Ocean Sci. Discuss., 5, S254–S259, 2009
www.ocean-sci-discuss.net/5/S254/2009/

Ocean Science Discussions

Interactive comment on "Molecular biology techniques and applications for ocean sensing" *by* J. P. Zehr et al.

J. P. Zehr et al.

Received and published: 19 February 2009

1. Describing the essence of many molecular techniques with numerous relevant references to follow. Jargon is inevitable, but the authors do a reasonable job of explaining several of the techniques, although an on-line encyclopedia was essential!

Nothing to add. Some jargon is inevitable.

2. Providing numerous examples of the questions in ocean sciences that these techniques can address. Here Figures 1 and 3 are particularly helpful.

No comment.

3. Pointing to those techniques with the potential to be incorporated within in situ sensors.

No comment.

Figure 1 is most useful. It would be enhanced if those techniques referred to later and without acronyms in the figure (e.g. automated rRNA intergenic spacer analysis) had their acronyms added at this early stage, improving the figure’s usefulness as an introduction to the paper. Its usefulness would be further enhanced if a few sentences at the end of section 1 pointed the reader to the relevant sections in the paper dealing with the techniques mentioned.

We can modify the text to aid the readers.

A few specific questions from this non-specialist: 1. Page 627 line 8 - UV radiation is mentioned as a process causing mutations, can the authors provide an example that might be more relevant to the marine environment away from the surface?

This is a large and general topic, which really is not relevant to molecular biology techniques for identifying organisms. Getting into this topic would distract the reader from the point of the paper.

2. Page 627 line 22 - ’most environmental organisms have not been cultivated’ - the reference is dated 1998, is this still the case?

Yes, this general knowledge and stated over and over (too much!) in various papers.

3. Page 633 - the heading for section 5 is cryptic - might a heading that was more general e.g. ... fingerprinting ... be appropriate?

Heading can be modified.

3. Page 638 line 25 - the He et al. paper (for which I am grateful for the reference) mentioned use of the device in marine sediments, not mentioned here.

I am unsure what is suggested here.

Full Screen / Esc Printer-friendly Version Interactive Discussion Discussion Paper 1) I

OSD

5, S254–S259, 2009

Interactive Comment

would endorse the observations made in an earlier comment by G. Griffiths and would encourage the authors to take on his suggestions. In particular his point about Figure 1 is extremely valid. It may be worth, as part of Fig 1, identifying the relevant section number alongside the description of the technique. For example ’Quantitative PCR (qPCR) [3]’ or ’Denaturing Gel-Electrophoresis [5]’ and so forth. This would certainly assist the non-specialist reader to relate the text and figure together.

We can modify the text to link the figure with text better.

2) The use of an on-line dictionary was mentioned in this first short comment as well. The authors might consider a brief glossary of terms at the end of their paper. Terms such as ’fosmid’ are not defined currently. If the authors intend this to be a comprehensive introduction to the subject then a short glossary would help an interested reader to follow the manuscript from start to finish without consulting external sources.

It is hard to imagine that with an online publication that it is worthwhile making an old fashioned glossary-google is just as quick and better covers the lack of background for individual readers.

3) The authors might also consider the inclusion of other summary figures to assist with the text explanations. The description of the basic PCR reaction and the concept of degenerate primers (page 629) might be assisted with a diagram? Similarly the description of real-time PCR using hybridisation probes may also benefit from a diagram. If the authors feel that this would make the overall manuscript too long then perhaps they could cite some general texts (books, book chapters) which might assist the reader in addition to the more general review articles cited.

We have cited general texts. This would be too general for this topic. This was not to be a molecular biology primer, but only a review of methods pertinent to marine ecology, that ultimately could be used in monitoring or remote instrumentation (link to Scholin

OSD

5, S254–S259, 2009

article).

4) Section 2 - PCR techniques. I think that this section is useful. Is it worth including the comment that the principle of PCR underlies many of the other techniques as well, rather than treating it as a separate process? My feeling is that this is not as apparent as it might be.

Good point. We can revise this section.

5) In the flow chart in Figure 2 the restriction digest steps are not identified. This figure should be modified to indicate at what point the restriction digests, which are identified within the text, take place to generate appropriate ’fingerprints’ 6) Page 632, line 20 onwards - I feel this section of text requires some clarification.

Full Screen / Esc Printer-friendly Version Interactive Discussion Discussion Paper 7) Summary section - ultimately not all of the techniques described within this review will be appropriate for automation and deployment on sensor platforms.

I was requested to write this paper (and give a presentation) to complement the Scholin presentation (which I also gave). It thus is not meant to be overlapping with the implementation on platforms (at least not today with current technology) but to provide the basis for what can be done with molecular biology (and might someday form basis of sensors-technology is developing rapidly, more rapidly than this Discussion has taken place!)

The authors do offer some insight into which of these techniques might be most appropriate for sensor development. I wonder if it is worth including a short discussion of any specific challenges that lie in sensor development (in addition to power supply); for example sample isolation, signal to noise ratios, the sensitivity and idiosyncrasies of microarray methods, problems associated with the limited lifespan of fluors used in fluorescence detection and so forth. Perhaps this is to be covered in another article within the special edition?

OSD

5, S254–S259, 2009

This is definitely within the realm of the topic of the Scholin article, and would double this manuscript if included here.

In conclusion this is a very useful article, presenting as it does an overview of the discipline which sensor developers will undoubtedly find of benefit when considering the future development of technology for the marine environment.

Specific comments: 1) It is perhaps beyond the scope of this review, but readers should be made aware of the use of protein-based methods that are being applied in the field of oceanography.

This is correct. Which is why we defined our topic in the beginning as pertaining to nucleic acid techniques.

Zehr et al highlight some of the issues relating to probe binding efficiency (PCR bias) that may be overcome using proteomic approaches (which again have been developed for medical applications).

Proteomics at current technology can only address 1)cultures and 2) the most abundant organisms with 3) the most abundant proteins. Of course if “proteomics” to the reviewer includes things like immunoassays then of course there are ways that protein targets can avoid bias-but then you lose the ability to detect species diversity.

2) Another challenge that could be stressed in this review is the need to know the microbial diversity of a community before many of the approaches listed can be applied. Many of the techniques listed rely on development on cultured organisms, which may not be representative of the real environmental community (Azam et al 1998). The Shot-gun cloning / metagenomic approaches will often need to be conducted prior to any sensor development for this reason.

This is a point that we could develop. It certainly is true for some cases, if not all. However, with many studies, including metagenomic ones having been performed, there is

OSD

5, S254–S259, 2009

a large number of targets that can be addressed now, without further analysis.

3) Owing to Zehr’s background in understanding nitrogen-fixation, some other examples could be used for the application of molecular techniques in oceanic systems e.g. phosphate metabolism, calcification etc.

We can add references for other targets, although it was just meant as an example. I wrote a chapter on this 10 years ago.

4) On page 644 the authors stress the need to define ecosystem targets – are these molecular or geographic? Will well-characterised oceanic regions such as BATS and HOTS be candidate locations for sensor development?

Geographic locations are a philosophical, political and technological point that really cannot be discussed in a manuscript such as this. I believe the first order of business is defining molecular targets that are useful. If this point did not come across then I will modify text to do so.

Interactive comment on Ocean Sci. Discuss., 5, 625, 2008.

OSD

5, S254–S259, 2009

Interactive Comment