flank this region on chromosome 16.

✓We will use a saline wash to remove some cells from the inside of our mouths.

✓Then we will use these washes to amplify our own DNA from our cells.

✓For PV 92, there are two alleles, and like every other trait, we may be homozygous dominant (+/+), homozygous recessive (-/-), or heterozygous (+/-) for this locus.

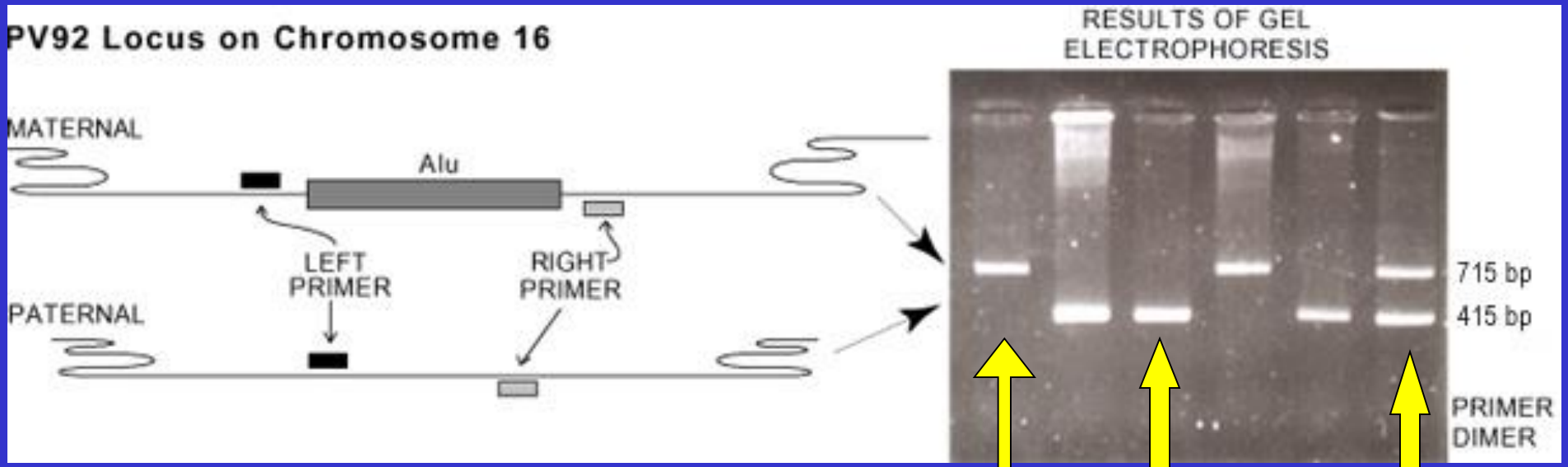
So if we are…
… we should see

Homozygous Dominant (+/+)…
… 715 bp of PV 92 that contains Alu on both alleles.

Homozygous Recessive (-/-)…
… 415 bp region from both alleles because we'd be lacking the 300 bp Alu repeat

Heterozygous (+/-)…
… 715 bp from one allele AND 415 bp from the other.

So what should our gel look like?



Homozygous for Alu

Homozygous w/o Alu

Heterozygous

Quiz Time!!!

1. Which lane(s) indicates someone who is homozygous for the lack of an *Alu* repeat?

Lane 2

2. Which lane(s) indicates someone who is heterozygous for *Alu*? Lanes 1 & 3

3. Which lane(s) indicates someone who is homozygous for the presence of an *Alu* repeat?

Lane 4