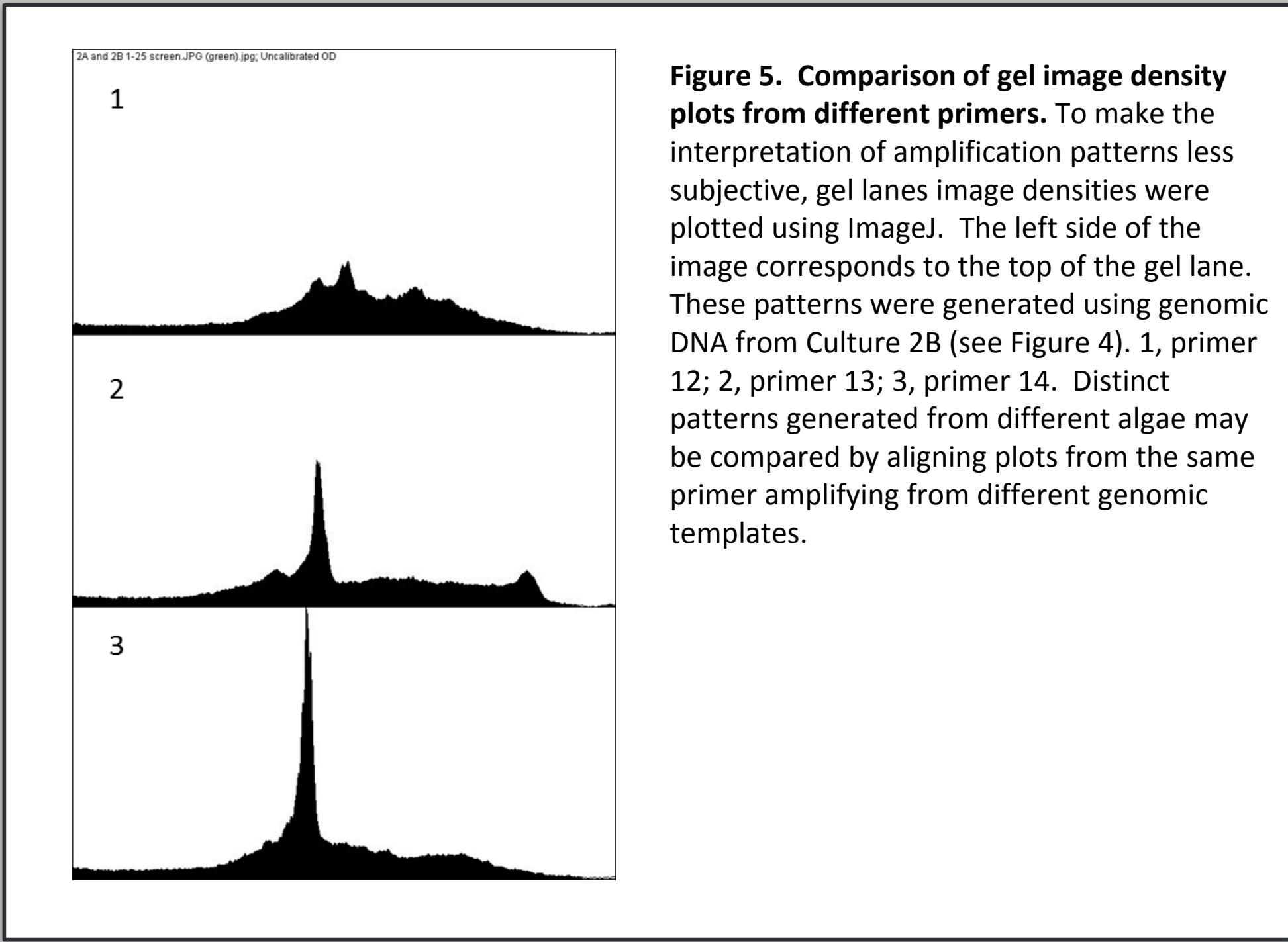
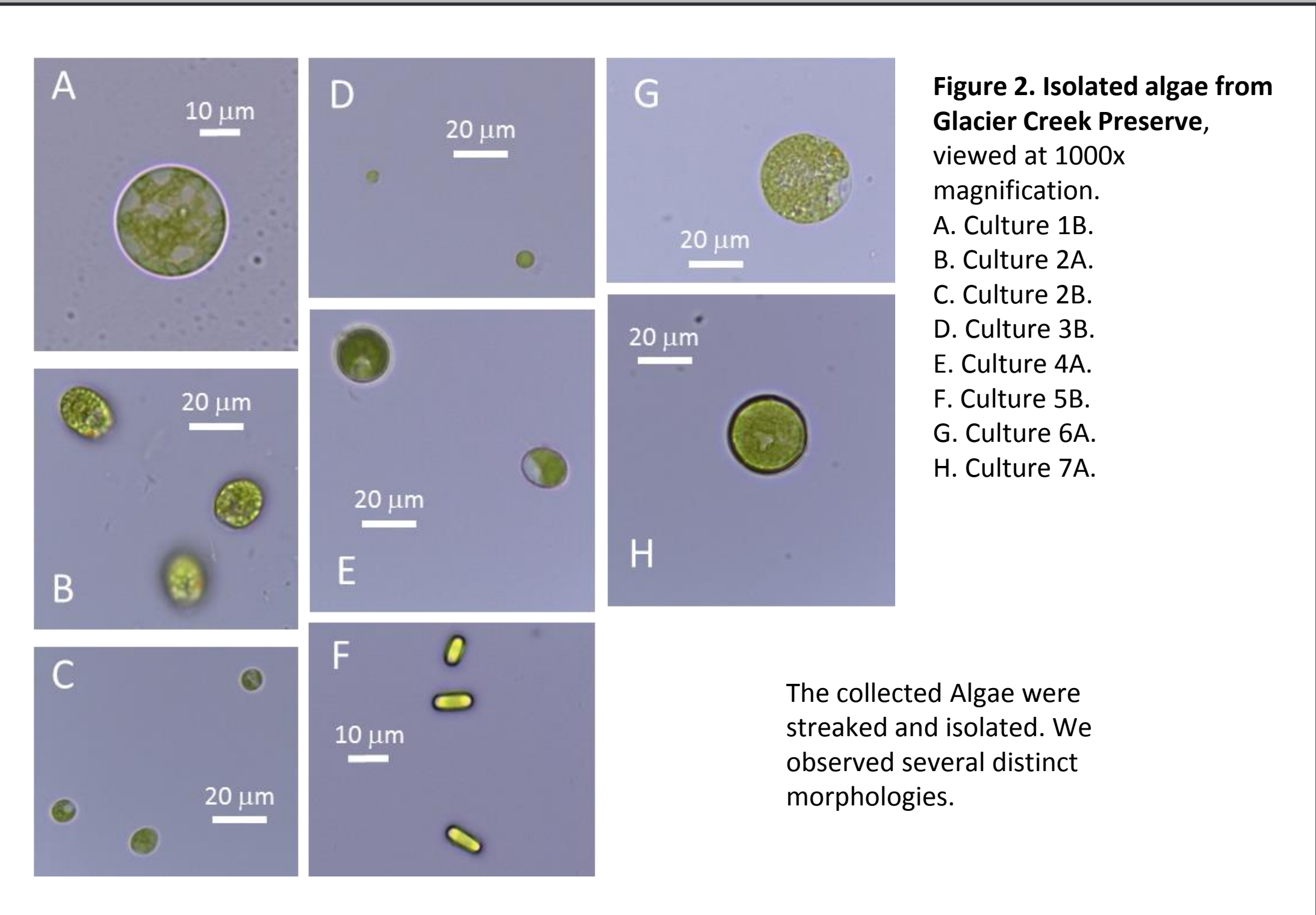


# Development of Molecular Tools for Identification of Prairie Terrestrial and Wetland Algae

Esther Mundembo Kapinga , Mark A. Schoenbeck,  
Biology Department, University of Nebraska at Omaha, Omaha, NE 68182

## ABSTRACT

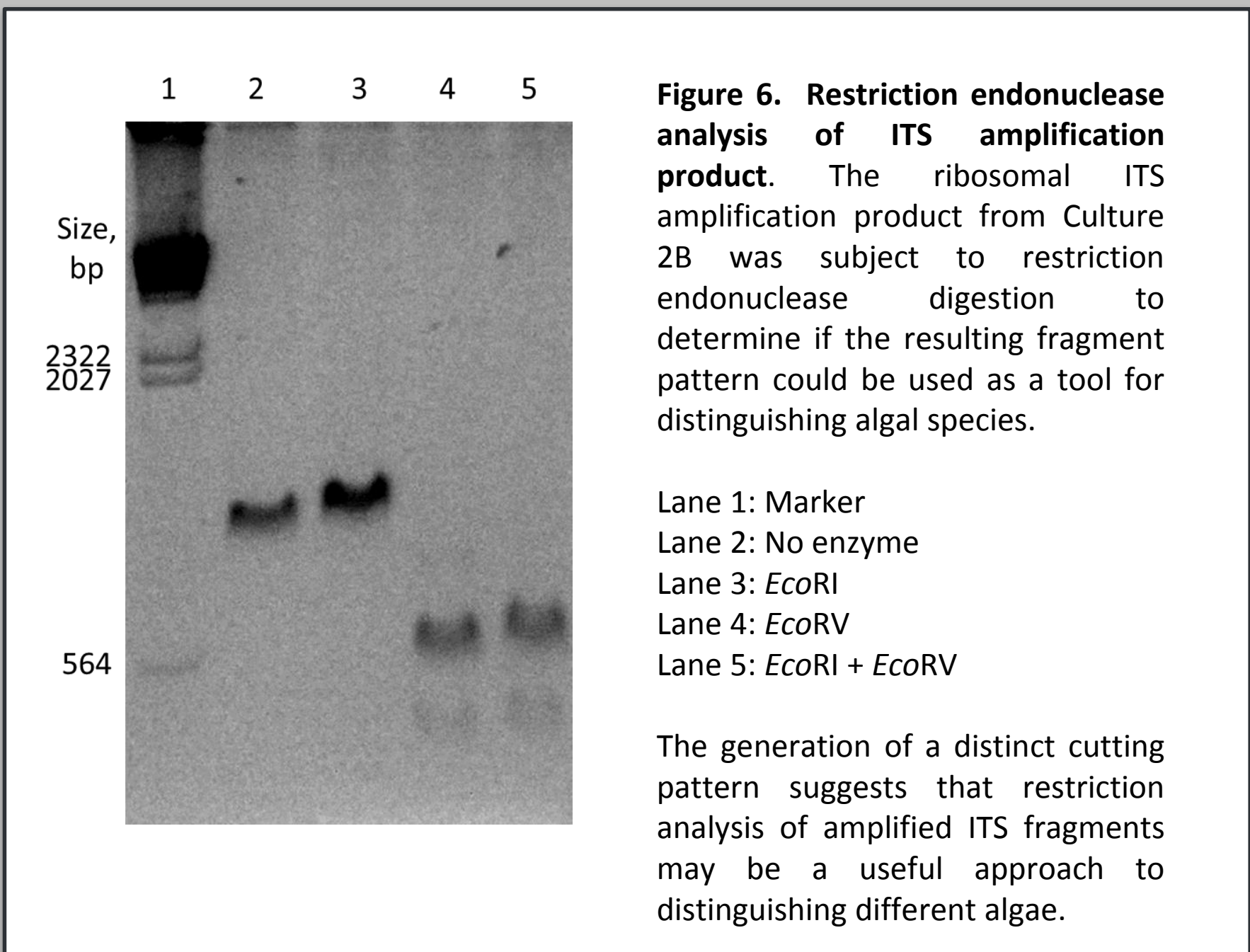
Algae are a diverse group of photosynthetic protists. Green algae are typically unicellular, though some may occur as multicellular colonies, and they are found across a wide range of habitats, including marine, freshwater, and terrestrial environments. As photosynthetic producers and sometimes symbionts, algae can occupy important roles in an ecosystem; in other circumstances they can have negative impacts on environmental quality. Estimations of the number of green algal species range from eight thousand to over fifteen thousand. The identification of individual algal species can be problematic due to their small size and typically simple cell anatomy. For this reason, molecular tools may be employed to distinguish between algae. We have examined the utility of inter-simple sequence repeat (ISSR) analysis to generate a “bar code” molecular phenotype for the identification of different green algae. Environmental samples were collected from UNO’s Glacier Creek Preserve and from other local sites. Algae from these sites were cultured and isolated. Genomic DNA from these algae was used as the template for PCR amplification, both with and without initial purification from the algae. Amplification of the high copy number ribosomal internal transcribed sequence was possible without initial DNA purification, yielding characteristic size products for different algae; this method did not reliably work for amplification using ISSR primers. Isolated algal DNA was tested with >50 ISSR primers to identify primers that could generate distinct amplification patterns that might be useful for rapid identification of algal species.



**Figure 1. Collection sites at Glacier Creek Preserve.** Water samples collected from moving and standing water on May 04, 2016. Numbers show collection sites. Numbers correspond to sites identified in Table I. Algae were cultured from water samples using a mineral nutrient medium. Image: Google Maps.

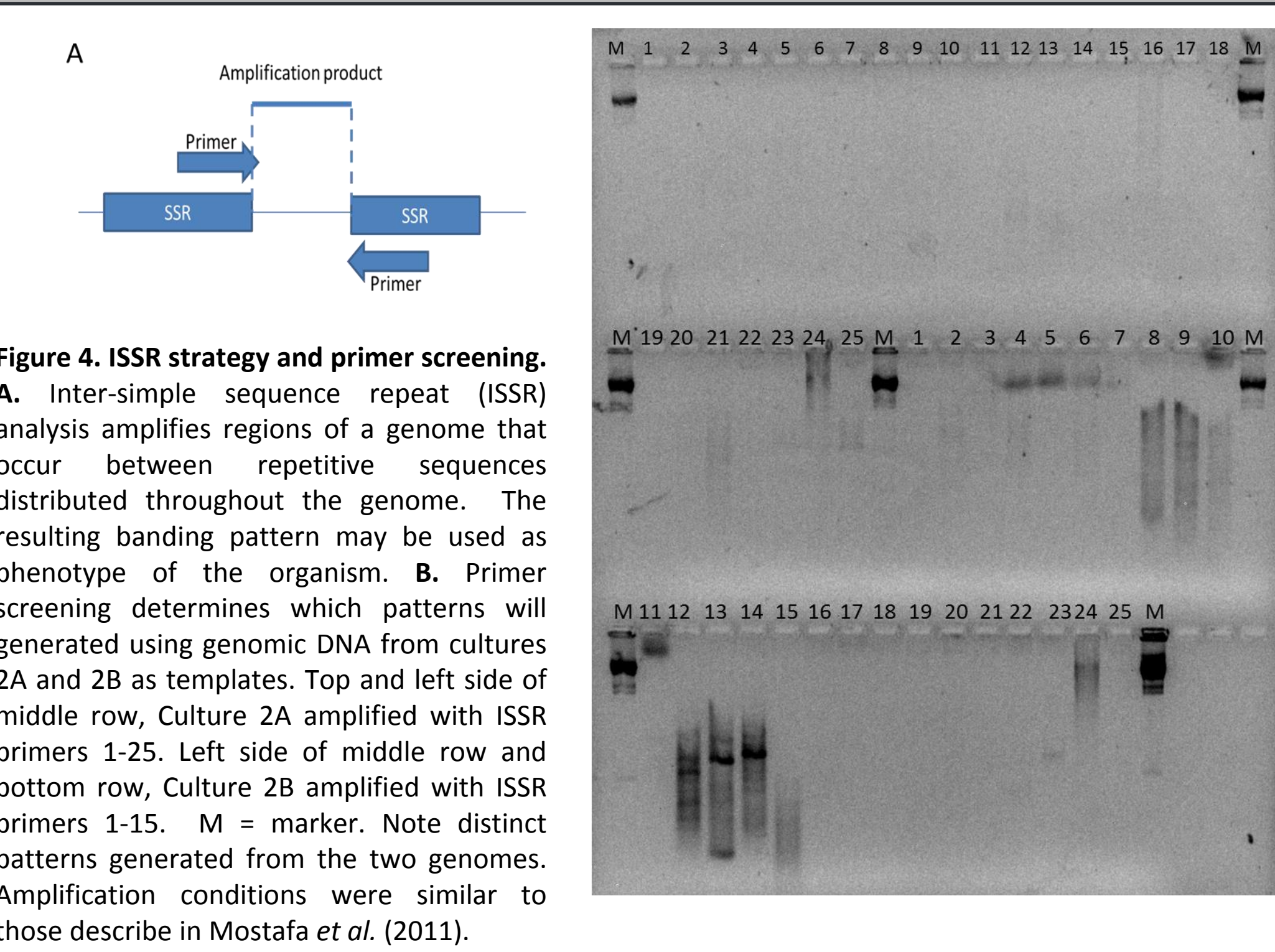
**Table II. ISSR primers used in this study.**

No.	Seq.	No.	Seq.	No.	Seq.	No.	Seq.	No.	Seq.
1	(AC)8C	6	(AG)8G	11	(ACC)6T	16	(AGT)6C	21	(CA)8A
2	(AC)8G	7	(AG)8T	12	(AGC)6	17	(AGC)6T	22	(CA)8G
3	(AC)8T	8	(ACC)6	13	(AGC)6C	18	(AGT)6G	23	(CA)8T
4	(AG)8	9	(ACC)6C	14	(AGC)6G	19	(AGT)6T	24	(CT)8
5	(AG)8C	10	(ACC)6G	15	(AGT)6	20	(CA)8	25	(CT)8A



**Table I. Environmental Conditions at Glacier Creek Preserve sampling sites.**

Sites	Oxygen (mg/L)	Temperature (C)
1. Wet Land	5.7	18
2. Glacier Creek Upstream in Woods	6.4	16
3. South Pond	5.0	14
4. Standing Water	5.3	20
5. Flowing water mid course	9.1	16
6. Standing water mid course	4.1	21
7. Downstream of Confluence	10.6	15



## Conclusions and Future Directions

Several morphologically distinct algae could be cultures from the Glacier Creek Prairie Preserve, suggesting a diverse community.

Amplification of the ribosomal Internal Transcribed Spacer from isolated algal genomic DNA yielded differently sized products, indicating that these isolates are genetically distinct from each other.

Application if ISSR primers to genomic templates also generated distinct amplification patterns that might be useful in identifying particular algal species when encountered.

Restriction endonuclease cutting of amplified ribosomal ITS products also generated distinct patterns that might provide an additional molecular phenotype for distinguishing between different algae.

## References

Mostafa, N., Omar, H., Tan, S. G., & Napis, S. (2011). Studies on the genetic variation of the green unicellular alga *Haematococcus pluvialis* (Chlorophyceae) obtained from different geographical locations using ISSR and RAPD molecular marker. *Molecules*, 16(3), 2599-2608.