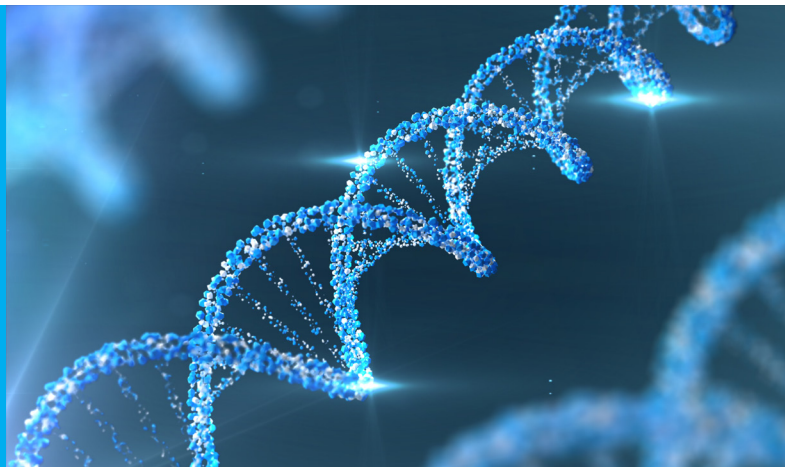
PYROSEQUENCING AT AGRF - FAQ

Single sample / single amplicon pyrosequencing, with a flexible variety of assays and the capacity to re-sequence an amplicon generated in one PCR multiple times



What can the PyroMark do?

1. Single sample / single amplicon Pyrosequencing (24 well format)
2. Flexible variety of assays including:
   - Sequencing short DNA fragments (eg 60-100bp Q-PCR amplicons)
   - Typing of difficult complex variable sites (eg multiple adjacent SNPs)
   - Quantitation of variants in a sample (eg levels of KRAS mutations in a tumour)
   - Quantitative CpG methylation analysis (Sequencing of bisulphite-treated DNA)
3. Re-sequence an amplicon generated in one PCR multiple times. Although the effective sequence length of pyrosequencing is up to 100bp, a 300-400bp amplicon can be sequenced with multiple internal sequencing primers to give full coverage of the fragment.

What can the PyroMark *not* do?

1. Massively parallel Pyrosequencing. For metagenomic studies, enquire about our Next Generation Sequencing service.
2. Sequence long reads. The effective length of a pyrosequence is up to 100bp. To screen long sections of DNA for methylation differences (eg 2-4kb) enquire about our AGENA Epityper service.

How does the PyroMark work?

The PyroMark runs single well pyrosequencing reactions on single stranded DNA templates that have been generated from a biotin-labelled double stranded amplicon. For efficient and effective pyrosequencing, one PCR primer needs to be labelled with 5'-biotin.This label is used to immobilise the amplicon on sepaharose-streptavidin beads allowing it to be treated to remove the unlabelled strand. The single stranded template is then annealed to a shorter internal sequencing primer and the sequence determined by sequentially adding nucleotides to the pyrosequencing reaction. An enzyme cascade in the pyrosequencing reaction involving luciferase generates light in proportion to the nucleotides used to synthesise the DNA in the reaction.

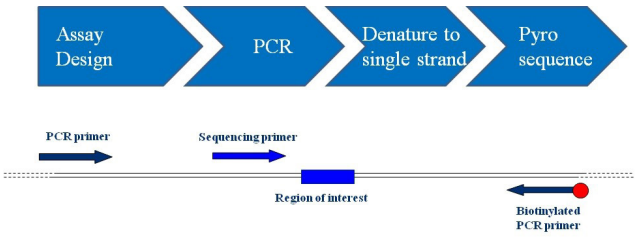


Figure 1. PyroMark Flowchart

What services does AGRF provide for Pyrosequencing?

1. Assay design, ordering and validation
2. DNA amplification for internally designed or client-provided assays
3. Bisulphite conversion of DNA (methylation quantitation projects)
4. Pyrosequencing (internally generated or client generated amplicons)
5. PCR bias test (optional, human and mouse controls available at this time for CpG methylation)

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How much does Pyrosequencing cost?

Pricing depends on two major factors:

1. Entry point to the service
2. Size of project

The below rates are for small scale and bulk projects. All projects awill require a quotation and there is no lower limit on project size.

- Assay design: Free
- Assay primer purchase and validation: $186/assay
- PCR bias test: $400/assay
- Bisulphite treatment: $31/sample (<95 samples)
- $12/sample (95 -189 samples)
- $7/sample (multiples of 189 samples)
- PCR amplification: $11/sample (<92 samples)
- $6/sample (>92 samples)
- Pyrosequencing: $15/sample (<92 samples)
- $8/sample (>92 samples)

How long does a Pyrosequencing project take?

Similar to the price, the time taken to complete a Pyrosequencing project is determined by the entry point to the service and the size of the project.

Some estimated turn-around times are:

- Design: 1-7 days (depending on complexity and number of assays)
- Primer purchase: 10 days
- Assay Validation: 3 days to 2 weeks (depending on complexity and number of assays)
- Bisulphite conversion: 2 days/1-378 samples.
- Amplification and Pyrosequencing: present maximum processing capacity of 276 assays/day (1380/week)

- A small methylation analysis project (30 samples, 1 assay) would take approximately 18 working days. A standard sized project (100-200 samples, 2-3 assays) would take approximately 4-6 weeks.

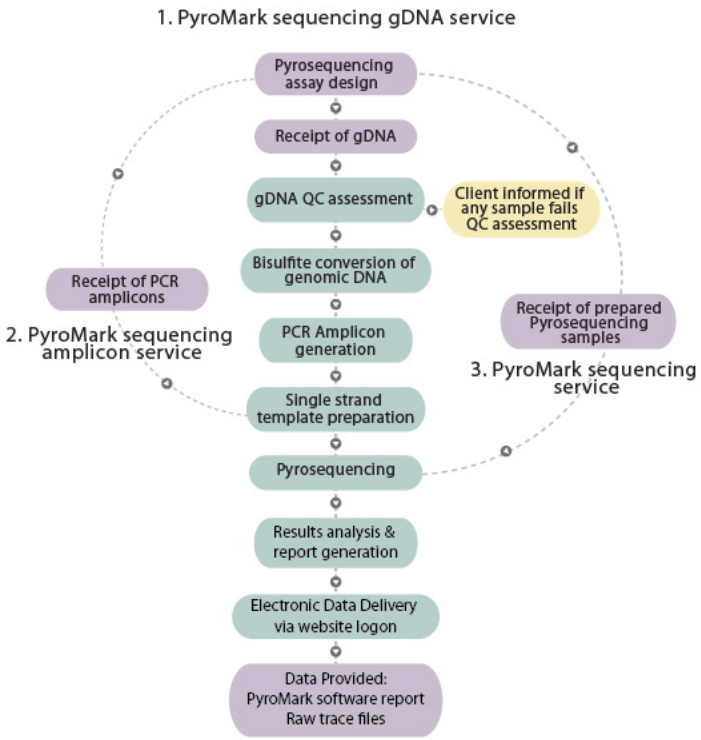

Figure 2. Pyrosequencing flowchart

Our funding partners

AGRF is a not-for-profit organisation supported by the Commonwealth Government infrastructure schemes administered through Bioplatforms Australia.

These schemes include NCRIS, EIF, Super Science Initiative CRIS and NCRIS 2.

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