Using PCR to analyse DNA samples in humans and ticks

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Overview :

PCR (polymerase chain reaction) is a method of DNA amplification in order to study the presence of the PTC taster allele in humans and to test DNA samples taken from ticks in an attempt to identify ticks carrying *Borrelia burgdorferi* spirochaetes.

Aims

- To use PCR to carry out a DNA assay of the PTC allele in humans.
- To use PCR as a method of amplifying tick and *Borrelia sp.* DNA to be used in gel electrophoresis.
- To gain an understanding of the process of PCR and to communicate this knowledge.

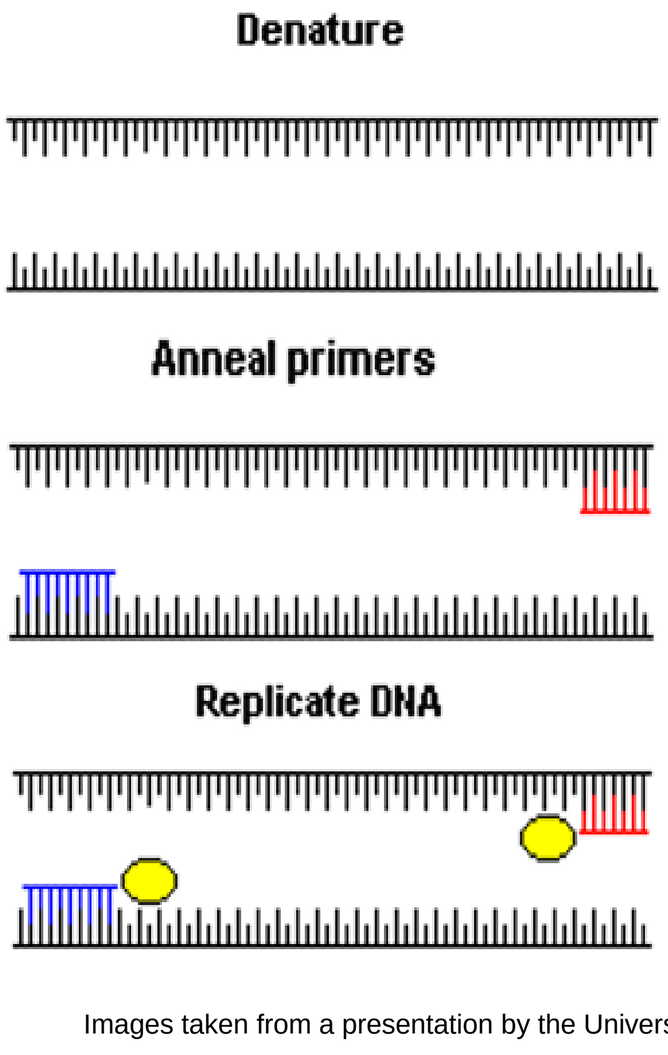
Techniques

- DNA extraction from humans and ticks.
- Use of restriction endonuclease enzymes.
- Carrying out PCR on human and tick DNA.
- Making and running electrophoresis gels.

Outreach

We have had several outreach opportunities. In December, we visited our partners at the University of Exeter, where we were able to participate in a PCR workshop to investigate the presence of the *Alu* genetic marker in our own DNA. We also were involved in a family science festival organised by the school and were able to discuss our work with members of the local community.

Fig. 1. Theory of PCR



Images taken from a presentation by the University of Exeter

PCR

Polymerase Chain Reaction is the process of using a primer complementary to a specific sequence of bases on DNA, along with a DNA polymerase enzyme – we used Taq polymerase in our experiments – to quickly produce large numbers of replicated DNA. In each cycle of PCR, the DNA is first heated to cause the unravelling of the strands. Then, once cooled to about 50ºC, the primers anneal to their complementary sequences. The sample is heated to 72ºC, the optimal temperature of the polymerase enzyme which then binds more nucleotides to these primers, replicating the DNA on each strand and therefore producing two copies of each original strand.
This means the quantity of DNA doubles with every cycle; after 35 cycles, this produces 34 billion copies of DNA from every one that was originally present. Running a set of DNA lasts about 3 hours with 35 cycles.

Conclusions

PCR was an invaluable scientific tool in the process of identifying PTC alleles in humans and *Borrelia* DNA in ticks.

References

The *Borrelia* primer sequences were taken from Hansford *et al*, '*Borrelia miyamotoi in host-seeking Ixodes ricinus ticks in England', Epidemiol. Infect.* (2015).

Method

The following method was used to carry out PCR on our different DNA samples.

- DNA was extracted from humans (in the PTC assay) or from ticks (when testing for tick and *Borrelia burgdorferi* DNA).
- Using an Eppendorf tube for each sample that we were testing the DNA sample being tested, along with a specific primer for the DNA we were testing (see references), Taq polymerase enzyme, and distilled water were placed.
- The proportions varied depending on the experiment and the primer in question.
- All prepared samples underwent 35 cycles of PCR as detailed below.

Fig. 2. Sample Reagents

Component	PTC assay volume /µl	Tick DNA volume /µl
DNA sample	1.25	2.5
GoTaq	6.25	6.5
Primer	1.25	2.5
Distilled Water	3.75	1.25

Results

We were able to successfully use PCR in all three of our original aims, to identify PTC alleles, to identify tick DNA and to test ticks for the presence of DNA from *Borrelia* spirochaetes.
The results to the right are the finished electrophoresis gels, with the DNA visible via the fluorescent Safe Blue dye.

Fig. 3: PTC assay (D="digested with restriction enzyme", U=not)
F**ig 4: Tick primer** showing there has been DNA extracted from the tick and that PCR is working.
Fig 5: *Borrelia* primer. Lane 2 shows a positive control. All tick samples were **negative** for the presence of *Borrelia* DNA.

Fig. 3

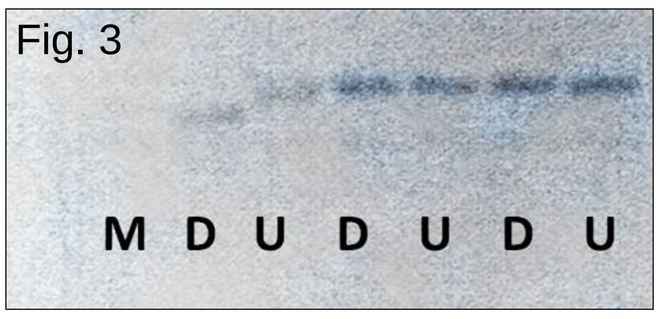


Fig. 4

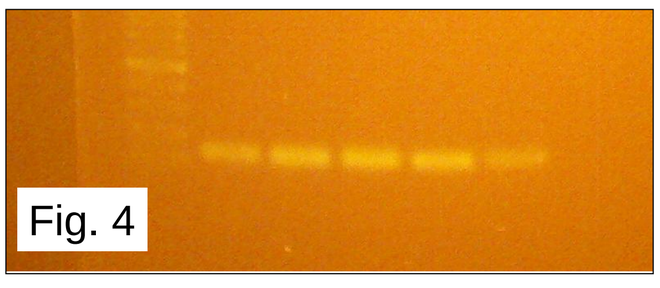
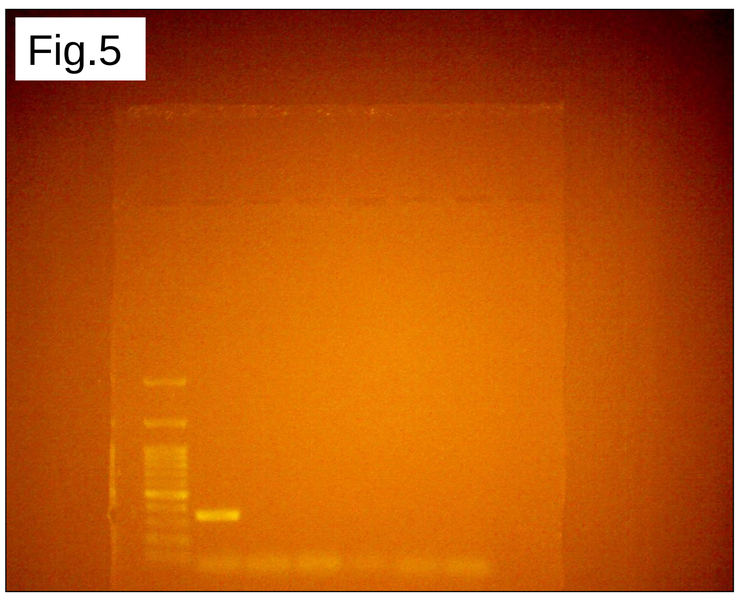


Fig.5



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