Different types of PCR
- **Standard PCR, DNA**
- **RT-PCR, RNA**
- **Real-Time PCR – RTQ-PCR (DNA or RNA)**
Standard-PCR
Nested-PCR

Human T Cell Lymphotrophic Virus Type 2
Multiplex-PCR assays for identification and epidemiological typing of *Pasteurella multocida* isolates

**Figures:** Agarose gel electrophoresis of multiplex PCR products with *Pasteurella multocida* strains carrying various combinations of virulence determinants. (1) *capA* = capsule type A; *toxA* = demonecrotxin; *capD* = capsule type D; *kmt* = *P. m.* specific (2) *exbB/tonB* = iron acquisition system; *oma87* = outer membrane protein; *hgbA/B* = hemoglobin binding proteins; *nanB/H* = neuraminidases; *ptfA* = type 4 fimbriae; *pfhaB* = *Pasteurella* filamentous hemagglutinin
DNA is cut and amplified using short single primers at low annealing temperatures, resulting in amplification at multiple loci. RAPDs lack specificity, due to low annealing temperatures and easier reaction conditions.
Long PCR used if the DNA amplification up to 27 kb fragments.

The method relies on a mixture of:
- Thermostable DNA polymerases (*Taq* DNA)
- Proofreading enzymes (e.g. *Pfu*)
PCR-RFLP analysis and automated sequencing of *MTHFR* C667T. *MTHFR* was restricted by *Hinfl*. Digestion resulted in a 400-bp fragment for the C allele, and 318 and 82 bp fragments for the T allele.
Amplified fragment length polymorphism (AFLP)

DNA is cut with two restriction enzymes to generate specific sequences, which are then amplified suitably. The mere addition or deletion of bases at the 3’ end determines the selectivity and complexity of the amplification.

<table>
<thead>
<tr>
<th>DNA sequence with EcoRI and MseI recognition sequences</th>
<th>DNA restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>//----GAATTC---//----TTAA---//</td>
<td>DNA restriction</td>
</tr>
<tr>
<td>//----CTTAAG--//--AATT---//</td>
<td>DNA restriction</td>
</tr>
<tr>
<td>EcoRI MseI</td>
<td>DNA restriction</td>
</tr>
<tr>
<td>---G AATTC---//--T TAA----//</td>
<td>DNA restriction</td>
</tr>
<tr>
<td>---CTTAA G---//--AAT T</td>
<td>DNA restriction</td>
</tr>
<tr>
<td>nnnA AATTC---//--T TACnnnn</td>
<td>DNA restriction</td>
</tr>
<tr>
<td>nnnTTTAA G//--//--AAT Gnnn</td>
<td>DNA restriction</td>
</tr>
</tbody>
</table>

Addition of adaptors. As nucleotides in red are different to the original ones (blue), restriction sites are not reconstructed.
In RT-PCR, reverse transcriptase (RT) is used to copy all of the mRNAs in an RNA sample into cDNA. This single stranded cDNA can then be amplified by PCR using primers that anneal to a specific cDNA (vis. mRNA).
Types of RT-PCR

• One step RT-PCR
• Two steps RT and PCR
Detection and the quantification of a fluorescent transmitter during the process of amplification.
1- Dye binding to the double-stranded-DNA (SybrGreen I).

2- Fluorescent probes (FAM, TAMRA, JOE, ROX,)
* TaqMan® analysis (Hydrolysis probes)
Correlation Coefficient: 0.999  Slope: -3.488  Intercept: 39.204  \( Y = -3.488 \times X + 39.204 \)
PCR Efficiency: 93.5 %

PCR Standard Curve: Data 27-Jan-03 12331eff.opd
PCR Applications
Forensic
Diagnosis
Studying evolution
Fossils
Gene cloning & expression

1a. F' plasmid ligates with itself

Electroporated into cells

1b. Colonies turn blue; LacZ Gene unbroken

2a. Gene of interest ligates with itself

Electroporated into cells

2b. Cells die; no antibiotic resistance
HBs Ag : surface antigen
HBc Ag : core antigen
Thrombin inhibitor effectively treats hematoma and inflammation (oedema, redness, hotness).
Creating mutation

Random or specific

ACCATCGG CCTG CATCA
TGGTAG CCTGACGT AGTC ATGCTGACCTGACT
PCR is just a tool. How to use it, is up to you.
Thank You!