# ZOOLOGY VIRTUAL LAB 3
# (VERTEBRATE MOUNTINGS PART – I)

## MOUNTING OF BARR BODY FROM BUCCAL EPITHELIUM

AIM: To mount and study the Barr body or sex chromatin in the somatic cell of a female.

BACKGROUND INFORMATION:

Murray Barr, a Canadian cytogeneticist and Bertram (1949) observed a condensed body in the nucleus of cells of female cats, which was not nucleolus, whereas male had none. They referred this body as sex chromatin that has been known as Barr body named after discoverer Murray Barr. British geneticist Mary Lyon suggested that this Barr body is an inactive X – chromosome formed during mammalian embryogenesis in cells with multiple X chromosomes, which becomes tightly coiled into heterochromatin and lies along the inside of the nuclear envelop in cells of females. Most of the genes of the X –chromosome that forms the Barr body are not expressed. However, small regions of that chromosome remain active.

Lyon hypothesis states that during early development, about the 100 cell stage in humans that is on about the $16^{th}$ day of embryonic life, one of the X chromosomes in a female gets turned off and this is maintained in all descendant cells of the clone. A number of evidences support Lyon hypothesis that only one X – chromosome is active in any cell. Some of the evidences can be listed as follows: (1) XXY males show a Barr body, XO females have none whereas XXX female have two Barr bodies. (2) Persons with abnormal number of X – chromosomes have Barr body one less than the number of X – chromosome per cell; (3) Any one X – chromosome becomes inactive at random, and once inactivated all the cells derived from it will maintain the same inactive X – chromosome. This inactivation of X-chromosome occurs when two X – chromosomes are present (inactivation of X – chromosome will not take place in males as they have only one X – chromosome).

Which of the two X chromosomes gets turned off in each of the 100 cells embryonic stage is purely a random event except where one of the X chromosomes is abnormal (deletion, insertion, inversion, etc.). An abnormal X is always turned off. However, if there is a translocation between an X chromosome and an autosome, the normal X is turned off and the translocation X remains active. No somatic cell will express both alleles.

From biochemical measurements there seemed to be roughly the same amount of gene product in both male and female. This is the phenomenon of "dosage compensation" and was explained by the discoveries of Mary Lyon. Only one gene product is produced in each cell of the male and only one gene product is expressed in each cell of the female but for some reason both X chromosomes remain active in female germ line cells. With this effective dosage of genes of the two sexes is made equal or nearly so.

Barr body testing was introduced in the 1966 Olympic games, in an effort to detect male athletes supposedly trying to 'pass' as females to gain a competitive advantage.

REQUIREMENTS:

1% Geimsa stain, 70% Alcohol, 0.9% Saline, Distilled water, Glycerine, Sterile cotton bud, Slide, Cover slip, Forcep, Dropper, Blotting papers, Compound Microscope, Discard bin, etc.

PROCEDURE:

1. Ask a girl student to rinse and gargle her mouth several times with water to get rid of all the food particles if present on her cheek pouch.

2. Take a sterile cotton bud and dip it in 0.9 % saline, in order to moisten it.

3. Then gently rub the cotton bud several times inside the mouth, on the cheek pouch to remove loosely bound epithelial cells lining the pouch.

4. The cells which are on cotton bud are uniformly spread on a clean and dry slide by just rolling it i.e. known as smear. Allow it to dry.

5. Add 1-2 drops of 70 % alcohol on the smear in order to fix the cells on slide.

6. After few minutes add 2-3 drops of Geimsa stain on the smear and allow it to stand for 7-10 minutes.

7. Gently with the help of dropper wash off the extra stain and allow the slide to dry.

8. Then add 1-2 drops of glycerine on the same slide for mounting.

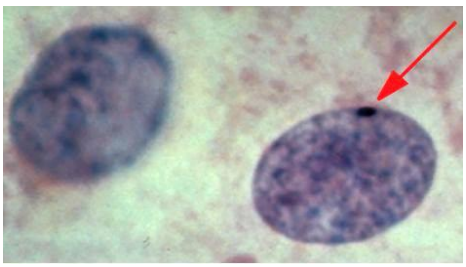
9. With the help of the forcep, hold a clean cover slip at its one corner and gently place it on the slide, without any air bubble. With the help of blotting paper blot extra stain and mounting medium.

10. First place the slide under compound microscope at low power objective i.e. 10X and with the help of rough and fine adjustment localize the cells.

11. Then adjust the microscope on high power at 45X and with the help of rough and fine adjustment observe the nucleus of a single cell. 'Barr body' which is darkly stained (bluish-green) spot like structure seen to attach to the nuclear envelop.

OBSERVATION AND RESULT:

Barr body stained as bluish-green and is found attached to the nuclear envelope.



EVALUATION:

1. Number of Barr bodies found in Normal male _____.
   a) One b) Two c) Nil d) Three
2. Number of Barr bodies found in Normal female _____.
   a) Nil b) Two c) Three d) One
3. Number of Barr bodies found in Klinefelter syndrome person _____.
   a) One b) Two c) Nil d) Three
4. Number of Barr bodies found in Turner syndrome person _____.
   a) Three b) Two c) Nil d) One
5. Number of Barr bodies found in Super female _____.
   a) Two b) Nil c) One d) Three
6. Number of Barr bodies found in Down syndrome male _____.
   a) One b) Two c) Three d) Nil
7. Number of Barr bodies found in Down syndrome female _____.
   a) Nil b) Two c) One d) Three
8. Dosage compensation phenomenon was explained by _____.
   a) Barr b) Lyon c) Bertram d) Murray
9. Barr body was found to be located _____.
   a) attached to the inner side of the nuclear membrane.
   b) attached to the outer side of the nuclear membrane.
   c) attached to the inner side of the cell membrane.
   d) attached internally of the nucleolus.
10. In _____ type of chromosomal aberration the normal X in a female gets turned off and then maintained in all descendant cells of the clone during early embryonic development.
    a) deletion b) insertion c) inversion d) translocation

ASSIGNMENTS:

1. Collect detail information regarding mosaicism in females.
2. Search for various examples of Lyon hypothesis.
3. Find the information regarding formation of "tortoise shell" pattern in cats.
4. Study the enzyme activity of G-6-PD in mammals.
5. Read various applications of Barr body testings.
6. Search for exceptions to the Lyon hypothesis.

REFERENCES:


1. Rao, V.; Krishnan, S.; Hambarde, M. and Sinkar, P. (2009): A handbook of practical in Zoology for S.Y.B.Sc., Himalaya publishing house, pp.106.
2. Bhattacharya, S. S. (2011): S.Y.B.Sc. Zoology Volume – II, Seth publishers Pvt. Ltd., Mumbai, pp. 108-109.
3. https://en.wikipedia.org/wiki/Barr_body
4. https://www.mun.ca/biology/scarr/Barr_Bodies.html
5. https://www.uic.edu/classes/bms/bms655/lesson10.html


# STORY BOARD

FRAME 1:

Rectangular Table with wash basin at on corner. On the table - Glass of water, Beakers one with 0.9 % Saline and other with Distilled water, Sterile Cotton buds in its container, 3 Glass dropper bottles one with 70% alcohol, second with 1% Geimsa stain and third with glycerine, Dropper, Slides in slide box, Cover slip in cover slip box, forcep, Blotting papers, Discard bin, Hand gloves, Microscope, Behind the table one girl is standing wearing apron.

FRAME 2:

Ask a girl student to rinse and gargle her mouth several times with water to get rid of all the food particles if present on her cheek pouch.

DESCRIPTION:

Girl will take a glass of water from the table in her right hand and then will take a sip of water in her mouth. Then she will gargle and spit off the water into the wash basin and will repeat the process 2 to 3 times so that mouth is cleaned and get rid of all the food particles if any. Then she will keep the glass back on the table.

FRAME 3:

Take a sterile cotton bud and dip it in 0.9 % saline, in order to moisten it.

DESCRIPTION:

She will pick sterile cotton bud from its container which is their on the table with her right hand. Then with her left hand she will take a beaker which is with 0.9% saline. She will dip the sterile cotton bud into the 0.9 % saline in order to moisten the cotton tip part. Finally she will keep the saline beaker back on the table.

FRAME 4:

Then gently rub the cotton bud several times inside the mouth, on the cheek pouch to remove loosely bound epithelial cells lining the pouch.

DESCRIPTION:

She will open her mouth and insert the moisten cotton bud in it which is in her right hand. Then she will rub and roll the moisten cotton bud several times on her cheek pouch so that loosely bound epithelial cells lining the pouch will stuck on the cotton bud.

FRAME 5:

The cells which are on cotton bud are uniformly spread on a clean and dry slide by just rolling it i.e. known as smear. Then allow it to dry.

DESCRIPTION:

She will remove cotton bud from her mouth. With her left hand she will pick a clean dry slide from slide box which is on the table. She will hold the slide in between her index finger and thumb. Then she will slightly roll the cotton bud on the slide with her right hand so that the cells on cotton bud will spread uniformly on the slide. Then she will keep the slide back on the table. She will wait for few minutes so that the smear on it will dry. She will discard the used cotton bud in the discard bin.

FRAME 6:

Add 1-2 drops of 70 % alcohol on the smear in order to fix the cells on slide.

DESCRIPTION:

After the smear is dried, she will take a dropper bottle containing 70% alcohol from the table with her right hand and will add 1 to 2 drops of it onto the smear and allow it to remain as it is for few minutes so that the cells on the slide will get fixed on it. Then she will keep the same dropper bottle back on the table.

FRAME 7:

After few minutes add 2-3 drops of Geimsa stain on the smear and allow it to stand for 7-10 minutes.

DESCRIPTION:

She will take 1% Geimsa stain containing dropper bottle from the table with her right hand and will add 2 to 3 drops of Geimsa stain on the slide and allow it to remain as is it for 7-10 minutes to get stained. Then she will keep the same dropper bottle back on the table.

FRAME 8:

Gently with the help of dropper wash off the extra stain and allow the slide to dry.

DESCRIPTION:

She will take a beaker of distilled water with her right hand and will place it in front of her on the table. She will take a dropper with her right hand and will fill it with water by putting it in the beaker. Then she will pick up the slide by holding it in her two fingers of her left hand at

one end of slide and gently wash off the extra stain on the slide by gently flushing the water through the dropper by pressing it. She will keep the slide on table and allow it to dry.

FRAME 9:

Then add 1-2 drops of glycerine on the same slide for mounting.

DESCRIPTION:

She will take a dropper bottle containing glycerine which is the mounting medium and will add 1 to 2 drops of glycerine on the slide.

FRAME 10:

With the help of the forcep, hold a clean cover slip at its one corner and gently place it on the slide, without any air bubble. With the help of blotting paper blot extra stain and mounting medium.

DESCRIPTION:

She will take a forcep from the table in her right hand. With the help of a forcep she will pick one clean, dry cover slip from cover slip box which is on the table. Then she will place the one end of the cover slip on the slide touching the drop of glycerine and gently lower down leave it from the forcep till it completely touches the glycerine, such that no air bubbles will be formed. Now she will take a blotting paper in her right hand and will blot extra stain and mounting medium.

FRAME 11:

First place the slide under compound microscope at low power objective i.e. 10X and with the help of rough and fine adjustment localize the cells.

DESCRIPTION:

She will take a compound microscope which is on table in front of her. She will set the microscope at low power objective i.e. 10X by keeping objective 10X and the eye piece in align condition and with the help of mirror will focus the light on stage by seeing under the eyepiece. She will fit the slide on the stage of the compound microscope. Looking into the eye piece she will move the slide with the help of stage screws to localize the cells. With the help of rough and fine adjustment knobs she will adjust for sharp image.

FRAME 12:

Then adjust the microscope on high power at 45X and with the help of rough and fine adjustment observe the nucleus of a single cell. ‘Barr body’ which is darkly stained (bluish-green) spot like structure seen to attach to the nuclear envelop.

DESCRIPTION:

Once she finds the cells under 10X objective, will adjust microscope on high power i.e. 45X by rotating objective disc. Looking into the eye piece of the microscope slightly moves rough and fine adjustment knobs for clear and sharp magnified image of the nucleus of a single cell. Now she could see a ‘Barr body’ which is darkly stained (bluish-green) spot like structure attached to inner side of the nuclear envelop.