

Program Number: 236

Human B-cell development is a regulated process characterized by the ordered differential expression of numerous cell surface and intra-cytoplasmic antigens, immunoglobulin gene rearrangements, and ultimately class switching. This process originates from self-renewing hematopoietic stem cells in the fetal liver and postnatal bone marrow and ends with the ultimate export of mature naive B-cells from the marrow into the peripheral blood circulation.

B-cell precursors have been extensively studied in mouse and human systems and there is general agreement that CD34 and CD38 help identify early B-cell progenitors. However, the relative order of CD19 and CD10 up-regulation is not well established, with numerous conflicting published reports.

Recently, a new type of modeling program has been developed that allows a detailed objective analysis of the relative order of antigen up- and down-regulation for high-dimensional cytometry data. In this study, Probability State Modeling was used to determine the relative order of CD38, CD19, and CD10 up-regulation for a number of uninvolved bone marrow specimens. It also provided detailed correlated information on other cytometric features such as forward angle light scatter (FSC), side scatter (SSC), CD26, CD9, and CD81.

Probability State Modeling Analysis of CD38, CD10, and CD19

Up-regulation in Early Human B-Cell Development

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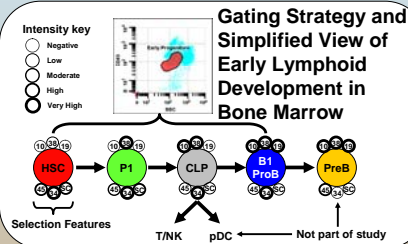
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Introduction

Early development of human B-cells occurs in distinct steps or stages in the bone marrow (BM). Many of these stages are defined by changes in the rearrangement status of immunoglobulin (Ig) heavy (H) and light chains (L), the expression of transcription factors, as well as specific phenotypic changes on the cell surface. Although much is known about B-cell development from a molecular genetics point-of-view, the functions of many of the surface and cytoplasmic proteins that appear and disappear as progenitor and B-cells develop remain unknown. Also, the literature contains a number of conflicting reports on the progression of many of these phenotypic changes. The exact timing of these cellular changes are important to document since many basic science studies are based on purification methods that use specific marker protein combinations. Also, many hematologic malignancies are defined in terms of having similar phenotypic patterns to these normal stages of differentiation.

This study examines very early lymphoid and B-cell phenotypic changes by the technique of Probability State Modeling (PSM). PSM uses a relatively objective and accurate modeling approach that orders events along a progression axis. Once ordered, the correlated changes of other cell features can also be studied. The cellular features examined are CD34, CD38, CD10, CD19, CD45, CD9, CD81, forward angle scatter (FS), and side scatter (SC).

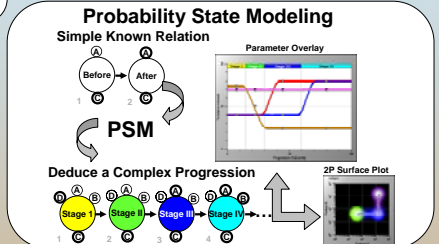
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A simplified consensus view of early lymphoid development is shown above along with the gating strategy used in this study. The selection features for the studied developmental progression were CD34 and the above early progenitor, CD45/SSC gating region. The progression features for the studied developmental progression were CD38 and CD19. The cells selected by feature C (intensity) are A, but later are A*. With this simple relation it is possible to model the progression of the cells selected by feature C (intensity) are A, but later are A*. With this simple relation it is possible to model the progression of the cells selected by feature C (intensity) are A, but later are A*. With this simple relation it is possible to model the progression of the cells selected by feature C (intensity) are A, but later are A*.

Many of these stages such as HSC have incomplete phenotypes and may not represent their true defined stage. Also, the existence of some of these stages is controversial.

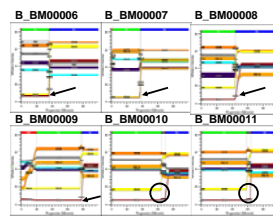
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Probability State Modeling (PSM) analyses were performed on 13 high-dimensional flow cytometry data files from clinical samples obtained by University of Washington's Cytometry Laboratory. Only very early progenitors and early B-cells were selected for the analysis (CD34+, gated on CD45/SSC). The model in this study. The only information provided in the model was that CD38, CD19, and CD10 up-regulate at some point in the progenitor progression. The progression was divided into four stages. The first progenitor stage (HSC, red) was defined as events that were CD38- or in transition to CD38-. The second progenitor stage (P1, green) ended when CD19 up-regulated. The third progenitor stage (CLP, grey) ended when CD19 up-regulated, and the fourth progenitor stage directly followed the third (B1, blue).

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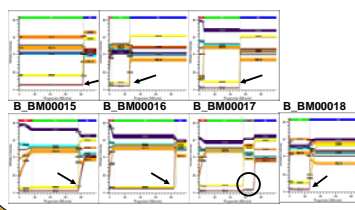
Probability State Modeling Results



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Probability State Modeling Results



In six of the thirteen samples, CD19 and CD10 apparently up-regulated nearly together (black arrows); whereas, in three samples, CD10 seemed to up-regulate slightly before CD19 (blue open circles). Clear partition observations were that PSM was highest in the P1 stage. CD34, CD45, and SSC stepped down during the HSC, P1, CLP, and B1 stages for many of the files.

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CD10 and CD19 Positions

Files	CD19 %	CD10 %	Diff	Cytometry Features and Modeled/Total Events
B_BM00006	36.83	36.38	0.45	CD45,19,34,38,45,46,54,56,58,62,64,66,68,70,72,74,76,78,80,82,84,86,88,90,92,94,96,98,100,102,104,106,108,110,112,114,116,118,120,122,124,126,128,130,132,134,136,138,140,142,144,146,148,150,152,154,156,158,160,162,164,166,168,170,172,174,176,178,180,182,184,186,188,190,192,194,196,198,200,202,204,206,208,210,212,214,216,218,220,222,224,226,228,230,232,234,236,238,240,242,244,246,248,250,252,254,256,258,260,262,264,266,268,270,272,274,276,278,280,282,284,286,288,290,292,294,296,298,300,302,304,306,308,310,312,314,316,318,320,322,324,326,328,330,332,334,336,338,340,342,344,346,348,350,352,354,356,358,360,362,364,366,368,370,372,374,376,378,380,382,384,386,388,390,392,394,396,398,400,402,404,406,408,410,412,414,416,418,420,422,424,426,428,430,432,434,436,438,440,442,444,446,448,450,452,454,456,458,460,462,464,466,468,470,472,474,476,478,480,482,484,486,488,490,492,494,496,498,500,502,504,506,508,510,512,514,516,518,520,522,524,526,528,530,532,534,536,538,540,542,544,546,548,550,552,554,556,558,560,562,564,566,568,570,572,574,576,578,580,582,584,586,588,590,592,594,596,598,600,602,604,606,608,610,612,614,616,618,620,622,624,626,628,630,632,634,636,638,640,642,644,646,648,650,652,654,656,658,660,662,664,666,668,670,672,674,676,678,680,682,684,686,688,690,692,694,696,698,700,702,704,706,708,710,712,714,716,718,720,722,724,726,728,730,732,734,736,738,740,742,744,746,748,750,752,754,756,758,760,762,764,766,768,770,772,774,776,778,780,782,784,786,788,790,792,794,796,798,800,802,804,806,808,810,812,814,816,818,820,822,824,826,828,830,832,834,836,838,840,842,844,846,848,850,852,854,856,858,860,862,864,866,868,870,872,874,876,878,880,882,884,886,888,890,892,894,896,898,900,902,904,906,908,910,912,914,916,918,920,922,924,926,928,930,932,934,936,938,940,942,944,946,948,950,952,954,956,958,960,962,964,966,968,970,972,974,976,978,980,982,984,986,988,990,992,994,996,998,1000
B_BM00007	38.10	38.21	-0.11	CD45,19,34,38,45,46,54,56,58,62,64,66,68,70,72,74,76,78,80,82,84,86,88,90,92,94,96,98,100,102,104,106,108,110,112,114,116,118,120,122,124,126,128,130,132,134,136,138,140,142,144,146,148,150,152,154,156,158,160,162,164,166,168,170,172,174,176,178,180,182,184,186,188,190,192,194,196,198,200,202,204,206,208,210,212,214,216,218,220,222,224,226,228,230,232,234,236,238,240,242,244,246,248,250,252,254,256,258,260,262,264,266,268,270,272,274,276,278,280,282,284,286,288,290,292,294,296,298,300,302,304,306,308,310,312,314,316,318,320,322,324,326,328,330,332,334,336,338,340,342,344,346,348,350,352,354,356,358,360,362,364,366,368,370,372,374,376,378,380,382,384,386,388,390,392,394,396,398,400,402,404,406,408,410,412,414,416,418,420,422,424,426,428,430,432,434,436,438,440,442,444,446,448,450,452,454,456,458,460,462,464,466,468,470,472,474,476,478,480,482,484,486,488,490,492,494,496,498,500,502,504,506,508,510,512,514,516,518,520,522,524,526,528,530,532,534,536,538,540,542,544,546,548,550,552,554,556,558,560,562,564,566,568,570,572,574,576,578,580,582,584,586,588,590,592,594,596,598,600,602,604,606,608,610,612,614,616,618,620,622,624,626,628,630,632,634,636,638,640,642,644,646,648,650,652,654,656,658,660,662,664,666,668,670,672,674,676,678,680,682,684,686,688,690,692,694,696,698,700,702,704,706,708,710,712,714,716,718,720,722,724,726,728,730,732,734,736,738,740,742,744,746,748,750,752,754,756,758,760,762,764,766,768,770,772,774,776,778,780,782,784,786,788,790,792,794,796,798,800,802,804,806,808,810,812,814,816,818,820,822,824,826,828,830,832,834,836,838,840,842,844,846,848,850,852,854,856,858,860,862,864,866,868,870,872,874,876,878,880,882,884,886,888,890,892,894,896,898,900,902,904,906,908,910,912,914,916,918,920,922,924,926,928,930,932,934,936,938,940,942,944,946,948,950,952,954,956,958,960,962,964,966,968,970,972,974,976,978,980,982,984,986,988,990,992,994,996,998,1000
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