OPTIMIZED PEGDA HYDROGEL MINIATURE GEL ELECTROPHORESIS FOR GENOMIC ANALYSIS

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OBJECTIVE

- To develop a miniaturized gel electrophoresis cartridge using Poly(ethylene glycol) diacrylate (PEGDA) hydrogel to analyze genomic expression.
- Quantify DNA band intensity via software analysis for comparison against qPCR values to investigate a relationship between gel electrophoresis and qPCR.

BACKGROUND

- Conventional gel electrophoresis uses agarose gel to separate DNA samples according to size.
  - A ladder and DNA samples are loaded into the gel.
  - The negatively charged particles migrate to the positively charged end of the gel electrophoresis chamber.
  - Samples are separated by molecular weight. Thus, smaller particles migrate further.
  - Base pair size of the samples can be verified by comparing the distance of sample migration against a standardized DNA ladder.

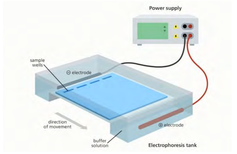

Figure 1. Gel electrophoresis apparatus.

- Limitations of agarose gel include long running time, low visibility of small sample sizes, large and thick gels are required for for accurate genomic analysis, and gel that appears cloudy.
- Poly(ethylene glycol) diacrylate (PEGDA)
  - Filters smaller DNA fragments more efficiently than agarose gel due to its porosity.
  - Can be molded and UV crosslinked to form a polymer gel of desired shape.
  - More affordable than agarose gel.
  - Appears clear at higher concentrations compared to agarose gels.

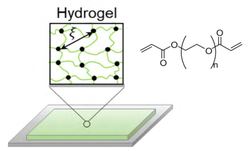

Figure 2. PEGDA gel is UV crosslinked and attached to glass surface.

ACKNOWLEDGEMENTS

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METHODS & MATERIALS

- The PEGDA cartridge was fabricated on a 24 x 60 [mm] microscope slide with two wells using 8% PEGDA-750, 0.1% photoinitiator, and 1X TBE. Solution was then UV crosslinked for 6 [min].

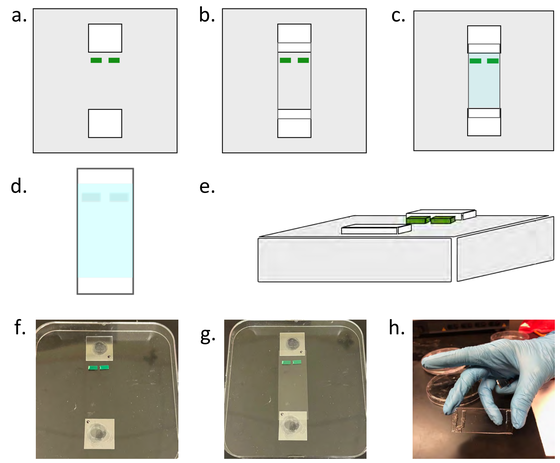
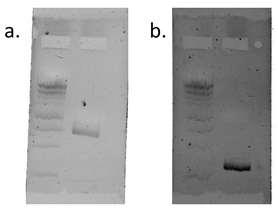


Figure 3. Construction of cartridge mold and PEGDA hydrogel fabrication. Process starts with a.) a mold with two wells, b.) a microscope slide treated with TMSPMA is then placed over the mold, and c.) PEGDA solution is pipetted into negative space. d.) PEGDA cartridge removed from mold. e.) 3D schematic diagram of mold. f.) Top view of mold. g.) Top view of mold with microscope slide. h.) PEGDA cartridge after crosslinking.

- Electrophoresis was conducted in 1X TBE with 4 [µL] of ethidium bromide. 9 [µL] of DNA samples were loaded with 6 [µL] of 1 kB ladder. Samples were electrophoresed for 60 [min] at 80 [V].
- DNA samples with known amplicon sizes were electrophoresed. The RDS wild type gene had a size of 300 [bp], and the Bouse gene had a size of 600 [bp].
- 2D cell culture of glioblastoma cell line LN229 was used for genomic analysis and screened for EGFR gene.
- DNA analysis of the EGFR gene was conducted using software analysis of photos taken with a phone. Pixel intensity of the DNA bands were quantified.

RESULTS

Figure 4. Electrophoresis of known amplicon sizes. a.) 1 kb ladder (left) Bouse gene at 600 [bp] (right). b.) 1 kb ladder (left) RDS wild type genotype at 300 [bp] (right).



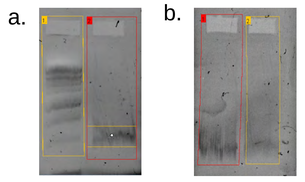
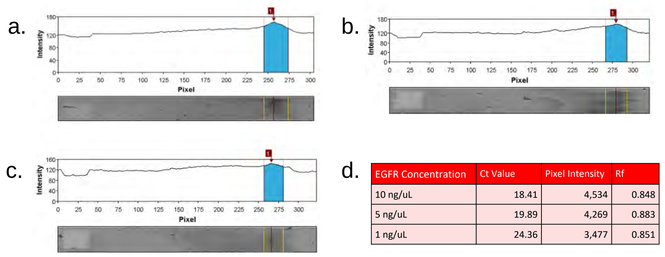


Figure 5. Electrophoresis of EGFR with varying DNA concentrations. a.) 1 kb ladder (left) EGFR concentration of 10 [ng/µL] (right). b.) EGFR at concentrations of 5 [ng/µL] (left) and 1 [ng/µL] (right). Amplicon size was verified to be 239 [bp].



EGFR Concentration	Ct Value	Pixel Intensity	Rf
10 ng/uL	18.41	4,534	0.848
5 ng/uL	19.89	4,269	0.883
1 ng/uL	24.36	3,477	0.851

Figure 6. DNA band analysis using GelAnalyzer software. a.) Pixel intensity analysis of EGFR with concentrations of a.) 10 [ng/µL], b.) 5 [ng/µL], and c.) 1 [ng/µL]. d.) Summary table shows the relationship between qPCR cycle threshold (Ct) results of EGFR amplification, pixel intensity, and Rf value for each concentration (a-c).

CONCLUSION

- 8% PEGDA gel separates DNA fragments 100-10,000 bp.
- The cartridge is compatible with traditional gel electrophoresis equipment to separate DNA samples by size.
- Gel electrophoresis results can be interpreted using gel analyzing software to identify and quantify the resulting DNA bands, but this does not produce a reliable or consistent relationship with qPCR results due to lack of standardization that relates Ct values with pixel intensity.