

Tips and tricks of the trade

Fast Lane to Microarray Probes

While some molecular biologists still design their PCR primers by eye, designing probes for microarrays without a computer is almost impossible. Thomas Kern's group at the Research Center Hagenberg and Bernhard Ronacher from the Austrian biotech company Anagnostics Bioanalysis together with Christof Sohn and Gerhard Gebauer from the University of Heidelberg have developed a new free-of-charge online probe design programme called "hybseek".

Lab Hint

Dear Editor,

After registration at www.hybseek.com, each user can use hybseek free of charge without any restrictions. Registration is necessary to administer user-specific data, for example configurations of different microarray experiments. A microarray experiment using hybseek is set up using the following simple steps:

First load the gene sequences (e.g. organisms) containing the desired DNA probes from the NCBI database. If you are configuring a microarray to typify human papilloma viruses (HPV) for example, you have to load gene sequences of HPV subtypes. To this end, you must prepare a list of accession numbers for the gene sequences. You can find these accessions at the NCBI CoreNucleotide database (www.ncbi.nlm.nih.gov, enter for example plain text 'HPV5' or 'HPV6' etc.). Of course, you may also use your own sequences from in-house sequencing, too.

Relevant gene sequences are compiled by simply checking a box in front of the displayed sequence. Adding and removing sequences by activating or deactivating the checkbox, immediately alters the composition and the influence of different sequences is easily calculated. Several sequences can be grouped to obtain a single consensus



Thomas Kern, Christian Frech and Karin Breuer (f.l.t.r) from the Hagenberg Research Center are responsible for software design and programming of hybseek, while Bernhard Ronacher from Anagnostics Bioanalysis is responsible for the hybseek concept and its validation. Rightmost: Christoph Reschreiter, Managing Director of Anagnostics Bioanalysis.

probe for the group (e.g. all high risk HPV types could be grouped). To adapt probes to different technologies, parameters like melting temperature or desired length of probes may be altered. Consideration of host genome is also feasible. You may choose between seven hosts ranging from bacteria to mouse and human. The calcula-



tion takes between a few seconds and several minutes, depending on the number of probes and the host genome selected. Calculation is done by the central server to avoid burdening client users' computers.

The results of any calculations are stored only temporarily on the server – until the user starts the next calculation or terminates the session. You should download and store all relevant results on your own computer. The number of hits (=suggested probes) can be quite numerous, especially when dealing with simple compositions. Therefore, hits are ordered along their calculated specificity (=quality) and some additional information like length, GC content, affinity to form homodimers or hairpins and uniqueness of 3' ending is shown. You can add a comment to each hit and store them in a table.

Please download the hybseek quickstart tutorial at www.hybseek.com for more details.

(Thomas Kern, Christian Frech, Karin Breuer, Upper Austria University of Applied Science, Hagenberg, Austria; Bernhard Ronacher, Anagnostics Analysis, Linz, Austria)

Highlight features of hybseek

- ▶ Consideration of host genome (Homo sapiens, Drosophila melanogaster, Caenorhabditis elegans, Escherichia coli K12, Mus musculus, Rattus norvegicus and Saccharomyces cerevisiae)
- ▶ Configuration of melting temperature and length of probes
- ▶ Calculation of specificity and GC content
- ▶ Guaranteed 3' uniqueness
- ▶ Affinity to form homodimers or hairpins
- ▶ Grouping of sequences and calculation of consensus probes
- ▶ Support of selection of forward/reverse sequences

Do you have any useful tips?

Contact us at:

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