

Research Experiences for Teachers

Employing TERRACE to identify circRNA molecules uniquely associated with varying grades of gliomas

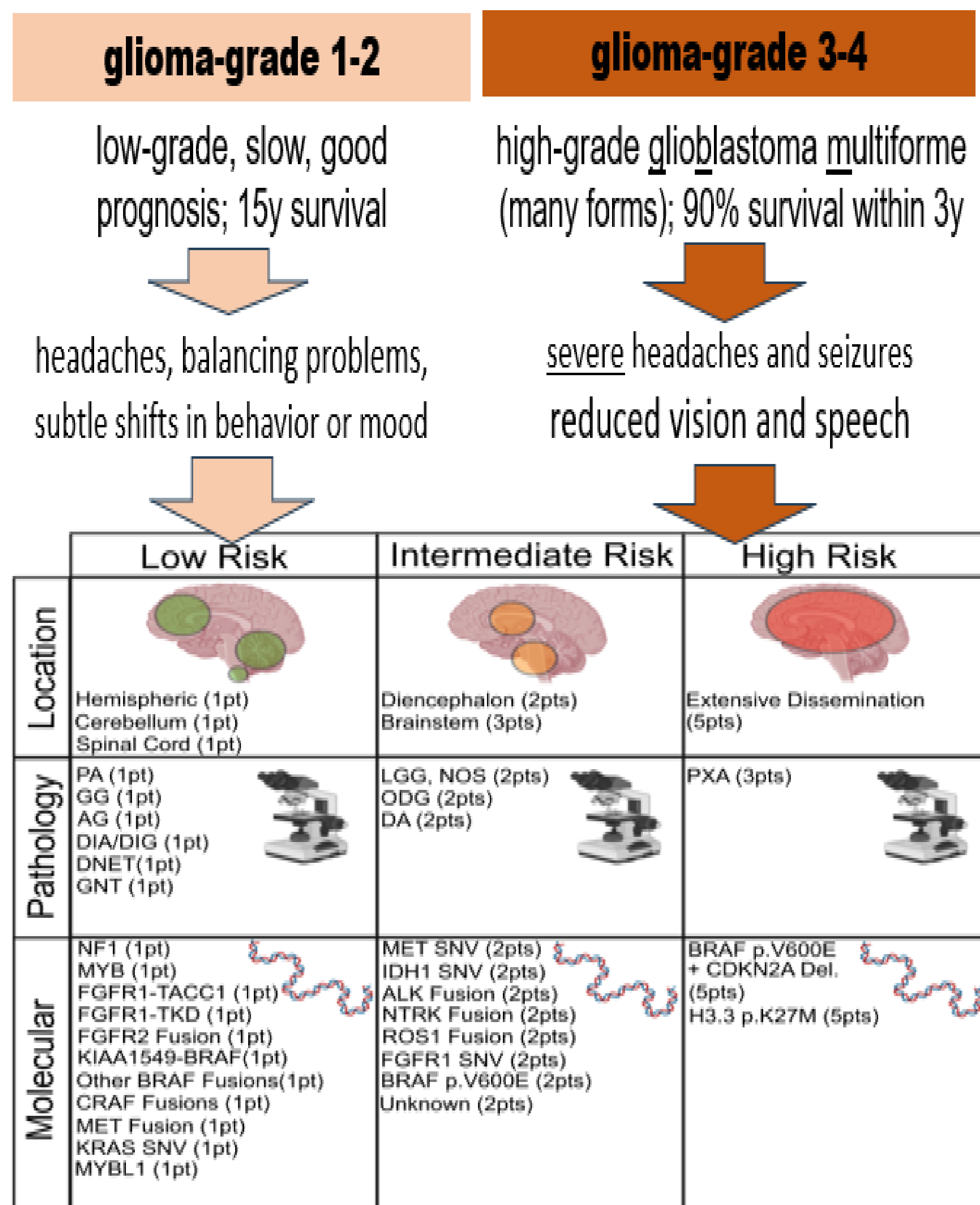
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Introduction

Brain cancer (glioma) is often considered incurable for reasons that symptoms (e.g., headaches, mood swings) experienced by victims surface too late for treatments to be effective because metastasis with resistance to apoptosis has already occurred. Developing a biomarker that allows for an early and accurate diagnosis of the varying grades of glioma would certainly improve the survival rate of sufferers.

Figure 1. Pathogenesis of gliomas and the available techniques currently available to detect, diagnose, and treat the varying associated grades.



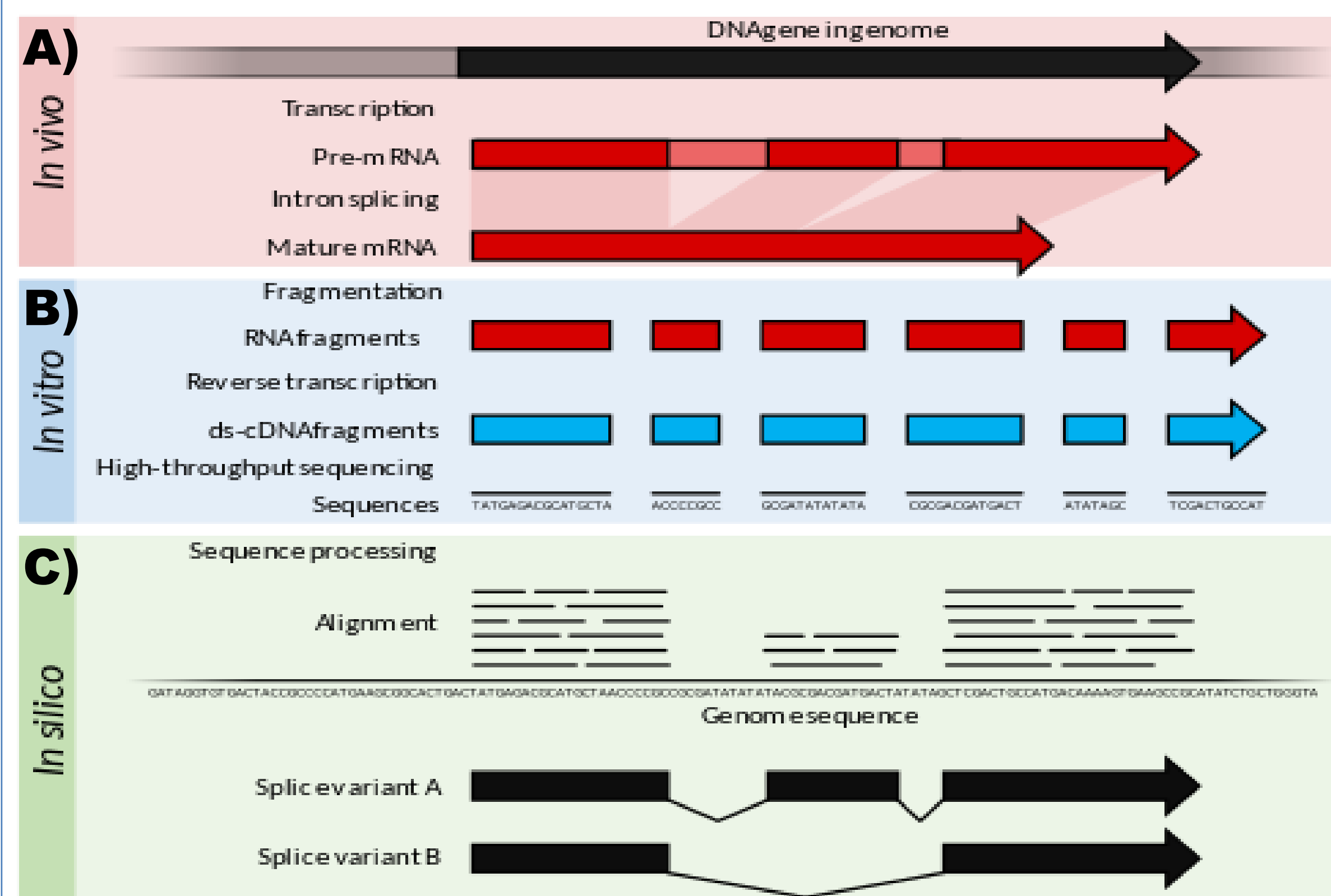
1) Surgery
2) IDH inhibitor (Vorasicenib);
activates apoptosis

1) Surgery
2) Temozolomide, alkylate-mutate-
induces apoptosis

Materials and Methods

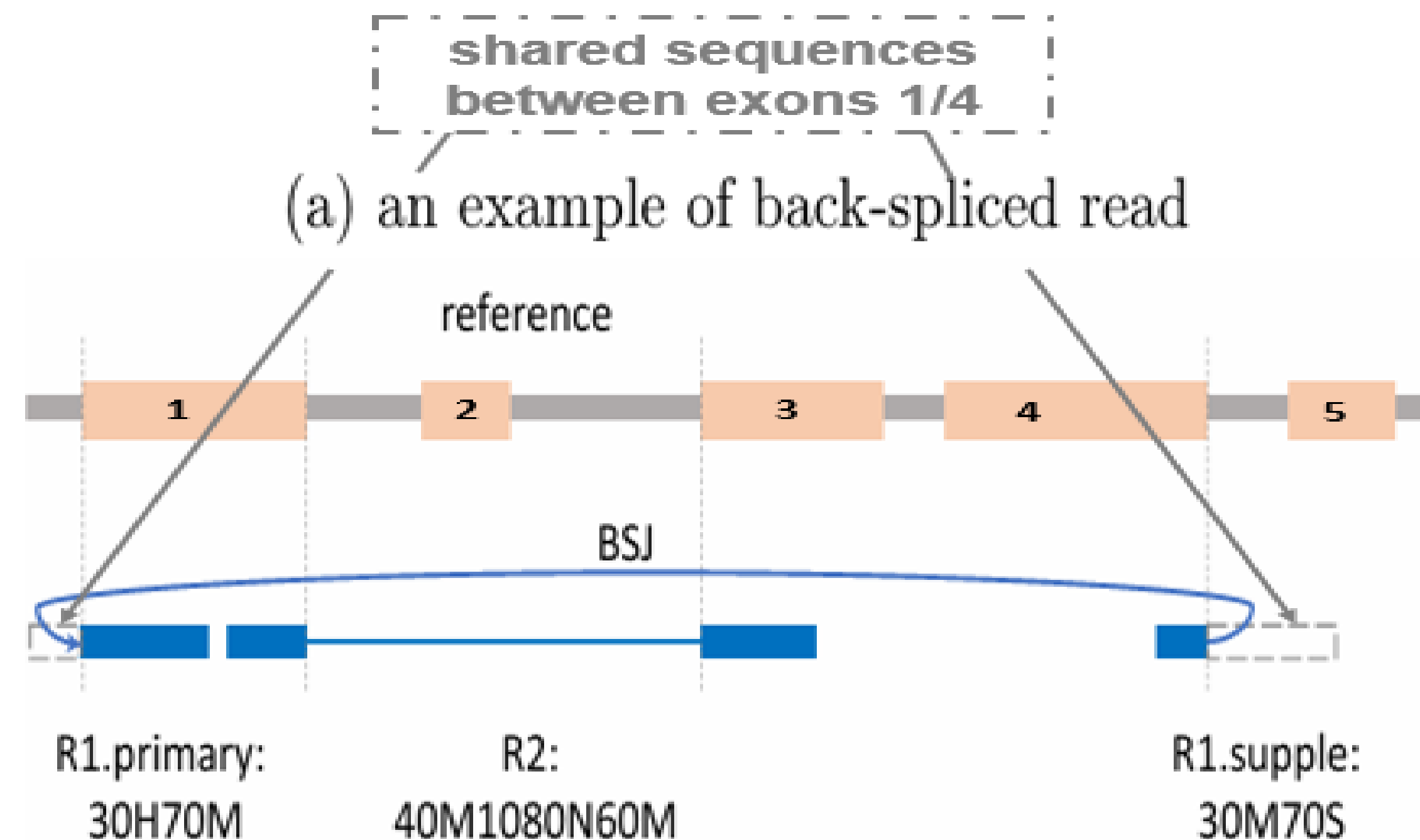
RNA/Sequencing – Computer modeling

Figure 2. Process involves **A)** extraction and fragmentation of a cell's transcriptome, **B)** reverse transcription of RNA into cDNA for subsequent sequencing (e.g., Illumina), **C)** followed by computer modeling to characterize various species of the cellular transcriptome.



TERRACE (accurate assembly of circRNAs using bridging and machine learning)

Figure 3. Assembles circRNAs accurately through identification of back-spliced reads (BSJs), represented by overlapping sequences (“bridging”) or shared ALU elements between distanced exons (1, 4).



Zahin, Shi, Zang, Shao Accurate Assembly of Circular RNAs with TERRACE bioRxiv preprint Feb 2024 <https://doi.org/10.1101/2024.02.09.579380>

Results

Figure 4. TERRACE generated 70 candidate biomarkers representing **grade-4** and 25 for **grade-1** gliomas. Common among **grades 1, 2, 4** gliomas are 6 circRNAs. Literature confirmed 12/70 **grade-4**, 3/25 **grade-1** are unique and upregulated; potential biomarkers!

GLIOMA-G1 (https://www.ncbi.nlm.nih.gov/gdv/)					GLIOMA-G2 (https://www.ncbi.nlm.nih.gov/gdv/)					GLIOMA-G4 (https://www.ncbi.nlm.nih.gov/gdv/)				
MATCH	MATCH	UNIQUE			MATCH	MATCH	UNIQUE			MATCH	MATCH	UNIQUE		
chr	start	end	cls	gene-func-lit	chr	start	end	cls	gene-func-lit	chr	start	end	cls	gene-func-lit
6	4,891,713	4,892,379	10	CDYL	5	38,523,419	38,530,666	10	LIFR	5	38,523,419	38,530,666	20	LIFR
11	33,286,413	33,287,511	13	HPK3	13	32,517,857	32,527,532	15	NBP2L2	6	4,891,713	4,892,379	46	CDYL
13	32,517,857	32,527,532	11	NBP2L2						11	33,286,413	33,287,511	32	HPK3
17	20,204,333	20,205,312	23	SPECC1						13	32,517,857	32,527,532	24	NBP2L2
1	7,777,160	7,778,163	10	VAMP3	1	169,704,568	169,704,753	12	SELL	1	8,541,214	8,614,666	20	PEPE
1	211,778,918	211,793,160	10	RNFSL344P	12	97,492,461	97,561,047	13	RMST	1	26,942,660	26,943,065	24	NUDC
1	231,795,242	231,818,517	10	DSC2	X	140,783,175	140,784,659	10	LINC00632	1	35,358,925	35,361,789	33	ZMYM

Figure 5. The circRNA, “ZNF609” is uniquely abundant to glioma grade-4, whose mechanism is well documented. Projected PCR primers, at 65C annealing temperature, will generate a 416 bp amplicon.

NCBI - Basic Local Alignment Search Tool – PCR primers

Primer pair 3	Sequence	Tm	GC%	Self compl
F-primer	CTAGCAAACCCACTCCAGGG (20)	43	60	4, 2
R-primer	ATTCTCCCCTTCTCTGGCT (20)	43	55	
Product length (amplicon)	416			

Conclusions and Future Work

These circRNAs are glioma grade specific, stable, feasible targets for PCR assay-based detection and diagnosis.

Optimize PCR protocol for in situ application of one or multiple circRNAs.

Perform “knock-in” studies to determine if circRNAs are causative and possible therapeutic targets.

References

- Zahin, Shi, Zang, Shao Accurate Assembly of Circular RNAs with TERRACE bioRxiv preprint Feb 2024 <https://doi.org/10.1101/2024.02.09.579380>
- Seyhan Circulating Liquid Biopsy Biomarkers in Glioblastoma: Advances and Challenges Int. J. Mol. Sci. 2024, 25, 7974. <https://doi.org/10.3390/ijms25147974>
- Pouyan Glioblastoma multiforme: insights into pathogenesis, key signaling pathways, and therapeutic strategies Molecular Cancer (2025) 24:58