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BD Accuri C6 应用

- 海洋
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Simple 简单安装、简单操作、简单维护

Multiplex 多重功能 - 激光多种模块、滤片多种规格、上样方式灵活多样 - 满足多种不同应用

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8. BD Accuri C6 在海洋研究中的应用

8.1 海洋藻类研究

8.1.1 Enhancement of BODIPY^{505/515} lipid fluorescence method for applications in biofuel-directed microalgal production

出处：Journal of Microbiological Methods 90 (2012) 137–143

摘要：This paper describes a microalgal cell lipid fluorescence enhancement method using BODIPY^{505/515}, which can be used to screen for lipids in wild-type microalgae and to monitor lipid content within microalgal production processes to determine optimal harvesting time. The study was based on four microalgal species (Dunaliella teteriolecta, Tetraselmis suecica, Nannochloropsis oculata, and Nannochloris atomus) selected because of their inherent high lipid content. An extended analysis was carried out with N. oculata due to the depressed fluorescence observed when compared with the other experimental strains. BODIPY^{505/515} lipid fluorescence was determined for two solvent pre-treatment methods (DMSO and glycerol) and four staining condition parameters (analysis time, staining temperature, dye concentration, and algal cell concentration). It was found that lipid fluorescence of thick cell-walled microalgae, such as N. oculata, is significantly enhanced by both the pre-treatment methods and staining condition parameters, thereby significantly enhancing lipid fluorescence by ca. 800 times the base autofluorescence. The lipid fluorescence enhancement method provides a quick and simple index for in vivo Flow Cytometry quantification of total lipid contents for purposes of species screening or whole culture monitoring in biofuel-directed microalgal production.

方法学：Evaluation of BODIPY^{505/515} lipid fluorescence characteristics

A 500 μM stock solution of BODIPY^{505/515} (4,4-Difluoro-1,3,5,7-Tetramethyl-4-Bora-3a-Diaza-s-Indacene; Life Technologies, Dublin, Ireland) was made by dissolving the dye in anhydrous dimethyl sulfoxide (DMSO) according to Cooper et al. (2010). Initially, a baseline staining method was established whereby aliquots of the stock solution were added directly to algal suspensions to achieve a final staining concentration of 0.12 μg mL^{-1} and 2% DMSO, which was then shaken gently by hand for 10 s and analysed immediately. The establishment of the baseline staining method permitted a comparison between the four experimental algal strains which were monitored over an 11 day growth period. Each sample was analysed for its background autofluorescence, BODIPY^{505/515} fluorescence, and chlorophyll fluorescence. Fluorescence measurements were taken in triplicate before and after staining by excitation with a blue laser at 488 nm and emission measured in the range of 530±15 nm for BODIPY^{505/515} and 670LP nm for chlorophyll. Each measurement duration was made for 60 s using a BD Accuri C6 Flow Cytometer (BD Accuri, Michigan, USA) while flow cytometry was initially used to isolate live algal cells from the measured chlorophyll fluorescence (i.e., cells with chlorophyll fluorescence were considered to be live (Marie et al. 1999), chlorophyll fluorescence >1000 mean fluorescence intensity (MFI)); these cells were subsequently analysed with CFlow and FCExpress software to determine the cell concentration, autofluorescence, and BODIPY^{505/515} fluorescence.

结果：

![Fig. 3. Bivariate histograms of BODIPY^{505/515} stained fluorescence for N. oculata for stain injection at 2 min into the analysis for (A) Staining (0.12 μg mL^{-1}) without pre-treatment, and (B) Staining (0.12 μg mL^{-1}) after 0.06 g mL^{-1} DMSO pre-treatment.](image)
8.1.2 High-efficiency homologous recombination in the oil-producing alga Nannochloropsis sp.

出处: PNAS | December 27, 2011 | vol. 108 | no. 52 | 21265–21269

摘要: Algae have reemerged as potential next-generation feedstocks for biofuels, but strain improvement and progress in algal biology research have been limited by the lack of advanced molecular tools for most eukaryotic microalgae. Here we describe the development of an efficient transformation method for Nannochloropsis sp., a fast-growing, unicellular alga capable of accumulating large amounts of oil. Moreover, we provide additional evidence that Nannochloropsis is haploid, and we demonstrate that insertion of transformation constructs into the nuclear genome can occur by high-efficiency homologous recombination. As examples, we generated knockouts of the genes encoding nitrate reductase and nitrite reductase, resulting in strains that were unable to grow on nitrate and nitrate/nitrite, respectively. The application of homologous recombination in this industrially relevant alga has the potential to rapidly advance algal functional genomics and biotechnology.

方法学: Screening and Analysis of Knockout Mutants. Initial screen. Zeocin-resistant colonies obtained by transformation with either NiR-KO or NR-KO were resuspended in 50 μL F/2 medium lacking any nitrogen source, and 6 μL was spotted on agar plates containing 1 mM KNO₃ or 1 mM NH₄Cl as a sole nitrogen source. Many clones started to bleach on plates containing nitrate, indicating starvation for a nutrient, whereas no signs of starvation were visible on plates containing NH₄Cl (Fig. 3).

PCR screen. Genomic DNA was isolated, and PCR with primers 5'-AGTAGGCGTAGCCTTGGAGTTTGT-3' and 5'-TCTGACAGCAGCAGCCT-3' on NR-KO mutants or with primers 5'-ACGGTGGAAGAGATGGTGAGAGAA-3' and 5'-AAGCTTAAGAAGGACGGCTCGGTA-3' on NiR-KO mutants was used to amplify the genomic DNA around the NR or NiR gene, respectively. PCR on genomic DNA isolated from the wild type was used as a control. Growth test. Wild type and two clones each of confirmed NR- and NiR-KO mutants (NR1, NR2, NiR1, and NiR2) were grown to mid-log phase in F2N medium containing 1 mM NH₄Cl. Cells were washed three times with 50% artificial seawater by centrifugation (5 min, 3,000 × g) and subsequent resuspension of the cells. Beakers with a clear lid containing 100 mL of F2N medium with no nitrogen source, 1 mM KNO₃, 1 mM NaNO₂, or 1 mM NH₄Cl were inoculated in triplicate with washed cells to a concentration of 4 × 10⁵ cells/mL and allowed to grow under 3% CO₂ atmosphere at 200 μmol photons·m⁻²·s⁻¹ for 4 d under constant shaking (80 rpm). At this time, wild-type cultures supplemented with 1 mM NH₄Cl reached stationary phase after exhausting the nitrogen source. Cells were counted with an Accuri C6 flow cytometer equipped with an Accuri C6 sampler in duplicate. Growth was estimated as % cells compared with wild-type cultures grown in F2N medium containing 1 mM NH₄Cl.

结果: Fig. 4. Growth of wild-type, NR-KO (NR1 and NR2), and NiR-KO (NiR1 and NiR2) strains with different nitrogen sources. Cells in mid-log phase were washed in nitrogen-free medium, resuspended in media with the indicated nitrogen sources, and allowed to grow to early stationary phase. Results are expressed relative to the wild type in 1 mM NH₄Cl, and SDs for three independent cultures of each strain are indicated.
8.1.3 Microflow Cytometer for optical analysis of phytoplankton

出处：Biosensors and Bioelectronics 26 (2011) 4263– 4269

摘要：Analysis of the intrinsic fluorescence profiles of individual marine algae can be used in general classification of organisms based on cell size and fluorescence properties. We describe the design and fabrication of a Microflow Cytometer on a chip for characterization of phytoplankton. The Microflow Cytometer measured distinct side scatter and fluorescence properties of Synechococcus sp., Nitzschia d., and Thalassiosira p.; measurements were confirmed using the benchtop Accuri C6 flow cytometer. The Microflow Cytometer proved sensitive enough to detect and characterize picoplankton with diameter approximately 1 μm and larger phytoplankton of up to 80 μm in length. The wide range in size discrimination coupled with detection of intrinsic fluorescent pigments suggests that this Microflow Cytometer will be able to distinguish different populations of phytoplankton on unmanned underwater vehicles.

方法学：Phytoplanktonic species Cultures of the three different algae were purchased from the Culture Collection of Algae (UTEX, Austin, TX). More information about the cultures is supplied in ESI. There are three classes of pigments present in these algae: chlorophylls, phycobilins, and carotenoids. Chlorophyll is a green pigment present in all phytoplankton, plants and cyanobacteria and can be used to discriminate phytoplankton from most other marine particles in a similar size range. Chlorophyll a is the primary photosynthetic pigment with a maximum absorption at 675 nm; however, chlorophyll also absorbs well at ~450 nm. Chlorophyll c couples photonic energy to chlorophyll a and is found in diatoms and dinoflagellates. Phycocyanin and phycoerythrin are present in cyanobacteria; phycoerythrin absorbs at 495 and 545–565 nm and fluoresces at 575 nm. Carotenoids are lipid-soluble pigments absorbing light in the blue to cyan region of approximately 450–490 nm. The phytoplanktonic species used for this work are shown in Table 1.

Prior to introduction into the Microflow Cytometer or Accuri C6, the algae were gently stirred to dissociate aggregates. Observation of the stirred samples under the microscope confirmed that most of the aggregates were dispersed and that there was visible heterogeneity among the cells in each population.

結果：

Fig. 4. Scatter plots of the phycoerythrin (y-axis) plotted vs. chlorophyll fluorescence (x-axis) for each individual cell are shown in (a and b). (c and d) depict scatter plots of chlorophyll fluorescence vs. side scatter, and (e and f) represent scatter plots of phycoerythrin fluorescence vs. side scatter. (a, c, and e) are the data obtained using the Microflow Cytometer and (b, d, and f) show the Accuri flow cytometer results. Synechococcus sp. is represented by red dots, Nitzschia d. by green dots, and Thalassiosira p. by purple dots. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
8.2 海洋药物学研究

8.2.1 New approach to modulate retinal cellular toxic effects of high glucose using marine EPA and DHA

出处：Nutrition & Metabolism 2011, 8:39

摘要：Background: Protective effects of omega-3 fatty acids against cellular damages of high glucose were studied on retinal pigmented epithelial (RPE) cells. Methods: Retinal epithelial cells were incubated with omega-3 marine oils rich in EPA and DHA and then with high glucose (25 mM) for 48 hours. Cellular responses were compared to normal glucose (5 mM): intracellular redox status, reactive oxygen species (ROS), mitochondrial succinate deshydrogenase activity, inflammatory cytokines release and caveolin-1 expression were evaluated using microplate cytometry, ELISA and flow cytometry techniques. Fatty acids incorporation in retinal cell membranes was analysed using chromatography. Results: Preincubation of the cells with fish oil decreased ROS overproduction, mitochondrial alterations and TNFα release. These protective effects could be attributed to an increase in caveolin-1 expression induced by marine oil. Conclusion: Marine formulations rich in omega-3 fatty acids represent a promising therapeutic approach for diabetic retinopathy.

方法学：Immunofluorescence for caveolin-1 expression The culture cells were harvested with trypsin-EDTA, pelleted, washed twice in PBS, and fixed in paraformaldehyde. After permeabilization, the cells were incubated with anti-caveolin-1 antibody for 45 minutes. After wash, the cells were incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibody for 45 minutes. Flow cytometric quantitation of fluorescence was measured using C6-Flow® cytometer (Accuri, France).

结果：

Figure 5 Evaluation of caveolin-1 expression. The RPE cells were incubated with culture medium (left chart) or YS-2636 oil (right chart) for 15 minutes. After the cells were rinsed with phosphate buffer, the cells were incubated with culture medium for 24 hours and fixed in PFA. The expression of caveolin-1 was quantified using flow cytometry. Figures are representative of three independent experiments.

8.2.2 Synthesis and biological evaluation of analogues of the marine alkaloids granulatimide and isogranulatimide


摘要：A series of pyrrolic analogues and two series of regioisomeric pyrazolic analogues of the marine alkaloids granulatimide and isogranulatimide were prepared. The synthesis of the two first ones was based on the condensation reaction of diversely 5-substituted 3-bromoindoles with pyrrole or pyrazole followed by addition of the intermediates on maleimide or dibromomaleimide respectively, the so-obtained acyclic adducts being finally photocyclized to the desired analogues. Compounds of the last series were obtained by reacting different 5-substituted-indole-3-glyoxylates with N-Boc-pyrazole-3-acetamide and subsequent photochemical cyclization of the adducts. All the compounds were evaluated for their in vitro growth inhibitory properties toward eight cancer cell lines. Several compounds were also assayed for their ability to abrogate the G2-cell cycle checkpoint or to inhibit a panel of Ser/Thr kinases. Lastly, computer-assisted phase-contrast microscopy (quantitative videomicroscopy) revealed that the three most potent compounds (4a, 9a, 9e), with IC50 growth inhibitory concentrations ranging between 10 and 20 μM, displayed cytostatic, not cytotoxic, anticancer effects.

方法学：Cell culture and checkpoint abrogation evaluation HCT116 p53-/– cells (a generous gift of B. Vogelstein) were grown in DMEM (Invitrogen) supplemented with 10% FCS. Etoposide (Sigma) was applied to the cells for one hour at a concentration of 40μM, before being washed extensively. Checkpoint recovery experiment was performed essentially as described previously [6]. The AZD7762 Chk1 inhibitor was generously provided by Dr Sonya Zabludoff (AstraZeneca). Cells were harvested and fixed with cold 70% ethanol. They were subsequently stained with either anti-phospho H3 Ser-10 antibodies or the monoclonal 3.12.I.22 [28] that were revealed using Alexa Fluor 488 conjugated antibodies. DNA was stained with Propidium Iodide (Sigma). DNA content and mitotic index were determined using an Accuri C6 flow cytometer.

结果：

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mitotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
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</tr>
<tr>
<td>4a</td>
<td>23.9</td>
</tr>
<tr>
<td>4b</td>
<td>13.9</td>
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<tr>
<td>4d</td>
<td>3.9</td>
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