Porcine circovirus infection

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SUMMARY

There are two porcine circoviruses. Porcine circovirus type 1 (PCV1) has not been associated with disease in pigs and was first reported as a persistent contaminant in PK15 cell lines. Porcine circovirus type 2 (PCV2) is considered by many virologists to be the aetiological agent of postweaning multisystemic wasting syndrome (PMWS). Both are members of the family Circoviridae, which are small (17 nm in diameter), non-enveloped DNA viruses with a 1767-1768 nucleotide circular viral genome.

Identification of the agent: Virus is usually identified using the polymerase chain reaction (PCR). Virus may be isolated in various cell cultures but does not produce a cytopathic effect (CPE) in cell culture. In tissues from pigs affected with PMWS, virus may be identified using immuno-histochemistry (IHC) or in-situ hybridisation (ISH)

Serological tests: Several serological tests to detect antibody, such as the enzyme-linked immunosorbent assay (ELISA) and cell culture-based immunofluorescence or immuno-peroxidase assays have been developed, but are little used due to the ubiquitous nature of PCV viral infections in pig herds.

Status of Australia and New Zealand: Both PCV1 and PCV2 are endemic in Australia and New Zealand and are widely distributed.[1,2] Serological evidence of the presence of PCV in New Zealand was first reported in 1991[3] and in Australia in 2000.[4] A serological survey was carried out in Australia in 2001, which showed the national prevalence of infection of PCV2 was 34.5%.[5] Disease due to these viruses, especially PCV2, is less well defined. Recently (October 2003) PMWS was diagnosed in a pig herd at

Waikato, New Zealand, and subsequently has been confirmed, or is suspected, on 16 farms (up to September 2004). Depopulation of these farms is being proposed by the industry but is not supported by the Ministry of Agriculture and Forestry, who consider eradication is impracticable.

In Australia PMWS has not been diagnosed, although other disease syndromes that may be associated with PCV2, such as porcine dermatopathy and nephritis syndrome (PDNS) and congenital tremor, have been reported.[1] The role of PCV2 in these syndromes remains controversial. It is possible that the status of PCV2-associated disease may change significantly in both countries over the next few years as greater knowledge about the role of the virus in these disease syndromes becomes available.

Diagnostic criteria for PMWS: For a diagnosis of PMWS to be made in Australia, certain criteria should be satisfied. These include the presence of suggestive clinical signs, especially wasting in conjunction with pneumonia or icterus, suggestive histological lesions and the demonstration of an abundance of PCV2 antigen in lesions using IHC or ISH.

INTRODUCTION

Aetiology

Porcine circoviruses are members of the family Circoviridae, genus Circovirus. They are small (17 nm diameter) non-enveloped DNA viruses with a unique single-stranded circular genome of 1767-68 nucleotides.[6] There are two porcine circoviruses, PCV1 and PCV2. PCV1 was first isolated as a contaminant of PK15 cells (ATCC CCL-33) and later of other pig cell lines. It has not been associated with any disease in pigs. In contrast, PCV2 has been associated with PMWS and several other disease syndromes in pigs.[6] Analysis of the full genome of PCV2 strains from PMWS cases and from sub-clinically infected animals has not detected any virulence factors that would account for a difference in pathogenicity.[7]

Epidemiology

PCV2 has been closely associated with PMWS and is considered the cause by many workers. Other co-factors appear to be needed to produce severe disease but some experimental infections have produced PMWS, albeit usually less severe than the natural disease. Immune system involvement appears likely.[6] PCV2 may also be implicated in other disease syndromes. These include porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC), proliferative and necrotising pneumonia (PNP), reproductive disorders and congenital tremors[6].

Weaner pigs (2-4-month-old) are most commonly affected with PMWS. Often other bacterial and viral infections are found on PMWS farms.[6]

Serological studies have shown that most pig herds have been infected with PCV2.

The virus is believed to be spread by several routes such as oro-nasal secretions, faeces, urine and semen. Wind-borne spread has also been suggested.

Clinical signs

Signs of PMWS include wasting, unthriftiness, skin pallor, respiratory distress, diarrhoea and occasional icterus. Lymph nodes may be enlarged. The clinical criteria used in New Zealand are:

> Non-responsive wasting in 6-12-week-old pigs, with many deaths. Mortality rates in this age group at least 15% soon after introduction of infection. This rate decreases in the recovery phase but is still twice that expected for the farm.

Gross pathology

The most striking gross lesions of PMWS are non-collapsed lungs, often with interstitial oedema, and enlarged lymph nodes, especially the superficial inguinal, submandibular, mesenteric and mediastinal nodes. Some animals have atrophic, discoloured livers and multifocal white foci in the renal cortex.[6]

Diagnostic Tests

A range of tests has been used to detect the viruses in tissues or in infected cell cultures. These include PCRs that differentiate between PCV1 and PCV2, immunohistochemistry (IHC) and in-situ hybridisation (ISH) to demonstrate the presence of viral antigen or nucleic acid in cells. Histopathological changes present in cases of PMWS are characteristic. Serological tests such as the immunoperoxidase monolayer

assay (IMPA) and various ELISAs been developed and used but are of little diagnostic value due to the high seroprevalence rate in pig farms and the cross-reactions between PCV1 and PCV2.

Histopathology

The preferred tissues to sample in suspected cases of PMWS are, in order of frequency of lesions: lymphoid tissues, lung, liver, kidney and colon.

The most characteristic lesions are found in the lymphoid tissues and are lymphoid depletion, infiltration of histiocytes, formation of syncytia (giant cells) and presence of botryoid cytoplasmic inclusions in histiocytic cells.

The usual lung lesion is a subacute interstitial pneumonia, sometimes with histiocytic and multinucleate giant cells present in thickened alveolar walls.

The histopathological criteria used in New Zealand with a significance ranking are:

In lymphoid tissue:

- depletion of lymphoid cells (a non-specific change),
- loss of cortico-medullary architecture (a suspicious change),
- infiltration of histiocytes into follicular and paracortical regions (a suspicious change),
- presence of multinucleated histiocytes (a suspicious change), and
- presence of botryoid inclusion bodies in areas of lymphocyte depletion/histiocyte infiltration (consistent with PMWS).

In other tissues signs are of secondary importance but contribute to the level of suspicion raised by assessment of the lymphoid lesions:

- granulomatous interstitial pneumonia,
- histiocytic infiltration and hepatocellular apoptosis in the liver, and
- granulomatous enteritis.

Viral antigen can be detected in specific cells by using either IHC or ISH.[9, 10, 11]

The criterion used in New Zealand is the demonstration of abundant PCV2 antigen within characteristic histological lesions.

Immunohistochemistry (IHC)

This test can be applied to standard paraffin-embedded tissues. Tissues can be stored in 10% neutral buffered formalin for up to 6 months but a prolonged time in fixative may influence results. McNeilly et al [9] and Rosell et al [10] have described the following technique: Sections (5 um) of tissue samples are cut, placed on slides that have been treated with 3-amino-propyltriethoxysilane, baked at 60°C for 30 min, dewaxed in xylene and rehydrated through graded alcohols. Endogenous peroxidase is blocked by treating sections with 0.5% H_2O_2 in methanol for 20 min. Sections are washed in distilled water then PBS or TBS. Antigen retrieval is achieved by treatment with a solution of either protease XIV or K at a concentration of 0.5 mg/mL at 37°C for 30 min. Following digestion the sections are washed in tap water and may be processed by either IHC or ISH.

For IHC a polyclonal rabbit antiserum[12] is applied at an optimal dilution in phosphate-buffered saline (PBS, pH 7.2) to tissues at 37°C for 1 h. In some laboratories monoclonal antibodies are used in the place of the polyclonal antibodies. The sections are then washed in PBS for 5 min and a biotinylated anti-rabbit IgG applied at 37°C for 30 min. The sections are then washed in PBS and a streptavidin

peroxidase conjugate applied at room temperature for 10 min. After a further 5 min wash in PBS, incubate the sections with the enzyme substrate diaminobenzidine tetrahydrochloride (or 3-amino 9-ethyl-carbazole-hydrogen peroxide substrate) at room temperature for 5 min. Then wash in running tap water and counterstain with haematoxylin at room temperature for 1 min. The tissue sections are then cleared through graded alcohols and xylene and mounted with a permanent mountant. Control tissues that are needed are known positive and negative tissues and the substitution of the primary antiserum with a negative rabbit antiserum on selected tissues.

Indirect immunofluorescence on acetone-fixed cryostat sections has also been used to detect PCV2 antigen using a PCV2-specific monoclonal antibody.

In-situ Hybridisation (ISH)

There is no standardised method but published methods involve hybridisation with specific PCV1 or 2 digoxigenin or biotinylated labelled DNA probes. [9, 10, 11] An anti-digoxigenin antibody conjugated to alkaline phosphatase is used to detect hybridisation. For biotinylated probes streptavidin conjugated with alkaline phosphatase or peroxidase is used followed by a suitable substrate.

Virus detection

Virus isolation can be carried out in various pig cell lines such as ST (swine testes ex ECACC) and PK13. Most PK13 cells are contaminated with PCV1 and thus should not be used for PCV2 isolation unless they have been tested and shown to be free of PCV1. A clone of PK15 cells free of PCV1 is available and is preferred by some laboratories.

Porcine circoviruses are not usually cytopathic and must be detected by other methods, usually by PCR, immunofluorescence or immunoperoxidase methods.

Polymerase chain reaction (PCR)

The PCR assay is widely used to detect viral DNA directly in tissues or in cell cultures. By using different specific primers PCV1 and PCV2 can be differentiated.[1, 2, 6, 7, 8] Increasingly, viral loads are being measured using the quantitative PCR. Sequencing of PCR fragments or the whole genome has been used to compare virus isolates from different countries and to compare PCV2 isolates from PMWS cases with those from subclinical infections. No genomic differences that could explain differences in virulence or differences in the disease expression seen in different countries have been detected.[7]

As PCV DNA can be found in the tissues of healthy pigs, a positive reaction cannot be used to indicate a diagnosis of PMWS.

A PCR assay for PCV1 and PCV2 is described in more detail in the appendix.

Serological assays

Although a number of serological assays have been developed to measure antibodies to PCV1 and PCV2, such as ELISAs [5,6,13], the immunoperoxidase monolayer assay (IMPA)[6,8] and the indirect immunofluorescence test [1,3], they are mainly used for research purposes. This is because the viruses are ubiquitous with an almost 100% herd seroprevalence and seroconversion does not differ to a significant extent between PMWS affected and non-affected farms. Also, there are often cross-reactions between PCV1 and PCV2. Negative results to serological assays may be of benefit in excluding PCV2 infections from syndromes such as foetal infections.

References

1. Buddle JR, Muhling J, Raye W, Raidal SR, Wilcox GE. Porcine circovirus in Australia. *Proceedings of Australian Association of Pig Veterinarians 2003-* Cairns Conference, 2003:67-77.
2. Tham K-M, Hansen M. Detection of porcine circovirus types 1 and 2 in abattoir-slaughtered pigs in New Zealand. *Surveillance* 2003; 30(1): 3-5.
3. Horner GW. Pig circovirus antibodies present in New Zealand pigs. *Surveillance* 1991; 18(5): 23.
4. Buddle JR, Warfe L, Raye W, Wilcox GE. Serological evidence for the occurrence of porcine circovirus in the Australian pig herd. *Proceedings of Australian Association of Pig Veterinarians*, Perth Conference, 2000: 47-48.
5. Buddle JR, Raye W, Palmer C et al. Status of Australian research on circoviruses. *Proceedings of the Australian Association of Pig Veterinarians*, Canberra Conference, 2004:39-44.
6. Segales J, Domingo M. Postweaning multisystemic wasting syndrome (PMWS) in pigs. A review. *Vet Quart* 2002; 24: 109-124.
7. de Boisseson C, Beven V, Bigarre L et al. A. Characterization and comparison of porcine circovirus sequences from post-weaning multisystemic wasting syndrome affected and non affected herds. *Proceedings of the 6th International Congress of Veterinary Virology*, St Malo, 2003:92.
8. Allan G, McNeilly F, Meehan B et al. Reproduction of postweaning multisystemic wasting syndrome in pigs experimentally inoculated with a Swedish porcine circovirus 2 isolate. *J Vet Diagn Invest* 2003; 15: 553-560.
9. McNeilly F, Kennedy S, Moffet D et al. A comparison of in situ hybridization and immunohisto-chemistry for the detection of a new porcine circovirus in formalin-fixed tissues from pigs with post-weaning multisystemic wasting syndrome (PMWS). *J Virol Methods* 1999; 80: 123-128.
10. Rosell C, Segales J, Plana-Duran J et al. Pathological, immunohisto-chemical, and in-situ hybridization studies of natural cases of postweaning multisystemic wasting syndrome (PMWS) in pigs. *J Comp Path* 1999; 120: 59-78.
11. Kim J, Chae C. Double in situ hybridization for simultaneous detection and differentiation of porcine circovirus 1 and 2 in pigs with postweaning multisystemic wasting syndrome. *Vet J* 2002; 164: 247-253.
12. Ellis J, Hassard L, Clark E et al. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. *Can Vet J* 1998; 39: 44-51.
13. Walker IW, Konoby CA, Jewhurst VA et al. Development and application of a competitive enzyme-linked immunosorbent assay for the detection of serum antibodies to porcine circovirus. *J Vet Diagn Invest* 2000; 11: 400-405.
14. Larochelle R, M. Antaya, M. Morin, R. Magar . Typing of porcine circovirus in clinical specimens by multiplex PCR. *J Virol Methods* 1999;80:69-75.
15. Mankertz A, Domingo M, Folch JM et al. Characterisation of PCV-2 isolates from Spain, Germany and France. *Virus Res* 2000; 66:65-77.

Appendix

Polymerase Chain Reaction assay for PCV1 and PCV2

1. Preparation of sample for DNA extraction

- On receipt of sample, identify each clinical sample and label all tubes and submission forms.
- Store samples at 4°C until ready for DNA extraction.

a) Unclotted blood samples
- Blood is collected in a tube containing an anticoagulant.
- Centrifuge the tube at 4 °C at 1500 *g* for 20 min.
- Draw off the buffy coat with a sterile Pasteur pipette and place in a sterile 1.5 mL tube.
- Add 10 mL of ice-cold 0.17 M NH_4Cl and shake to suspend cells. Stand at room temperature for 10 min to lyse any red blood cells.
- Centrifuge at 4°C at 1500 *g* for 10 min.
- Discard supernatant and add 15 mL ice-cold PBS and mix.
- Centrifuge at 4°C at 800 *g* for 10 min.
- Discard supernatant and resuspend cell pellet in 1 mL PBS.

b) Clotted blood
- Take about 1 cm^3 clot and break up manually in a sterile petri dish with forceps and scissors.
- Place the homogenate in a sterile 15 mL tube and add an equal volume of Tris EDTA (TE) buffer containing 2% v/v Tween-20.
- Shake vigorously to homogenise the clot.
- Stand at room temperature for at least 30 min and shake again.
  NB: Sterile sand will aid the homogenisation.

For DNA Extraction: Dispense 200 uL into a sterile, labelled 1.5 mL tube and proceed, using a minicolumn DNA extraction kit.
The remaining homogenate can be stored for future extractions if required.

c) Serum, plasma and other body fluids
Serum and other body fluids often contain very low numbers of cells, bacteria or viruses. One option is that samples can be concentrated from volumes of up to 3.5 mL to a final volume of 200 uL using a vacuum concentrator. Centrifugation of up to 6 h may be necessary.
- Dispense 200 uL of serum into a sterile, labelled 1.5 mL tube.
- Alternatively, serum may be ultracentrifuged as follows: Transfer 5 - 6 mL serum to an ultraclear ultracentrifuge tube. Add sterile deionised water until the volume is 2 - 3 mm from the top of the tube. Cap with parafilm and mix by inversion. Prepare negative controls (5 mL negative serum and 6 mL sterile deionised water) and positive control (5.9 mL sterile deionised water + 100 uL sonicated high titre

positive sample). Balance pairs of tubes to within 0.002 g of each other. Centrifuge in a swing-out head at 110,000 *g* for 2 h. Transfer the supernatant to a 10 mL disposable tube (and store at 4^oC), leaving 300 - 500 uL with the pellet. Digest the pellet and remainder of supernatant in the ultraclear tube with Proteinase K as follows: add 0.01M Tris/0.001M EDTA pH 8.0 to make a final volume of about 700 uL. Add 11.6 uL of 6M NaCl, 14 uL of 10% SDS and Proteinase K to a final concentration of 340 - 680 ug/mL, depending on the initial pellet volume. Incubate at 56^oC overnight.

For DNA Extraction: Add three volumes of Digestion Buffer (100 mM NaCl , 10 mM Tris HCl pH 8.0, 25 mM EDTA pH 8.0, 0.5% SDS) and proceed with extraction using Phenol:Chloroform:Isoamyl alcohol (PCIA) method. Alternatively, using the minicolumn DNA extraction kit, add 200 uL of lysis buffer to 200 uL of the serum sample in a sterile 1.5 mL tube and proceed.
The remaining serum can be stored for future extractions if required.

d) Animal tissue samples

- Take a 200 mg to 1 g (up to about 1 cm^3) section of tissue using a sterile scalpel (or as much as is available). The remaining tissue is stored at -70^oC for future extractions.
- Mince the tissue as fine as possible using either sterile scissors or a scalpel in a petri dish, a mortar and pestle, a glass grinder or a microfuge grinder (maximum 25 to 30 mg of tissue).
  NB: Sterile sand will aid in homogenisation.

For DNA extraction: Place homogenate in a sterile tube and add 1.2 ml of Digestion Buffer per 100 mg of tissue and proceed with an extraction using the PCIA method. Alternatively, place 25 to 50 mg of the tissue homogenate in a sterile microfuge tube and proceed with an extraction using a PCR Template Purification Kit.

e) Embedded tissue samples

- Tissue samples are fixed in 10% formalin then embedded in paraffin. From this, 10 x 5 um slices are taken and place into a sterile polypropylene microfuge tube (xylene degrades polystyrene tubes).
- Add 1 mL xylene to 1.5 mL microfuge tube, shake lightly and stand at room temperature for 30 min.
- Centrifuge at 18,000 *g* for 5 min in a microcentrifuge.
- Pour off xylene into the xylene waste discard in a fume cupboard.
- Add 1 mL xylene, shake lightly and stand at room temperature for 30 min.
- Centrifuge at 18,000 *g* for 5 min in a microcentrifuge.
- Pour off xylene into xylene waste discard.
- Add 1 mL absolute ethanol and shake lightly to mix.
- Centrifuge 18,000 *g* for 5 min and discard the supernatant.
- Add 500 uL of 95% ethanol and shake lightly to mix.
- Centrifuge 18,000 *g* for 5 min.
- Pour off ethanol and dry in air or using a vacuum concentrator, thus leaving dry tissue pellet.

For DNA Extraction: Suspend the pellet in 200 uL Tissue Lysis Buffer and proceed with an extraction using a PCR Template Purification Kit.

f) Swabs

- Add 1 mL of TE containing 2% v/v Tween-20 to the bottle containing the swab.
- Shake vigorously.
- Stand at room temperature for at least 30 min and shake again.
- Squeeze all liquid from swab using sterile forceps and discard the swab.

For DNA Extraction: Dispense 200 uL into a microfuge tube, and add equal volume of Tissue Lysis Buffer. Proceed with an extraction using a PCR Template Purification Kit. Alternatively, using a minicolumn DNA extraction kit, add 200 uL of lysis buffer and proceed with extraction according to the kit instructions.
The remaining suspension is stored for future extractions.

g) Tissue culture

- Scrape the cells off the tissue culture flask with a cell scraper and pour the contents into a sterile centrifuge tube.
- Centrifuge at 4°C at 1500 *g* for 10 min. Pour off and discard the supernatant.

For DNA Extraction: Resuspend the cell pellet in 500 uL sterile PBS and remove 200 uL cell suspension for DNA extraction. Add 200 uL Tissue Lysis Buffer or 200 uL tissue lysis buffer and proceed with an extraction using a PCR Template Purification Kit or a minicolumn DNA extraction kit. The remaining cell suspension is stored at -70°C for future extractions.

2. DNA extraction

DNA is extracted from clinical specimens using either a minicolumn DNA extraction kit or a PCR Template Purification Kit.
Swine testis (ST) cells free of PCV are extracted as a negative control.
Ensure all tubes are clearly identified with a sample number and date of extraction.

Make a note of the DNA yield of each sample extracted using a spectrophotometer.

3. Polymerase Chain Reaction

Primers for PCV1, PCV2 or PCV1/PCV2 are reconstituted to 1 ug/uL with TE buffer (from which aliquots of 200 ng/uL are dispensed and stored at -20°C).
Record details of the PCR assay and identify each reaction tube with a PCR and tube number.
Include the negative controls ST cell DNA and water blank (sterile distilled water in place of templates) in each PCR assay.
Include two strong and weak positive controls (PK15 and/or pc127 DNAs for PCV1 and PCV2, respectively) in each PCR assay.

PCV1 DNA is prepared from PCV1-contaminated PK15 cells. PCV2 DNA is prepared from a field isolate obtained from the tonsil of an abattoir-slaughtered healthy pig and cultured in PCV-free ST cells.

PK15	strong positive = 10^{-5} dilution weak positive = 10^{-6} dilution
pc127	strong positive = 10^{-1} dilution weak positive = 10^{-2} dilution

Amplification Reaction

- For the standard PCV PCR amplification using PCV1 and PCV2 or PCV1-specific or PCV2-specific primers see below:

MASTERMIX FOR PCR AMPLIFICATION

REAGENT	VOLUME per reaction	FINAL CONCENTRATION per reaction
Sterile distilled water	31.5 uL	
10x High Buffer *	5.0	1x
2 mM dNTPs	5.0	200 uM
25 mM MgCl2	1.0	2 mM
200 ng primer, forward	1.0	250 ng
200 ng primer, reverse	1.0	250 ng
5 U/uL Taq Polymerase	0.5 uL	2.5 Units

* Supplied with Taq Polymerase

- Prepare a master-mix for n + 1 tubes, where n = number of diagnostic and control samples.
- Dispense 45 uL of the master-mix into each 0.5 mL thin-walled PCR tube.

  Add 5 uL of template DNA to the appropriate tube (5 uL of sterile distilled water for the negative blank). Total volume = 50 uL.

Cycling Conditions are shown below.

PRIMERS *	SEQUENCE +	NUCLEOTIDE LOCATION
F41	5' - ATACGGTAGTATTGGAAAGGTAGGG	441 - 465
B42	5' - ACACTCGATAAGTATGTGGCCTTCT	1129 - 1106
PF2	5' - TTGCTGAGCCTAGCGACACC	597 - 616
PR2	5' - TCCACTGCTTCAAATCGGCC	945 - 926
CF8	5' - TAGGTTAGGGCTGTGGCCTT	1323 - 1342
CR8	5' - CCGCACCTTCGGATATACTG	1586 - 1567
F66	5' - GGTTTGTAGCCTCAGCCAAAGC	172 - 193
B67	5' - GCACCTTCGGATATACTGTCAAG	587- 564
MCV1	5' - GCTGAACTTTTGAAAGTGAGCGGG	508 - 531
MCV2	5' - TCACACAGTCTCAGTAGATCATCCCA	749 - 724
CFS	5' - AACTGCTGTCCCAGCTGTAG	844 - 864
CRS	5' - AGGAGGCGTTACCGCAGAAG	1723 - 1704

Program (primers): PCV L1 (PF2/PR2; CF8/CR8; MCV1/MCV2; CFS/CRS)
PCV L3 (F41/B42; F66/B67)

Cycling Conditions:	PCV L1	PCV L3	
	95°C for 1 min	1 min	
Denaturation:	95°C for 1 min	95°C for 1 min	x 30 cycles
Annealing:	65°C for 1 min	55°C for 1 min	
Extension:	72°C for 1 min	72°C for 1 min	
	72°C for 5 min	72°C for 5 min	
	4°C Hold	4°C Hold	

* Custom oligonucleotides supplied commercially
\+ Primer sequences are based on the published nucleotide sequences of PCV1 and PCV2. [13,14]

Expected sizes of amplicons for PCV1 and PCV2 PCR

PCV Type	PCR program	Primers	Expected size of PCR amplicons (bp)
PCV1	PCV L3	F41/B42	688
PCV1	PCV L1	PF2/PR2	349
PCV2	PCV L1	CF8/CR8	263
PCV2	PCV L3	F66/B67	416
PCV1/PCV2	PCV L1	MCV1/MCV2	243
PCV1/PCV2	PCV L1	CFS/CRS	894

4 Agarose Gel Electrophoresis

To evaluate a PCR reaction, products are separated by agarose gel electrophoresis and visualised by staining with ethidium bromide and illumination under ultraviolet light.

- Make up a 1.5 % agarose gel in the appropriate electrophoresis tank to run all PCR products and a molecular weight marker.
- Stained gel is examined and photographed using the gel documentation system

5 PCR product sequencing

Preparation of PCR products
PCR products are purified using a PCR purification kit or a gel extraction kit, according to the manufacturer's instructions.

Sequencing primers
Both the forward and reverse primers are diluted to the required concentrations specified by the DNA Sequencing providers, for example, 5 pmol/uL of sequencing primer per sample is required by the Waikato University DNA Sequencing Facility.

Submitting PCR products for sequencing
The purified PCR products and the sequencing primers, diluted to 5 pmol/uL, are labelled and placed in a container.
Fill in the appropriate DNA sequencing submission form.
The Waikato DNA Sequencing Facility has submission form available in the website**:**
<http://www.bio.waikato.ac.nz/sequence>.
In Australia sequencing facilities will vary between States, being available on site or being contracted out.