

Extraction of gapdh gene from grass species



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The extraction of the GAPDH gene from grass species, for sequencing, to determine which grasses will be most suitable for the stimulation of hydrocarbon-degradation

Introduction:

Glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) is a house keeping gene for a crucial enzyme which catalyses an important step in glycolysis, found in all phylogeny. This gene can be extracted and isolated from plant gDNA through the use of PCR. Once cloned, the GAPDH gene will be sequenced and ultimately analysed through bioinformatics for further research. The grasses that will be investigated throughout this experiment are as follows.

Cymbopogon Ambiguus, also commonly known as lemon scented grass, mainly grows in NSW, QLD, NT and WA. This grass is a tufted perennial, which has thin dark green coloured leaves, growing 60-100cm tall approximately.

As the common name for this grass states, when this grass is crushed it gives off a distinct lemon smell, allowing it to be easily identified. This grass is also quite a hardy grass, it is very tolerant to wind, drought and frost conditions, allowing it to be grown in a variety of different areas.

Themeda Triandra, known as Kangaroo Grass is native to Australia, found to be growing in every state and territory (Unkown, n. d. Native Seeds). This grass is a tufted perennial, growing up to 1.5 m tall and 0.5 m wide. This particular grass is quite coarse, with a grey/green leaves, which produce a

very distinct red/brown spikelets (Jennifer Liles, 2004) with the head containing the seeds.

Themeda Triandra is considered a good grass for occasional grazing; however it does not tolerate heavy or continuous grazing. Meaning it can be a good indicator for the level of animal activity in a certain grassland area, as if grazed too frequently, the grass has been known to disappear, and not re-grow.

Dicanthemum Carrisium, known as Queensland Blue Grass is a native Australian Grass, grown not only in Queensland, but all parts of Australia. This Grass was originally very popular in Southern Queensland, due to its optimum performance in the weather conditions of the area.

Dicanthemum Carrisium is a perennial, with an upright tufted appearance. Growing from 30-80cm tall, and 10-15cm wide with flat broad leaves, It is quite a soft texture with typically blue/dark green colour, making it quite distinguishable from many other grasses.

Microlaena Stipoides, known as Weeping Grass, is native to Australia and is found in many of the damper regions from Cape York Town to Tasmania (Unknown, n. d. Native Seeds).

Microlaena Stopoides is a slowly spreading perennial, growing to approximately 70cm tall, with weeping seed heads. This is a very tough and tolerant grass, suited to many different pH soil levels <5. 5. And a very high tolerance to drought, frost, salt and soil aluminium, although its growth is most abundant in damp shaded areas.

This grass has a variety of applications, from grazing to domestic lawns.

Once established, *Microlaena Stopoides* has been seen to be one of the best native grasses used for lawns, because of its tolerable nature, especially its tolerance of deep shade.

Pennisetum Alopecuroides, also commonly known as Fountain Grass, is not native to Australia, it is native to Northern and Eastern Africa. But has found its way into Australia, with limited distribution in NSW, but it has naturalised itself in QLD, VIC, SA, WA and NT.

Pennisteam Alopercuroides grows in a mound of foliage, growing to 10mm wide, and up to 150 cm tall. (Jim Brockmeyer, 2014). This grass type contains bottle-brush like seedheads, which are on stems reaching to the tallest point of the plant. Due to these seed heads, the plants seed can be distributed in a variety of directions very easily by wind. Allowing for the plant to spread quickly and easily. It is a very tough and hardy species.

Poa labillardieri, also known as Tussock Grass is native to Australia and found mostly in the south-eastern regions of Australia (Unknown, n. d. Native Seeds). It is a thick perennial grass with bright blue/Green soft weeping foliage, which grows to approximately 45cm tall, and 45cm wide. *Poa labillardieri* is tolerant to being exposed to full sun, but grows best in damp areas.

These Australian Grasses can be used in a variety of ways, some being investigated for their suitability for Bioremediation. Bioremediation is the use of biological organisms to treat soils containing organic chemicals (Government of SA, 2005). This technology is an environmentally sound, and

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cost-effective way of potentially eliminating contaminants in soils. Gaskin (2010) investigated into Australian grasses and their individual ability to stimulate the removal of aliphatic hydrocarbons from a mine site soil (Shayrn E. Gaskin, 2010). In this study three species were further investigated, two which are included in our study, *Cymbopogon Ambiguus* (Lemon Scented Grass), and *Microlaena Stipoides* (Weeping Grass). It was found in this study, that both *C. Ambiguus* and *M. Stipoides* stimulated the hydrocarbon-degradation the most. However the way in which these plants were stimulating this effect was unknown.

The aim of this experiment is to obtain a sequence of GAPDH gene from one of the 6 grass species discussed, and determine their suitability for bioremediation. This will be achieved through using PCR on the extracted gDNA from the grass sample. Once the initial PCR has been done, a second set of primers is used to amplify the PCR products, this technique is called nested PCR. Once the PCR products have been cleaned, using size exclusion column chromatography; separating the DNA from the proteins, primers and nucleotides, a ligation is performed on the DNA, and Bacterial *E. coli* cells will be used for transformation. The transformed colonies will be inoculated into miniprep LB ampicillin to be cultured (Flinders University, 2014), and the Bacteria is grown. Once this is done, the plasmid DNA will be purified, and cut with Not I, allowing for it to be prepared for sequencing, and analysis. Once sequenced it may be possible to see a difference between the grasses GAPDH gene, which may indicate as to which Australian grasses will be effective when it comes to bioremediation.

Words: 978

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