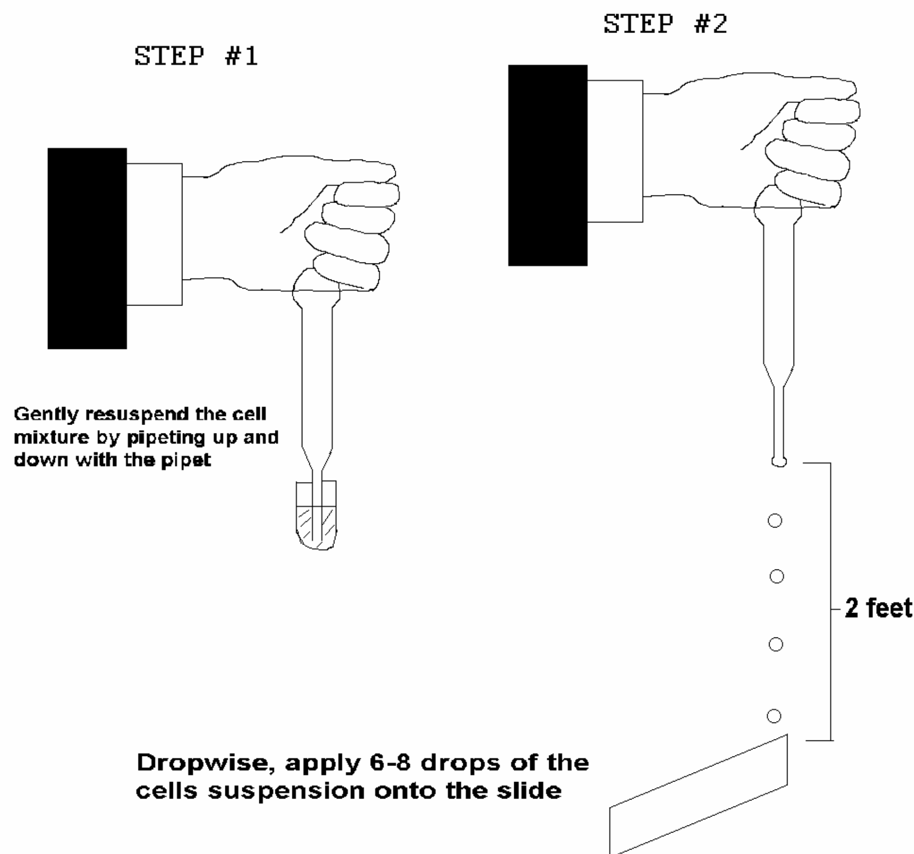
Karyotype of HeLa Cells

Procedures

1. The slides used can be either:
    a. Dry and at room temperature, or
    b. Placed in room temperature water prior to use.
2. Place the wet or dry slide vertically at a 45º angle.
3. With a pipette, **Gently Resuspend** the cells in the tube provided. Remove a small sample of cell suspension with a pipette and hold the pipette 2 feet above the slide. Allow **one drop** of cell suspension to "splat" onto the slide about ¾ inch from the upper end and tumble down the slide. Carefully apply 6-8 more drops from various heights, **One drop at a time, suspension one drop-at-a-time. Do not expel all of your cell suspension in one squirt, or you will obtain poor results.** Gently blow across the slide for 2-3 seconds. The drying will help "spread" the chromosomes.



4. Allow the cells to AIR DRY COMPLETELY.
5. Dip the slide into the tube containing STAIN #1 for **1 Second only.**
   Remove the slide and dip into STAIN #1 again for **1 Second only.**
   Remove the slide and dip into STAIN #1 again for **1 Second only.**
6. Drain off stain and dip the slide into tube containing STAIN #2 for **1 Second only**.
   Remove the slide and dip into STAIN #2 again for **1 Second only.**

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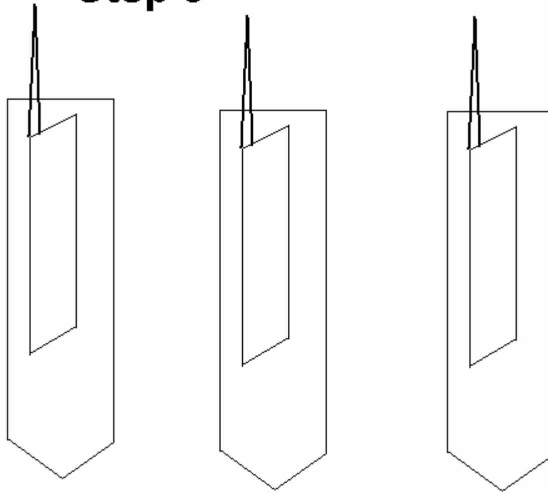
Caution should be taken to avoid carryover of stains (wipe the bottom of slide with a paper towel before transferring).



7. Remove slide from stain am thoroughly rinse with distilled water.
8. Allow slide to AIR DRY COMPLETELY. A stream of warm air or blowing may help speed up the drying process. Incomplete drying will result in very poor resolution when the mounting medium (Permount) is added.
9. Place 2 drops of Permount on the stained area of your slide and place a #1 coverslip over the Permount. Apply gentle pressure to the coverslip to spread the Permount evenly under the coverslip. You may with to place 2 coverslips side by side so as to allow viewing of the entire microscope slide. Once the Permount has dried the slide is ready for viewing.
10. Find one good “splat” and draw the chromosome spread
11. Count number of “splats” and determine the average number of chromosomes present in these cancerous cells.

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How is it really done?

Preparing a Karyotype

Metaphase cells are required to prepare a standard karyotype, and virtually any population of dividing cells could be used. Blood is easily the most frequently sampled tissue, but at times, karyotypes are prepared from cultured skin fibroblasts or bone marrow cells. None of the leukocytes in blood normally divide, but **lymphocytes can readily be induced to proliferate, providing a very accessible source of metaphase cells.**
There are many protocols for preparing a karyotype from peripheral blood lymphocytes, but a rather standard series of steps is involved:

- A sample of blood is drawn and coagulation prevented by addition of heparin.
- Mononuclear cells are purified from the blood by centrifugation through a dense medium that allows red cells and granulocytes to pellet, but retards the mononuclear cells (lymphocytes and monocytes).
- The mononuclear cells are cultured for 3-4 days in the presence of a mitogen like phytohemagglutinin, which stimulates the lymphocytes to proliferate madly.
- At the end of the culture period, when there is a large population of dividing cells, the culture is treated with a drug such as colcemid, which disrupts mitotic spindles and prevents completion of mitosis. This greatly enriches the population of metaphase cells.
- The lymphocytes are harvested and treated briefly with a hypotonic solution. This makes the nuclei swell osmotically and greatly aids in getting preparations in which the chromosomes don't lie on top of one another.
- The swollen cells are fixed, dropped onto a microscope slide and dried.
- Slides are stained after treatment to induce a banding pattern as described above.

Once stained slides are prepared, they are scanned to identify "good" chromosome spreads (i.e. the chromosomes are not too long or too compact and are not overlapping), which are photographed. The photos then are given to kindergarten children, who cut out the images of each chromosome and paste them to a backing sheet in an orderly manner. Alternatively, a digital image of the chromosomes can be cut and pasted using a computer. If standard staining was used, the orderly arrangement is limited to grouping like-sized chromosomes together in pairs, whereas if the chromosomes were banded, they can be unambiguously paired and numbered.

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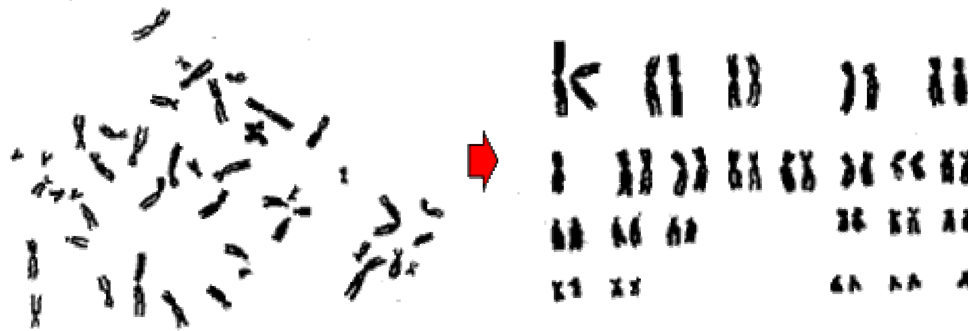

Karyotypes are presented in a standard form. First, the total number of chromsomes is given, followed by a comma and the sex chromosome constitution. This shorthand description is followed by coding of any autosomal abnormalities. A few (simple) examples of this format are:

- A normal male cat: 38, XY
- Horse with three X chromosomes (trisomy X): 65, XXX
- Female dog with increased length of the short (p) arm of chromosome 2: 78, XX, 2p+
- Male pig with a deletion from the long arm (q) of chromosome 10: 38, XY, 10q-

Generally, several metaphases are processed because its not uncommon for a single spread to artifactually have extra chromosomes or be missing chromosomes. This is particularly important if one is to diagnose an abnormality in an individual. It also allows one to diagnose cases of mosaicism, in which an individual has multiple, cytogenetically-distinct populations of cells.

One final point. The discussion above has focused on initial evaluation of an individual's cytogenetic status. If abnormalities are found in peripheral blood, it is sometimes desirable to determine whether that abnormality is present throughout the individual, and further studies with tissues other than blood can be performed. Also, analysis of diseased tissues can often provide useful information. **A prime example of this is the cytogenetic evaluation of cancers, which is not only used diagnostically, but has provided valuable understanding of the pathogenesis of certain types of neoplasia.**

References:

Nardone, Richard *Karyotyping Kit* National Institute of Health 2005

Bowen, R. *Biomedical Sciences Hypertextbook*, http://arbl.cvmbs.colostate.edu/hbooks/genetics/medgen/index.html accessed 5/10/05