Lucerne University of
Applied Sciences and Arts

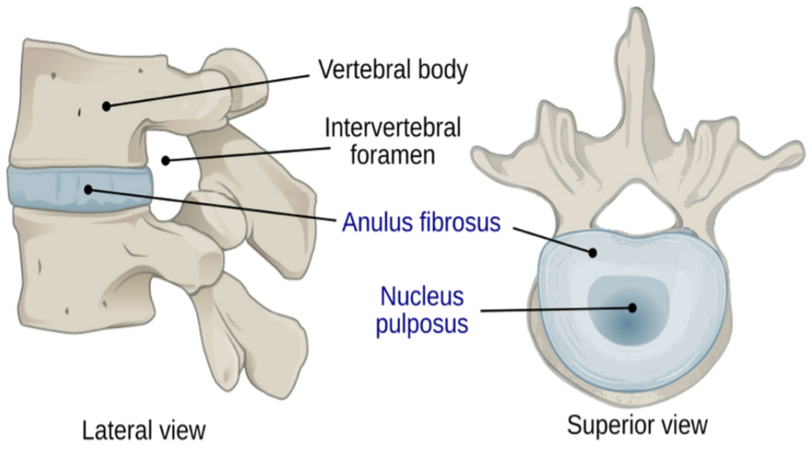
HOCHSCHULE
LUZERN

Technik & Architektur
FH Zentralschweiz

Competence Center for Bioscience and Medical Engineering
Obermattweg 9, 6052 Hergiswil

Bachelor-Thesis Medizintechnik

Genetic Validation of a Specific Intervertebral Disc Cell Population



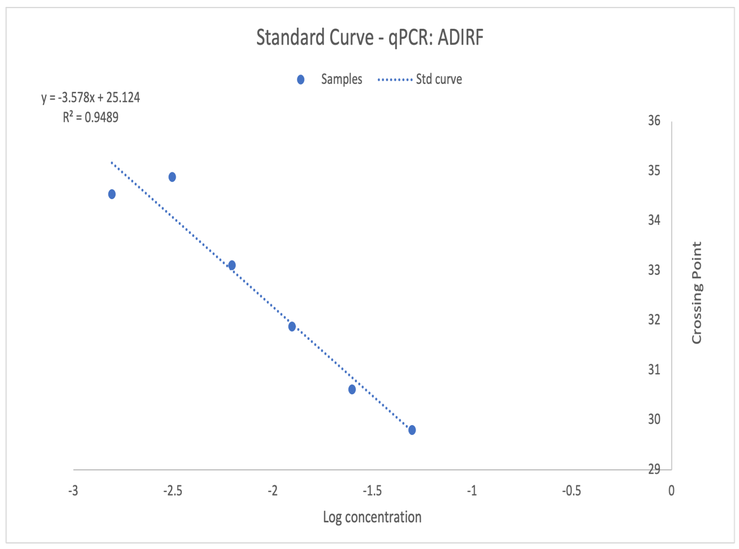
Methodology

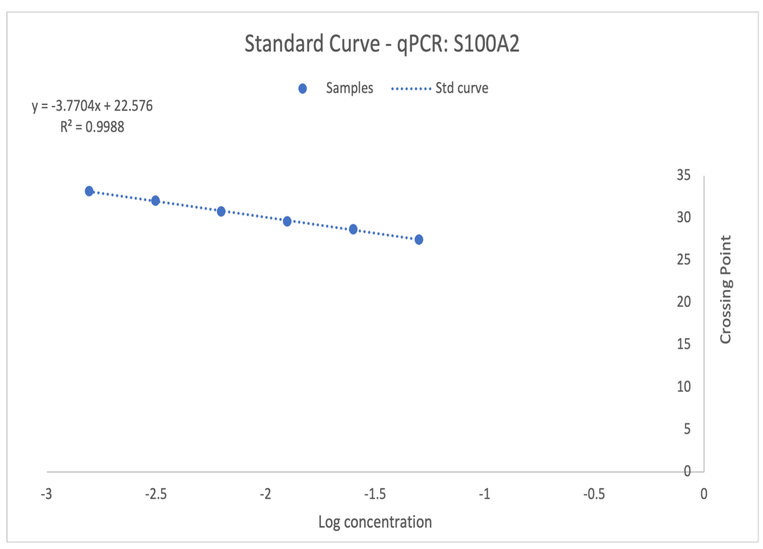
- **Assay Design**
  - Sequence Specificity
  - Secondary Structure analysis
- **Sample Acquisition**
  - RNA extraction
  - Reverse Transcription
- **qPCR**
  - Three replicates
  - NTC
- **Validation**
  - Specificity Analysis
  - Efficiency Analysis
- **Data Analysis**
  - Relative Quantification

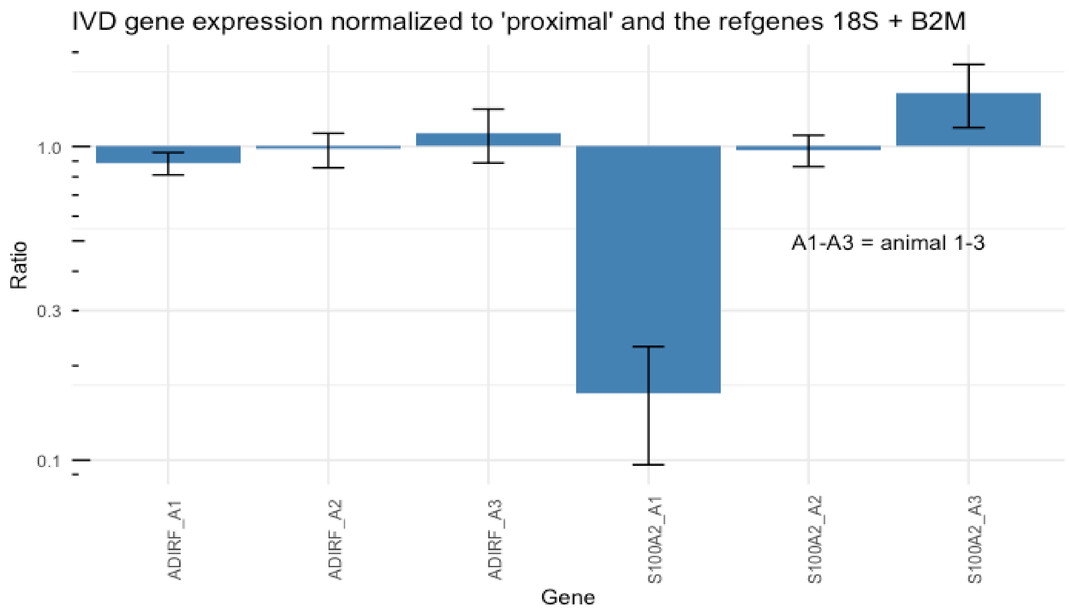
Results

qPCR Primers used to assess gene expression

Gene	Accession number	Sequence	bp	T_m (°C)	G/C %	Amplicon length	Primer dimer (kcal/mole)		
							Hairpin	Self-dimer	Hetero-dimer
SFRP5	NM_174461.3	FP: AACGACCTCTGCATCGCTGT	20	62.16	55	137	- 1.5	- 7.05	- 6.53
		RP: ACCACGAAGTCGCTGGAACA	20	62.02	55		- 3.16, - 1.67	- 5.19	
ADIRF	NM_001114513.2	FP: ACCCTTAACCACTCGCAGACC	21	62.05	57.14	145	-0.15, -0.01, 0.01, 0.15	- 4.85	- 6.37
		RP: TGCTTCTGTGGCCTGATCCA	20	61.49	55		-0.66, -0.5, -0.3	- 9.28	
S100A2	NM_001034367.2	FP: GAGTCTCTCTCTGCCCTCTGG	21	61.02	61.9	97	-1.46, -0.47	- 3.17	- 6.21
		RP: GAGTACTTGTGGAAGGTGGCG	21	60.94	57.14		-1.14	- 6.84	







Problem & Aim
The intervertebral disc (IVD) is known as the most extensive avascular tissue in the body consisting of 3 structures: the Annulus Fibrosus (AF), Nucleus Pulposus (NP), the cartilaginous endplate. Degeneration of the intervertebral disc (IVDD) and its medical repercussions remains an issue in today's society. Recent experimental studies have explored various biological strategies to address IVDD. However, a lack of knowledge of the cellular composition of the IVD hinders advances in regenerative therapies. To assist in this matter, Calió et al. (2021) determined the cellular composition of the NP and AF. Five uniquely expressed genes that are exclusively expressed in cell cluster two out of the identified NP cells, were selected for validation through gene expression analysis using quantitative polymerase chain reaction (qPCR). This project aimed to design qPCR primers for the five uniquely expressed genes: SFRP5, ADIRF, S100A2, ACTG1 and TUBB.

Methodology
Firstly, the primers were designed to meet a certain list of requirements and then analysed for secondary structures. Thereafter, the primers were tested on RNA that had been extracted and reverse transcribed into complementary DNA. Specificity analysis via melt curve analysis and gel electrophoresis and efficiency analysis using a standard curve were used to validate the genes. Lastly, relative quantification was performed to determine the difference in expression of the genes in distal IVD compared to proximal IVD.

Results
The outcome indicated that it was feasible to establish primers for SFRP5, ADIRF and S100A2 but not for ACTG1 and TUBB. During the qPCR SFRP5 amplified non-specific products and was disregarded as a non-working primer. The dilution series was only conducted with ADIRF and S100A2. The accepted range of qPCR efficiency is between 90-110%. The efficiency of ADIRF was determined to be 90.32%. This primer satisfied the requirement. S100A2, on the other hand, had an efficiency of 84.17% and hence failed to fulfil the requirement. Furthermore, an R2 greater than 0.985 is acceptable. ADIRF linearity was determined to be 0.9489 and indicated that the replicates are non-reproducible. The condition was not met by this primer. S100A2, on the other hand, met the criteria with a linearity of 0.9988 and indicated that the replicates were reproducible. The final qPCR determined the difference in expression of ADIRF and S100A2 in distal IVD compared to proximal IVD through relative quantification. These results indicated that distal IVD from animals 1 and 2, expressed ADIRF at a 0.88-fold and 0.97-fold lower level than proximal IVD. However, distal IVD from animal 3 expressed ADIRF at a 1.10-fold higher level than proximal IVD. In regards to S100A2, distal IVD from animals 1 and 2 expressed the gene at a 0.16-fold and 0.97-fold lower level than proximal IVD. Lastly, distal IVD from animal 3 expressed S100A2 at a 1.48-fold higher level than proximal IVD. All of the research questions could be answered during the project. Subsequently, the outcome signifies the occurrence and activity of ADIRF and S100A2 during the gene expression analysis. Hence, the presents of ADIRF and S100A2 in RNA from bovine IVD is validated.

Frunz Sarah Leigh
Supervisor:
Prof. Dr. Fabian Ille