HSLU Hochschule Luzern

Technik & Architektur

Bachelor Thesis Medical Engineering

Establishment of qPCR primers for diagnostics in the intervertebral disc model

Results Primer Testing

Gene	ASPN	COL1A2	LUM	MMP2	PCOLCE
CT values	28.15	22.81	21.59	25.17	25.09
	27.91	22.84	21.53	25.03	24.92
	28.04	22.78	21.56	24.91	24.71
CT average	28.04	22.81	21.56	25.04	24.91

A CT value of less than 29 indicates a strong positive reaction or a good result. CT values between 30 and 37 indicate a positive reaction or an ok result and a CT value above 37 indicates a weak reaction or a bad result.

Since all resulting CT values of the primer testing were lower than 29, this leads to the conclusion that all primers worked as intended.

Results Dilution Series

Genes	ASPN	COL1A2 adjusted	LUM	MMP2	PCOLCE
Efficiency	84.9%	88.7%	96.2%	95.4%	86.1%
R^2 Value	0.932	0.995	0.997	0.987	0.994

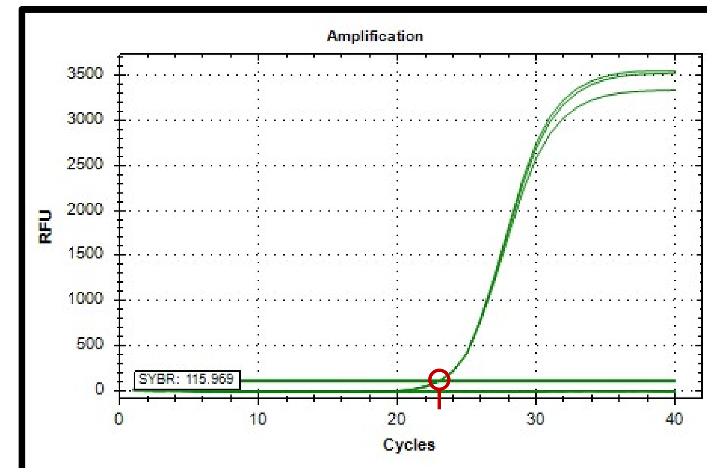
A primer efficiency of 90% - 110% is desired. The genes LUM and MMP2 fulfil this condition. However, the genes ASPN, COL1A2 (adjusted) and PCOLCE do not. They are at efficiencies between 85% and 89%. This is not in the desired range, but it is not far off.

The R^2 value indicates how well the standard curve matches the measured datapoints. An R^2 greater than 0.985 is desirable. All primers except for those of the gene ASPN meet this requirement.

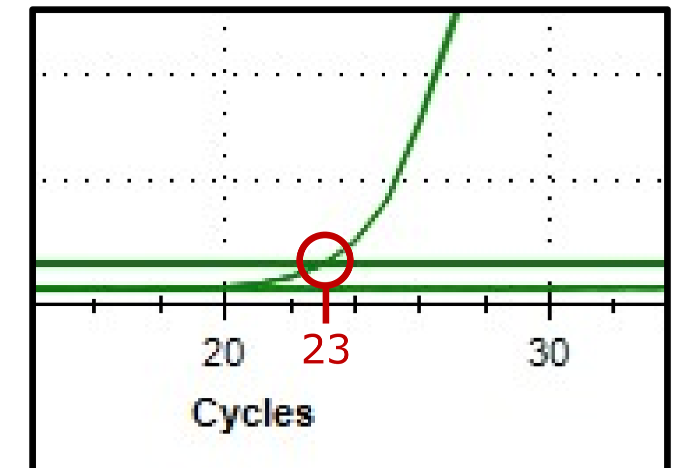
Explanation of the key terms

CT Value (= Cycle Threshold value)

Amplification Plot (COL1A2)

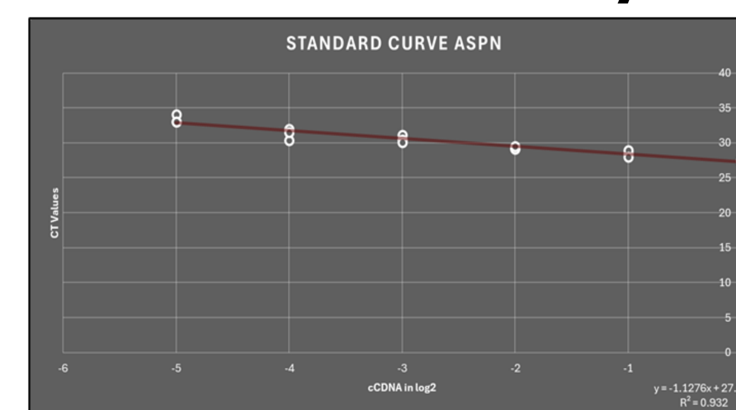


AP zoomed in (COL1A2)



In qPCR, a target sequence of a cDNA sample is duplicated during each qPCR cycle. The qPCR machine is able to measure the amount of cDNA present, above a minimum detection threshold. The number of cycles needed until the cDNA amount first raises over this minimum threshold is known as the CT value (as shown in the images above). A low CT value indicates a high initial concentration of cDNA and vice versa.

Primer Efficiency



Part of the primer validation is a dilution series, where the cDNA sample is diluted five times by a factor of 2. Each dilution stage gets tested and, in theory, diluting the cDNA by the factor of 2 should increase the CT value by exactly 1. If the CT increases by more than 1.0 across all stages, this indicates that the primers are operating at less than 100% efficiency, since a doubling of the cDNA takes longer than intended. Plotting the results of the dilution series in a graph and overlaying a standard curve (as shown above) allows for an accurate calculation of the efficiency of these primers.

Research Problem

In a prior research project the entire cell population of the nucleus pulposus (core) and the annulus fibrosus (fibrous ring around the core) of the intervertebral discs of cows was established.

For further research on this topic it would be very useful if qPCR tests could be performed on those cell populations. For this to be possible, qPCR primers need to be established and tested for each of the 51 genes discovered in the prior research study.

The goal of this thesis is to establish qPCR primers for five of these genes:
ASPN, COL1A2, LUM, MMP2 and PCOLCE (originating from the nucleus pulposus)

Methods

To establish and validate these primers, the following steps were performed:

- ◊ Selection of suitable primers
- ◊ Acquisition of cDNA samples
- ◊ qPCR assay - primer testing
- ◊ Gel electrophoresis
- ◊ qPCR assay - dilution series

Results

Primer testing results indicate that all five primers are capable of performing a polymerase chain reaction.

The melt curves of the 5 target genes all show a single common peak. This indicates that the PCR reaction was working as intended, with no unwanted by-products.

The gel electrophoresis results all agree with the putative amplicon lengths suggested by the primer research. This again confirms the absence of unwanted by-products.

The primers for the genes LUM and MMP2 meet the criteria for efficiency and R^2.

The primers for the genes ASPN, COL1A2 and PCOLCE do not or only partially meet the criteria for efficiency and R^2. However, by adjusting the qPCR process, an improvement of those values should be possible.

David Liam Fenner

Main supervisor:
Prof. Dr. Fabian Ille

Expert:
PD Dr. Philipp Stämpfli

Partner:
Competence Center in Biomedical Space Research and Medical Engineering