Acute lymphoblastic leukemia (ALL) is characterized by the uncontrolled proliferation of lymphoblasts which have been arrested in an early stage of B-cell differentiation. A key obstacle in identifying biomarkers of malignant B-cells in ALL patients has been constructing a cDNA library of normal B-lineage committed lymphoblasts from pediatric bone marrow for comparison. In order to identify unique biomarkers, a relatively quick method for isolating B cell subsets from pediatric bone marrow for RNA expression profiling is in development. B cells were magnetically sorted from bone marrow aspirate by immunophenotype into pro-B, pre-B, and immature B-cell subsets using antibodies covalently linked to paramagnetic microbeads. RNA was isolated and amplified from the subsets then probed using RT-PCR for expression of B-lineage marker (PAX5), myeloid-lineage marker (MPO), terminal deoxynucleotidyltransferase (DNTT), and immunoglobulin µ-heavy chain (IGHM) to determine the efficacy of the separation. Both PAX5 and IGHM expression were directly proportional to B-cell maturity with PAX5 expression residing between the positive (RL) and negative (U266) controls. Expression of DNTT was inversely related to B-cell maturity. Expression of MPO in all B cell subsets was higher than in RL, but parallel to the AML cell line, KG-1. The expression patterns of PAX5, IGHM, and DNTT were consistent with successful separation of the B-cells into pro-B, pre-B, and immature B subsets, but further validation of this method with flow cytometry is necessary.