

Schedule of Major Meetings of the American Chemical Society

Meeting	Year	Dates	Location
208th	Fall 1994	August 21-25	Washington, D.C.
209th	Spring 1995	April 2-6	Anaheim, CA
210th	Fall 1995	August 20-24	Chicago, IL
1995 INTERNATIONAL CHEMICAL CONGRESS OF PACIFIC BASIN SOCIETIES		December 17-22	Honolulu, HI
211th	Spring 1996	March 24-29	New Orleans, LA
212th	Fall 1996	August 18-23	Boston, MA
213th	Spring 1997	April 13-18	San Francisco, CA
214th	Fall 1997	September 7-12	Las Vegas, NV
1997 FIFTH CHEMICAL CONGRESS OF NORTH AMERICA		November/December	Cancun, Mexico
215th	Spring 1998	March 29 - April 3	St. Louis, MO
216th	Fall 1998	August 16-21	Orlando, FL
217th	Spring 1999	March 21-26	Anaheim, CA
218th	Fall 1999	August 22-27	New Orleans, LA
219th	Spring 2000	March 26-31	Las Vegas, NV
220th	Fall 2000	August 20-25	Washington, DC
221st	Spring 2001	April 1-6	San Francisco, CA
222nd	Fall 2001	August 19-24	Chicago, IL
223rd	Spring 2002	April 7-12	Orlando, FL
224th	Fall 2002	September 8-13	Boston, MA
225th	Spring 2003	March 23-28	New Orleans, LA
226th	Fall 2003	September 7-12	New York, NY

ABSTRACTS OF PAPERS

Part 1

207th ACS National Meeting

0-8412-2774-8

American Chemical Society

San Diego, CA.

March 13-17, 1994

interaction modes of the energy refined antibody to its antigen using novel docking techniques. We present an application of this protocol to the anti-renin monoclonal antibody R1.20.5, which was sequenced in our laboratory, and derived its binding mode to the crystal structure of human renin. The optimal binding mode, in which the H2 loop of the complementarity determining regions inserts into the active cleft of the renin molecule, is presented.

- 117. TRANSPLANTATION OF BONE MARROW CULTURES INTO RATS USING BIORESORBABLE POLYMER TEMPLATES.** B. A. Naughton, J. San Román, B. Sibanda, and V. Kamali. Hematology Laboratory, Advanced Tissue Sciences, Inc., La Jolla, California 92037.

A transplantable bone marrow (BM) tissue was developed by co-culturing BM stroma with various hematopoietic cell populations of Long-Evans rats on templates composed of randomly arranged fibers of polyglycolic acid (PGA), a bioresorbable material. BM stromal cells were inoculated onto PGA felt and seeded ~ 7 days later with Thy 1.1⁺ cells (comprising the stem and progenitor cell populations of rat BM) or unselected BM hematopoietic cells. After 2 weeks in culture, the constructs were grafted subcutaneously or into vascular folds of the omentum and mesentery. Grafts of BM stroma co-cultured with both Thy 1.1⁺ cells and unselected BM hematopoietic cells displayed active hematopoietic foci at all implantation sites when examined 30, 60, and 110 days post grafting. Differentiation of immature BM cells *in situ* was implied by the clusters of Thy 1.1⁺, OX-33⁺ (B cell), MOM/3F12/F3⁺ (myeloid), and ED-1⁺ (monocyte/macrophage) cells which were present. The potential advantages of transplanting BM in this manner include: associations of BM stromal and hematopoietic cells that form *in vitro* are maintained, the relative populations of stem and progenitor cells can be controlled, and lyophilized growth/regulatory factors can be incorporated into PGA and other bioresorbable materials during their synthesis, thus allowing for the time release of these factors locally for the putative benefit of the transplanted hematopoietic cells. This method may be applicable to the treatment of those with microenvironmental BM defects.

- 118. ENGRAFTMENT OF LIVER TISSUE GROWN IN VITRO.** B. A. Naughton, B. Sibanda, J. San Román, and V. Kamali. Hematology Laboratory, Advanced Tissue Sciences Inc., La Jolla, California 92037.

Adult rat hepatic parenchymal cells (PC) and stromal cells were co-cultured on nylon filtration screens (NS) or biodegradable polyglycolic acid (PGA) templates. Stromal (vascular and biliary endothelial cells, fat storing cells, fibroblasts, and Kupfer cells) were isolated by gradient centrifugation after liver perfusion, expanded in monolayer culture, and seeded onto either NS or PGA felt-like constructs. A second inoculum of freshly isolated PC was applied after the stromal cells became established. PC proliferated in association with stroma on NS until all available space for expansion within the template was exhausted; after 4-5 weeks 7-9 "layers" of PC filled the 140 µm deep template. Dioxin-inducible cytochrome P450 enzyme activity was detected for at least 56 days in culture and the synthesis of albumin, fibrinogen, transferrin, soluble and matrix-associated fibronectin, collagen type III, and laminin was evident based on ELISA assays of the spent medium and immunohistochemistry of sections through the cultures. PC and liver stromal cells were co-cultured on bioresorbable PGA felt constructs for ~ 24 days *in vitro* and grafted into sites in the mesentery, omentum, and subcutaneous tissues of adult Long-Evans rats. The growth of hepatocytes after 30 days *in situ* was evident by histological analysis and grafts of co-cultures regenerated a liver-like architecture consisting of sinusoids and biliary structures. In addition, PC at these extrahepatic graft sites were positive for albumin, transferrin, and fibrinogen synthesis by immunohistochemistry. Graft survival was enhanced when recipients were subjected to ~ 40% hepatectomy. Hepatic PC:stromal cell co-cultures may prove useful in the restoration of liver function either by direct transplantation using PGA or similar templates or as extracorporeal devices, using nylon screens.

- 119. RANDOM PRIMER LABELING OF DNA USING THERMOSTABLE POLYMERASES.** L. S. Basehore, C. J. Hansen, J. C. Braman. Stratagene, 11099 N. Torrey Pines Rd., La Jolla, CA 92037

Through the use of a novel thermostable polymerase and dehydrated reaction components, a random primer labeling kit, that produces high-specific activity radioactive DNA probes can now be shipped and stored at room temperature. The Prime-It[®] RmT kit generates probes with specific-activities greater than 1×10^9 dpm/µg in just 5 minutes. These probes are capable of detecting 1.2 picograms of target in 0.5 µg of human genomic DNA after overnight exposure to X-ray film. Individually aliquotted reactions can be conveniently stored at room temperature and are guaranteed to maintain full functional activity for at least 6 months.

- 120. IMPROVED METHODS FOR NUCLEIC ACID PURIFICATION.** W. Callen, C. Hansen, J. Anderson. Stratagene, 11099 N. Torrey Pines Rd., La Jolla, CA 92037

The need for rapid and efficient procedures for purifying nucleic acid has been met by the development of improved systems for isolating DNA and RNA. Plasmid DNA can be quickly isolated from lysed bacterial cultures by binding the DNA to high silica resin. This 20 minute procedure requires no organic extraction or precipitation and yields DNA suitable for cloning, sequencing, transformations, etc. Total RNA can be isolated from small samples of cells or tissue through a 30 minute, two-phase separation system. This procedure yields high amounts of nuclease-free intact RNA which can be used for a hybridization template or cDNA conversion/PCR amplification. Alternatively, messenger RNA can be isolated directly from homogenized cells or tissue through affinity binding to a solid support medium which contains oligo-dT bound to its surface.

- 121. DNA SEQUENCE DETERMINATION USING EXONUCLEASE-DEFICIENT *Pfu* DNA POLYMERASE IN A CYCLE SEQUENCING FORMAT.** Valerie Hedden, Jeff Braman and Keith Kretz. Stratagene, 11099 N. Torrey Pines Road, La Jolla, CA 92037.

Cycle sequencing utilizes a DNA polymerase and dideoxynucleotide triphosphates to generate chain termination sequencing ladders in a thermal cycling profile. These characteristics allow smaller amounts of DNA template to be sequenced with less template preparation. We have chosen to use 3'-5' exonuclease deficient *Pyrococcus furiosus* (*Pfu*) polymerase because it shows no discrimination against α-thionucleotides. This allows the efficient labeling of the sequencing reactions with ³⁵S-dATP. In addition to the standard sequencing templates (plasmids and M13), we demonstrate that PCR products, purified lambda and cosmid DNA, as well as lambda or M13 phage DNA in plaques will serve as templates. Cycle sequencing greatly expands the ability to generate sequence data from non-traditional templates while utilizing a more time efficient protocol.

- 122. CHEMILUMINESCENT DETECTION OF DNA AND RNA IMMOBILIZED ON MEMBRANES.** M. Jerpseth, S. Ransom, J. Braman, H. Huang, J. Anderson and J. Moores. Stratagene, 11099 N. Torrey Pines Rd., La Jolla, CA 92037.

The cost and difficulty of radioactive disposal is increasing and this necessitates the development of non-isotopic alternatives. We have developed a non-isotopic detection system for nucleic acids immobilized on membranes. The kit is designed to be used in conjunction with Stratagene's Prime-It[®] Fluor fluorescence labeling kit which incorporates fluorescent nucleotides into randomly primed DNA probes. After hybridization to nucleic acid targets bound to membrane, the fluorescein labeled probe is detected with anti-fluorescein antibody conjugated to alkaline phosphatase and a dioxetane substrate for the enzyme. Upon cleavage, the substrate generates a chemiluminescent signal which can be detected on X-ray film. By Southern analysis, the kit allows detection of 0.1 pg of plasmid target DNA in a 30 minute exposure of the processed filter to X-ray film or, in a similar exposure time, 1 pg of a single copy gene can be detected in less than 0.5 µg of human genomic DNA. This system can also be used for rapid and sensitive northern blot analysis of RNA.

- 123. VECTOR SYSTEMS FOR DIRECTIONAL AND BIDIRECTIONAL CLONING OF PCR-GENERATED DNA FRAGMENTS AND HIGH-LEVEL PROTEIN EXPRESSION.** Peter Vaillancourt, Gina L. Costa and Michael P. Weiner. Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037.

We report the development of one-hour procedures for the efficient and high yield cloning of blunt-ended PCR-generated DNA fragments. Bidirectional cloning is accomplished by incubation of a PCR fragment in a solution containing the restriction enzyme *SrfI* (target sequence 5'-GCCCGGGC-3'), *SrfI*-linearized plasmid vector DNA [pCR-Script SK(+)], and T4 DNA ligase. Directional cloning is achieved by enzymatically processing a linearized vector to contain a single terminal 5' phosphate. A PCR fragment, also containing a single 5' phosphate (created by