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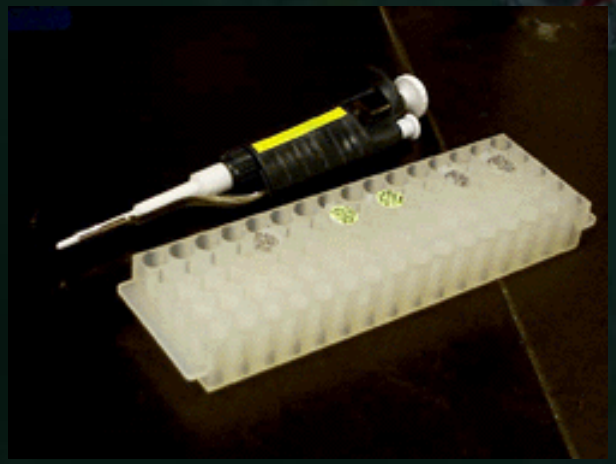
Invent the Future

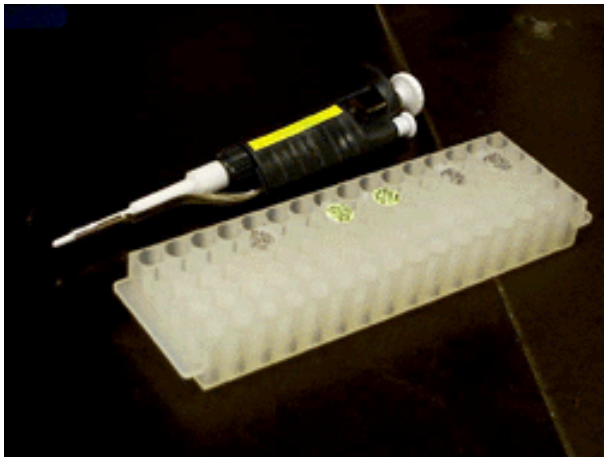
Trouble shooting of PCR

Basic Experimental Design



- A well-designed experiment can keep you from getting into trouble!
- A poorly-designed experiment is asking for problems!!!!

Basic Experimental Design



- Main point: Always use **CONTROLS**
- **Positive control**
  - So you'll know what a successful result looks like.
- **Negative control**
  - Lets you know if you have contamination.

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1- No products

1. No marker
2. Marker +ve but no product
3. No positive control
4. Positive control

Annealing temperature

Extention (time and temp)

DNA

PCR contaminants

Something missing (primers, sample, enzyme)

Wrong primers

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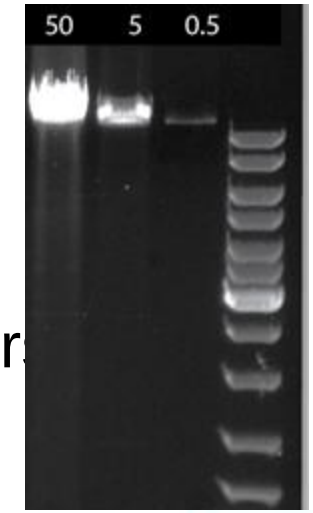
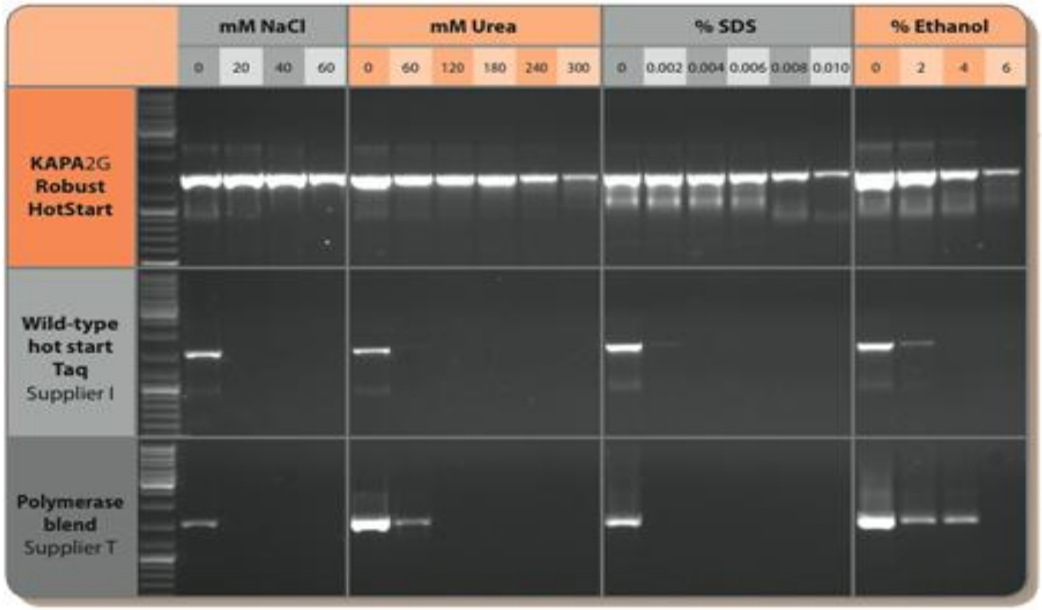
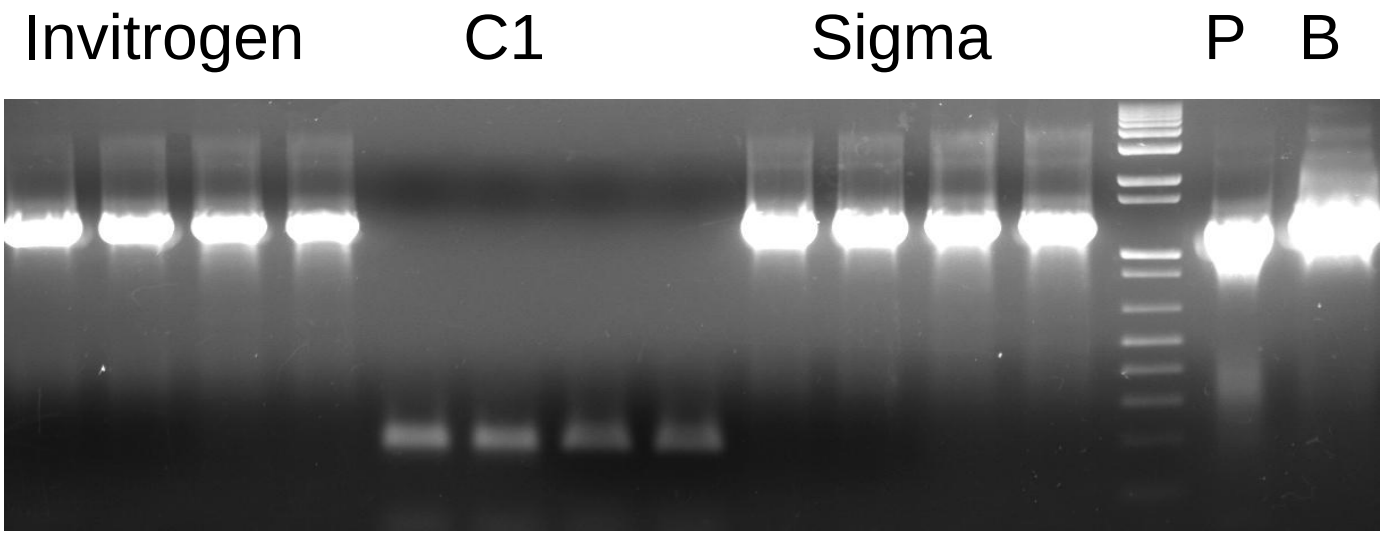
2- Low quantity

1. Increase DNA
2. Increase cycle
3. Change enzyme (next slide)
4. Decrease annealing temp (2-5)
5. High or low GC content (use PCR enhancer
6. PCR inhibitors



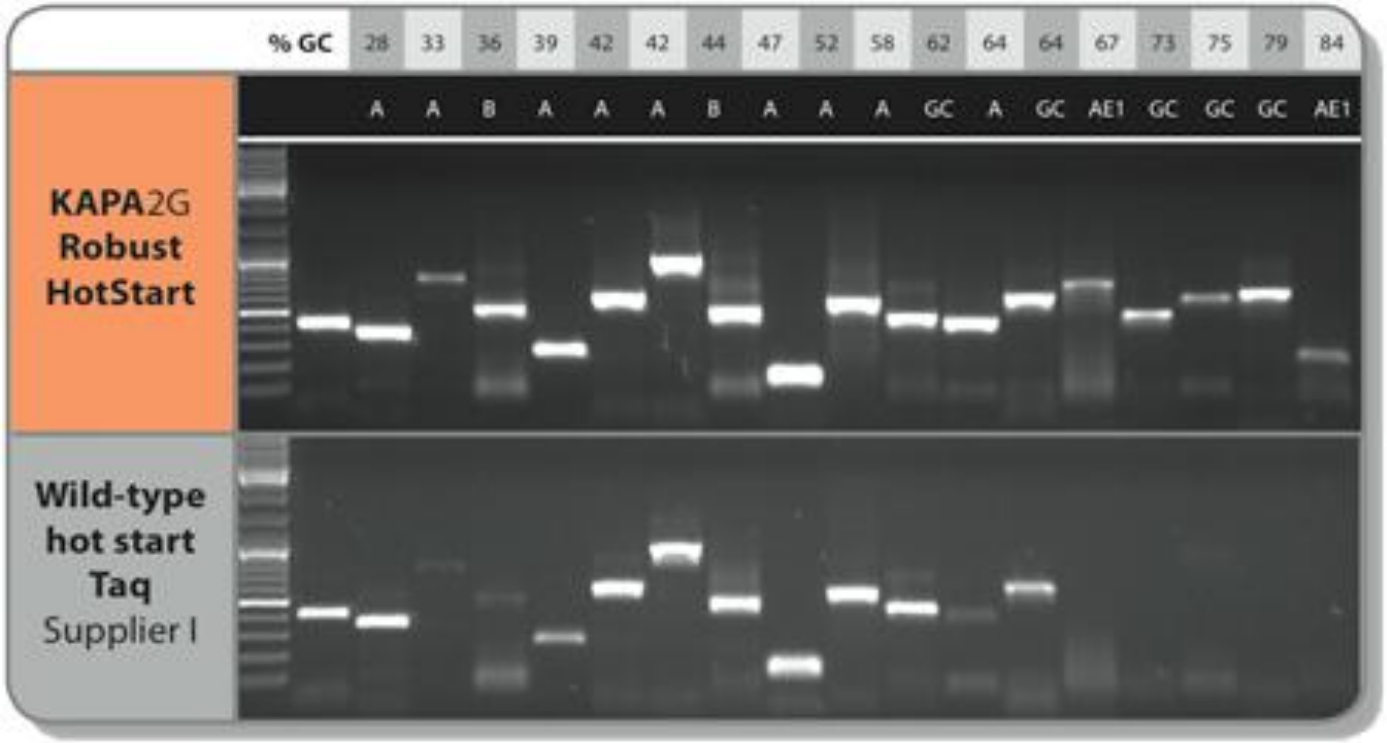


2- Low quantity



2- Low quantity

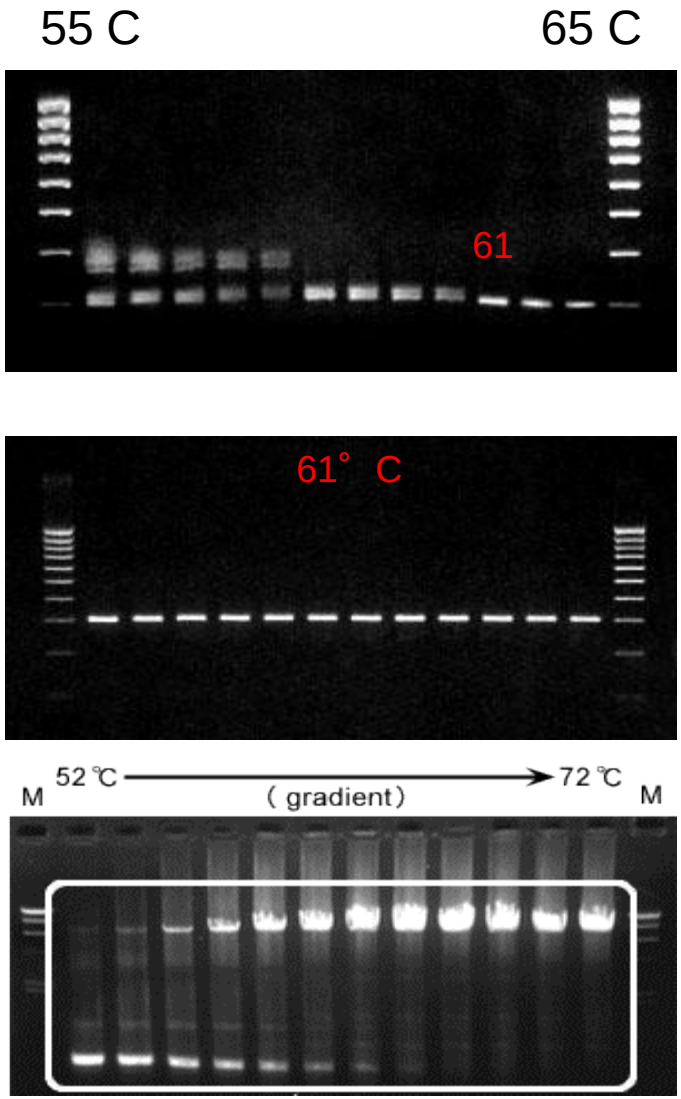
GC content



formamide, glycerol, DMSO, Tween-20. Varadaraj K and Skinner DM 1994

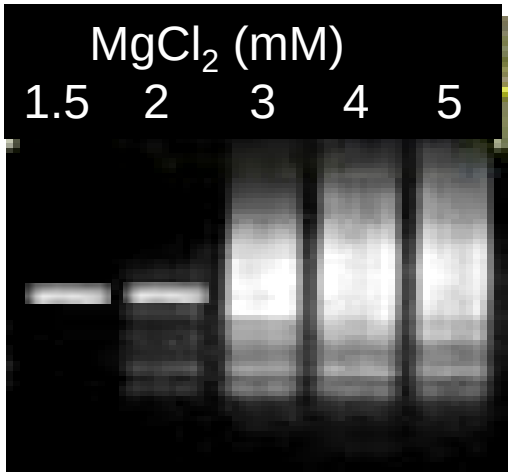
3- Non specific bands

1. Increase annealing temp
2. Prepare your reaction on Ice
3. ***Taq* 1.5 nt/sec at 37 ° C in 5 minutes 450bp**
4. Contamination
5. Wrong primers short
6. Decrease cycles
7. Hot start



4- Diffuse smearing

1. Decrease $MgCl_2$
2. Decrease DNA
3. Decrease polymerase
4. DNA degradation

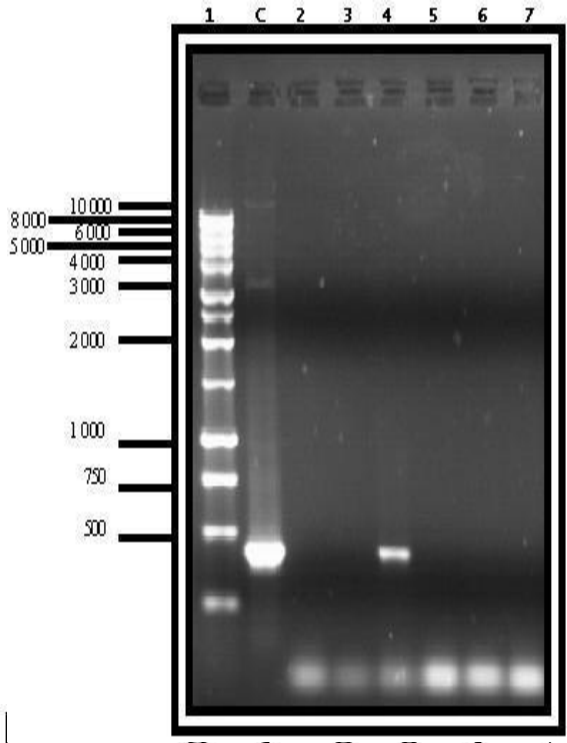


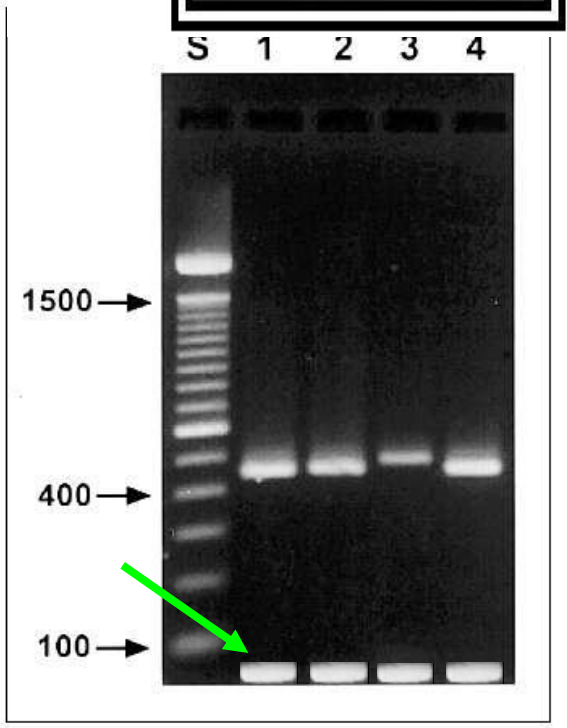
5- Primer Dimers

• Pair of Primers
5’-ACGGATACGTTACGCTGAT-3’
5’-TCCAGATGTACCTTATCAG-3’

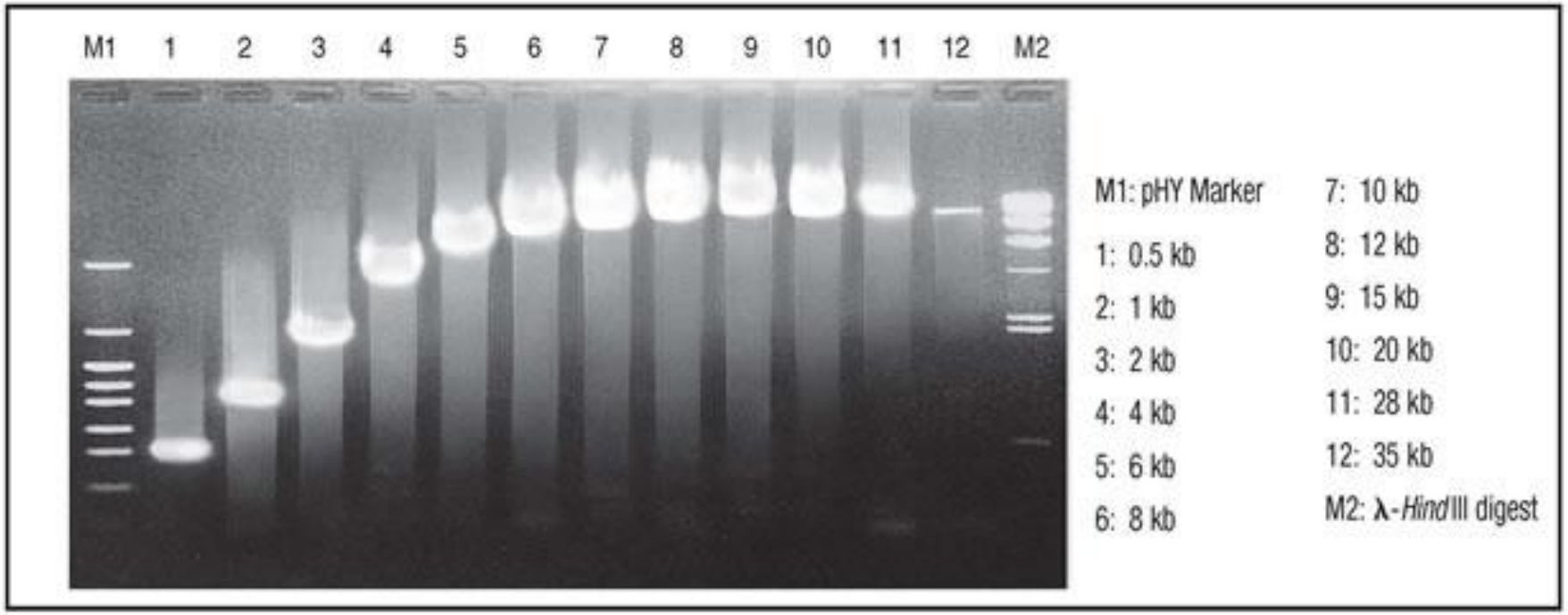
• Complementarity of primer 3’ ends
5’-ACGGATACGTTACGCTGAT-3’
3’-GACTATTCCATGTAGACCT-5’

• Results in PCR product
Primer 1
5’-ACGGATACGTTACGCTGATAAGGTACATCTGGA-3’
3’-TGCCTATGCAATGCGACTATTCCATGTAGACCT-5’
Primer 2



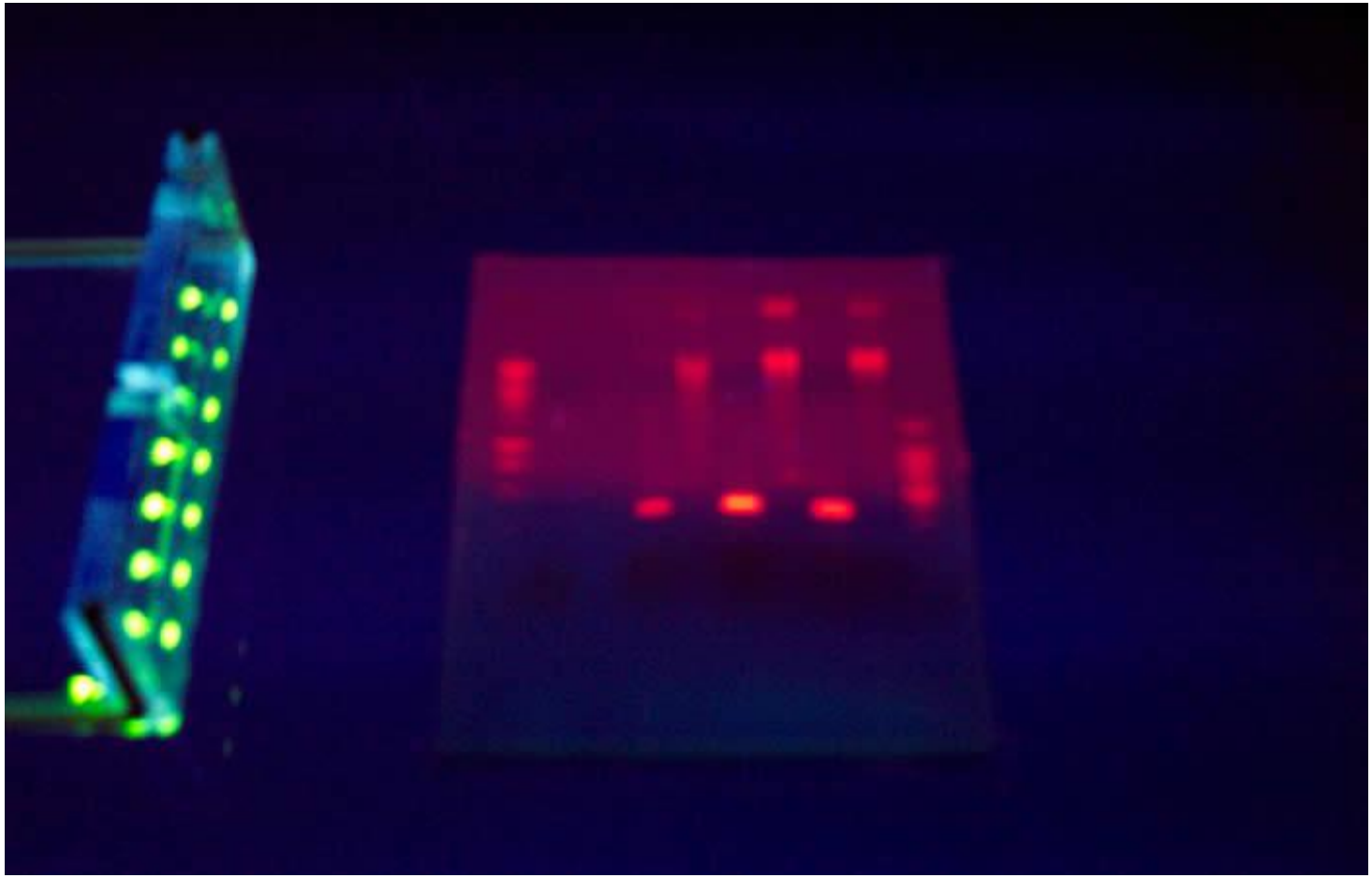


Long PCR



Amplification of DNA Fragments from 0.5-35 kb in Size (different primer sets) using *LA Taq*™. *LA Taq*™ DNA Polymerase was used to amplify the various fragments and generated high product yields, even with very long (28 kb) fragments.

mixture of *Taq* Polymerase with a proofreading polymerase optimized for amplification of long DNA templates. 20 kb and up to 48 kb is possible



Common PCR additives

BSA (usually at 0.1 to 0.8 µg/µL final concentration)

Stabilize *Taq* polymerase & overcome PCR inhibitors

DMSO (usually at 2-5% v/v, inhibitory at ≤ 10% v/v)

Denaturant - good at keeping GC rich template/primer strands from forming secondary structures.

Glycerol (usually at 5-10% v/v)

Increases apparent concentration of primer/template mix, and often increases PCR efficiency at high temperatures.

Stringency enhancers (Formamide, Betaine, TMAC)

Concentrations used vary by type

Enhances yield and reduces non-specific priming

Non-ionic detergents (Triton X, Tween 20) (0.1–1%)

Stabilize *Taq* polymerase & suppress formation of 2º structure

Common PCR inhibitors

SDS (denature polymerase. 0.01% SDS cuts *Taq* activity to ~10% of normal)

Ca (calcium ions inhibit PCR by competing with the magnesium ions as a cofactor for the DNA polymerase).

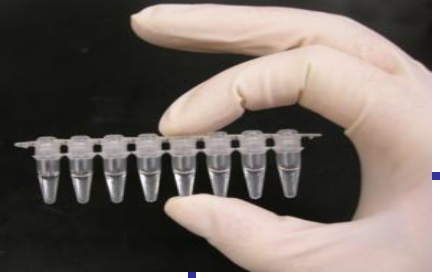
EDTA (chelating the Mg2+ necessary for the activity of DNA polymerase. Therefore, use of an increased magnesium ion concentration has been employed to maintain PCR activity in the presence of chelating agents.)

Blood (PCR inhibitors originating from the starting material include heparin (>0.15mg/mL), proteins such as hemoglobin and lactoferin (>1mg/mL), immunoglobulin

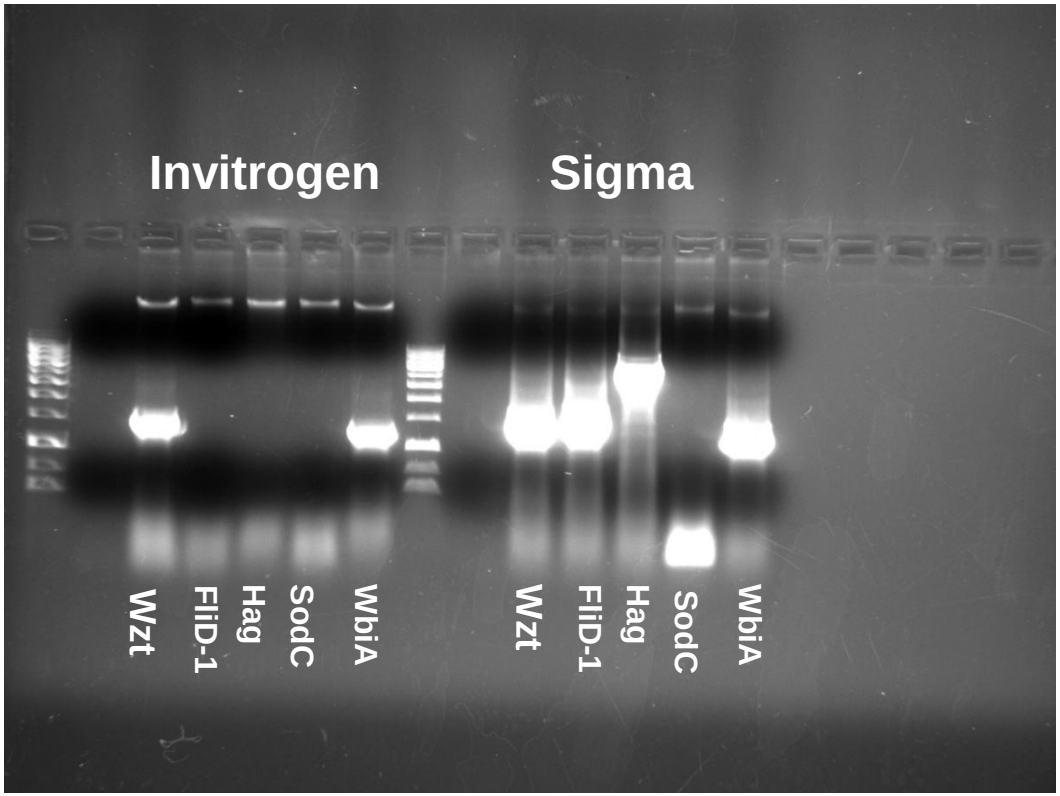
polysaccharides, chlorophylls, melanin, humic acids, etc. Contaminants from the nucleic acid extraction phase include SDS (>0.01% w/v), phenol (>0.2% w/v), ethanol (>1%), proteinase K, guanidinium, and sodium acetate (>5mM).

BSA, provides some resistance to inhibitors

Diluting your sample can solve the problem



Supermix Comparison-PCR



Courtesy of Bettina Heid, Virginia Tech

Questions

If we knew what it was we were doing, it would not be called research, would it?"
- Albert Einstein