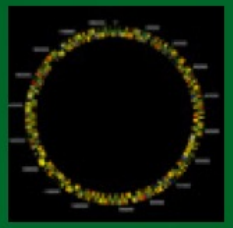
the Redfield Lab



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What We're Planning

The best way to find out what experiments we're planning is to read our grant proposals.

Here are links to our latest proposals. The first two are those we have just submitted to NIH and to CIHR (The Canadian Institutes for Health Research, the Canadian equivalent of NIH). These proposals aim to investigate transformation recombination (NIH) and the mechanisms of DNA uptake (CIHR). We are all currently working on prelimary data for these proposals.

- Summary of recombination proposal
- Complete recombination proposal
- Summary of uptake proposal
- Complete uptake proposal

The third proposal addresses the role of the Sxy transcriptional activator, considering both how the sxy gene is regulated and how the Sxy protein works with CRP to activate genes with CRP-S sites in their promoters. Its title is "Regulation of CRP-S promoters in *H. influenzae* and *E. coli*.". This proposal was successfully funded in 2007. We are currently pursuing most of the goals outlined in this proposal.

- Summary of Sxy proposal
- Complete Sxy proposal

Here are links to two other successful previous proposals:

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Arsenic-associated bacteria (NASA's claims)

By Rosie Redfield on Saturday, December 04, 2010

Here's a detailed review of the new paper from NASA claiming to have isolated a bacterium that substitutes arsenic for phosphorus on its macromolecules and metabolites. (Wolfe-Simon *et al*. 2010, A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus.) NASA's shameful analysis of the alleged bacteria in the Mars meteorite made me very suspicious of their microbiology, an attitude that's only strengthened by my reading of this paper. Basically, it doesn't present ANY convincing evidence that arsenic has been incorporated into DNA (or any other biological molecule).

What did the authors actually do? They took sediment from Mono Lake in California, a very salty and alkaline lake containing 88 mg of phosphate and 17 mg of arsenic per liter. They put the sediment into a similarly alkaline and hypersaline defined medium containing 10 mM glucose as a carbon source, 0.8 mM NH_4SO_4 as a nitrogen and sulfur source, and a full assortment of the vitamins and trace minerals that might be needed for bacterial growth. Although this basic medium had no added phosphate or arsenate, contamination of the ingredients caused it to contain about 3 µM phosphate (PO_4) and about 0.3 µM arsenate (AsO_4). For bacterial growth it was supplemented with arsenate or phosphate at various concentrations.

The interesting results came from sediment originally diluted into medium

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Heresy about Ethidium Bromide

By Rosie Redfield on Wednesday, October 11, 2006

Recently I've received several copies of an email warning of the dangers of ethidium bromide (a chemical used to visualize DNA in agarose gels) and advertising a seminar about a safer alternative. The email originated from Invitrogen, the company that sells the alternative DNA stain ("SYBR safe"*) and is sponsoring the seminar. Here's the first two paragraphs of the email:

> A real risk of lab science is accidental exposure to hazardous chemicals, especially ones that may be fatal if inhaled, harmful if swallowed or absorbed through skin, causing irritation to skin, eyes and the respiratory tract and causing heritable damage.
>
> Ethidium bromide has just such characteristics and is frequently in use at many UBC labs. A safer alternative is available that is non-toxic, non-mutagenic and is not classified as cytotoxic waste when disposed. To alert the UBC research community to this safer alternative called SYBRSafe Gel Stain, the Department of Health Safety and Environment and the UBC Sustainability Office are sponsoring a seminar presentation by Invitrogen on the product comparison and practical application of SYBRSafe.

This would be fine if ethidium bromide (EthBR) really was a serious hazard. But it's not. And it's actually *less* toxic than this alternative.

Ethidium (also called homidium) was developed as a treatment for trypanosomiasis (African sleeping sickness), and is still used in Africa where resistance to it is not a problem. The following quote is taken from the

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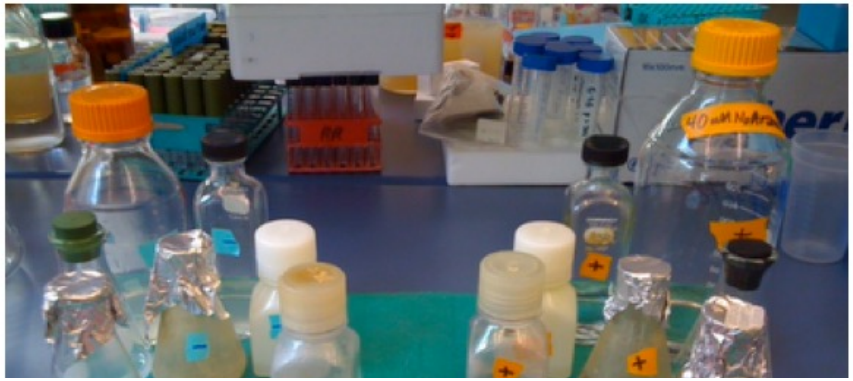
No growth in 40 mM arsenate in ANY container!

By Rosie Redfield on Monday, October 17, 2011

And excellent growth without arsenate in every container...

As I had planned, I mixed the cells (about 10^5 cfu/ml) with 500 ml of medium, then split the medium in tow, adding arsenate to one half and the same volume of water to the other. Then I distributed the cultures among all the containers and put them in the 28 °C incubator for about 36 hr (sitting on the shelf, not agitated in any way). (The biggest bottles, the ones I mixed the initial cultures in, were in the 30 °C room.)

The containers with the blue '-' labels have no arsenate, those with the orange '+' labels have 40 mM arsenate.



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Controlling for contamination in the uptake sequence set

By Rosie Redfield on Sunday, June 19, 2011

The postdoc gave me the actual numbers for the fragments with single mismatches at position 7: The input set contained 5940 of these, and the recovered (uptake) set had only 215. If we hypothesize that all of these 215 arise from contamination, then 3.6% of the fragments in the recovered pool come directly from the input pool. Because we know the exact sequence distribution of the uptake pool fragments (we sequenced 10^7 of them) we can correct the distributions in various subsets of the recovered pool for this possible contamination.

The plan is to do the main analyses with and without the correction. We don't actually know how much contamination there is, but 3.6% is the upper limit. Any results that don't change when the correction is applied are robust.

The analysis I'm most concerned about is the test for interactions between bases at different positions in the uptake sequence. The measure of interactions between positions that don't have big effects on uptake is likely to be robust, as these samples are large and removing 3.6% is unlikely to make much difference. For positions with very strong effects (6, 7, 8 and 9), the contamination correction will definitely reduce the ability to detect any interactions (because the sample size will get much smaller)...

What we see when we ignore possible contamination: When all the sequences with a mismatch at a weak position (e.g. 5) are aligned, we see

RRResearch

Not your typical science blog, but an 'open science' research blog. Watch me fumbling my way towards understanding how and why bacteria take up DNA, and getting distracted by other cool questions.

Progress at the bench

By Rosie Redfield on Wednesday, May 18, 2011

1. I made lysates of all four phages. Making lysates of HP1 phage is an act of faith, because inducing the prophage doesn't cause most of the cells in the culture to lyse. When prophage lambda is induced in a lysogenic *E. coli* culture, the culture density increased for an hour or so and then the cells all lyse (the culture goes clear, with bits of threadlike cell debris visible). But the induced HP1 lysogens just continue to get more dense, and never really appear lysed (though sometimes some debris is visible).

I've only titered the wildtype lysate because the 34°C and 41°C incubators can't be moved to our lab until next week. Despite the lack of obvious lysis, the lysate contains about 5 x 10^10 plaque-forming units per ml, which is just fine. If the temperature-sensitive mutants behaved similarly I'll have lots of phage for my recombination assays (the titer of the phage needs to be about ten times that of the cells, to get the necessary multiplicity of infection).

2. I've finished transformation assays on three of our new competence-gene knockouts. Two of the genes, *comA* and *comC* are in the big *comABCDEF* operon; both mutations are 'unmarked' deletions and not expected to interfere with expression of the downstream genes in the operon. The RA tells me that all of the genes in the homologous *Neisseria meningitidis* operon (*pilMNOPQ*) are needed for functioning of the *pilQ*-encoded secretin pore through which DNA is taken up (I haven't looked at the paper(s) yet), so we expect knockouts of these genes to completely eliminate transformation.

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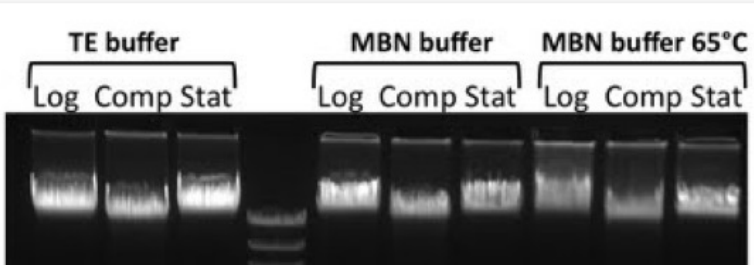
Clean boring results

By Rosie Redfield on Saturday, May 14, 2011

OK, I've done the third of the experiments I planned here, and the results cleanly show that nothing interesting is going on (at least nothing interesting that warrants investigation).

I wanted to know if the peculiarities I had long-ago noticed in DNA of competent cells were reproducible. So yesterday I grew some wildtype cells in rich medium. Some of them I collected during exponential growth ('log phase', cell density about 2 x 10^9 cells/ml), some when the culture was approaching its final density ('stationary phase', about 10^10 cells/ml), and some I transferred to the competence-inducing starvation medium while they were in log phase. I prepared chromosomal DNA from all three treatments, and then incubated it either in the standard DNA buffer TE (10 mM Tris pH 8, 1 mM EDTA) or in the mung bean nuclease buffer that had previously given anomalous results for competent-cell DNA. DNA in the mung bean nuclease buffer sat at room temperature for about an hour, with and without being heated to 65 °C for 10 minutes. Then I ran all the DNAs in a gel to check their condition.



You can see that

Archive

► 2011 (139)

▼ 2010 (143)

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Could we do the serum-survival experiment here?

Writing the recombination tract paper

FedEx, why oh why do you hate us so?

Brian Cox and Jeff Forshaw explain the big bang

What is infinity? Is the Milky Way omlette-shaped? Readers ask particle physicists to unscramble some of the universe's mysteries

How do you feel about scientists who blog their research rather than waiting to publish their final results?
Stephen Marks via email

BC: The peer review process works and I'm an enormous supporter of it. If you try to circumvent the process, that's a recipe for disaster. Often, it's based on a suspicion of the scientific community and the scientific method. They often see themselves as the hero outside of science, cutting through the jungle of bureaucracy. That's nonsense: science is a very open pursuit, but peer review is there to ensure some kind of minimal standard of professionalism.

JF: I think it's unfair for people to blog. People have overstepped the mark and leaked results, and that's just not fair on their collaborators who are working to get the result into a publishable form.

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News › Science › Brian Cox

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JF: [illegible] for people to blog. People have overstepped the mark and leaked results, and that's just not fair on their collaborators who are working to get the result into a publishable form.

RRResearch is not an attempt to circumvent peer review.

Collaborators always know in advance about the openness of my research.

The research benefits of open communication usually outweigh the risk of being scooped.

The public should be able to see how research is really done.