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B95-8 cell line thawing, expansion, supernatant harvesting and cryopreservation.

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This protocol describes the tissue culture procedures employed to expand cryopreserved B95-8 cell line, to harvest Epstein-Barr virus (EBV) supernatant and to re-cryopreserve expanded cells. The B95-8 cell line is derived from cotton-top tamarin (*Saguinus oedipus*) lymphocytes, a new world (Colbian) monkey also known as bichichi or tití. The B95-8 cell line is commonly used in the field of virology and immunology as a continuous source of Epstein-Barr virus (EBV), also known as human herpesvirus 4 (HHV-4), to transform human and primate B cells into immortalized B-lymphoblastoid cell lines (BLCL's) as a means to preserve genomic DNA traits of interest as well as to study EBV life cycle, immune responses to EBV and its associations with infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinoma (Miller et al, PNAS, 1972). The continuous and indefinite propagation of the B95-8 and BLCL's ensures and indefinite supply of the cell line, of EBV supernatant and of BLCLs and of their genome (DNA banking applications). This cell line was distributed through the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) as catalogue number ACC 100 as well as through the American Type Culture Collection (ATCC) as catalogue number VR-1492. The European Collection of Authenticated Cell Cultures (ECACC) employed B95-8 derived EBV-transformed BLCL's to generate in excess of 10,000 cell lines to establish the HLA typed Collection, Human Random Control DNA panels and for use in the UK human genome mapping project. EBV-transformed human cell lines remain in a state of latency producing negligible amounts of virus, the B95-8 cell line possesses the ability to re-enter the phase of active viral replication and produce large amounts of EBV particles into the tissue culture (supernatant). The EBV particles generated by this cell line are viable and can infect human lymphocytes. EBV infection in humans can cause hematological malignancies or immune disorders. EBV is a risk group 2 pathogen and handling of B95-8 cell line, of EBV-transformed BLCL's and tissue culture supernatant should only be carried out within a Class II biological safety cabinet (GSB) in adherence to biosafety level-3 (BSL-3) guidelines for viral production scale cultures.

Preparations (24 hours in advance)

1. Open CO_2 supply to incubator by opening main cylinder valve until hi-pressure manometer reads at minimum 1000 PSI.

2. Regulate low-pressure manometer until it reads 3 PSI.

3. Open downstream CO_2 regulator dial (small black dial under low-pressure dial).

4. Decontaminate incubator with mild detergent and ethanol, fill water tray with sterilized water.

5. Verify that CO_2 incubator is on, set at 37°C and 5% CO_2.

6. Make sure water bath is filled to brim with tap water (supplemented with copper sulphate to prevent proliferation of bacteria or fungi). Turn water bath on and set at 37°C.

7. Charge automatic pipettor.

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Preparations (same day as thawing)

1. Document CO_2 cylinder pressures, incubator and BSL-3 lab conditions in the "Tissue culture CO_2 incubator log" (http://www.genomica.uaslp.mx/Admin/Lab/CO2_Incubator_Log.pdf).

2. Obtain the required amount of T25 tissue culture flasks (column F on table below) and 12 ml round bottom tubes (one per cryovial) based on number of B95-8 vials to thaw (see table below). Vials prepared at our lab normally contain between 4 and 7 $x10^6$ cells/ml in 1.6 ml of volume.

3. Remove the required amount of 20% FBS tissue culture media (20%TCM) from BSL-3 refrigerator and place in water bath at 37°C for 5 to 10 minutes (Column D in table below). See table below and notes for preparation at the end of this document.

Table 1. Tissue culture media volume requirements calculations table.

Source cryovial		20% Tissue Culture Media				E	F	G
		A	B	C	D			
$x10^6$ cells/ml	ml	DMSO wash (ml)	Suspension vol (ml)	50 ml conical tube (ml)	Total required 20%TCM (ml)	ml for $0.5x10^6$ cells/ml	Number of T25s	ml per T25
2	1.6	4	4	2.4	10.4	6.4	1	6.4
2.5	1.6	4	4	4	12	8	2	4.0
3	1.6	4	4	5.6	13.6	9.6	2	4.8
3.5	1.6	4	4	7.2	15.2	11.2	2	5.6
4	1.6	4	4	8.8	16.8	12.8	2	6.4
4.5	1.6	4	4	10.4	18.4	14.4	3	4.8
5	1.6	4	4	12	20	16	3	5.3
5.5	1.6	4	4	13.6	21.6	17.6	3	5.9
6	1.6	4	4	15.2	23.2	19.2	4	4.8
6.5	1.6	4	4	16.8	24.8	20.8	4	5.2
7	1.6	4	4	18.4	26.4	22.4	4	5.6
7.5	1.6	4	4	20	28	24	5	4.8
8	1.6	4	4	21.6	29.6	25.6	5	5.1
8.5	1.6	4	4	23.2	31.2	27.2	6	4.5

A: Discarded after spin
B: For cell resuspension
D: To calculate how much 20%TCM to warm per cryovial
E: Total B95-8 cell suspension at $0.5x10^6$ to be prepared
F: Number of T25 flasks needed
G: Volume of B95-8 cell suspension at $0.5x10^6$ to be distributed into each T25 flask

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4. Turn refrigerated centrifuge on and place S41 swinging rotor and baskets fitted with 12 mL round bottom tube adaptors. Set appropriate rotor radius, centrifuge temperature at 4°C and spin cycle of

5. Turn biological safety cabinet on, decontaminate with ethanol and prepare work supplies.

Left of work area	Central work area	Right of work area
Decon bottle with 100 mL 0.1% $NaOCL_2$	Cryovial rack	5x 2 mL serological pipettes
Lab wash-bottle with 0.1% $NaOCL_2$	Paper towels	5x 5 mL serological pipettes
Spary bottle with 70% ethanol	T25 tissue culture flasks	5x 10 mL serological pipettes
Paper towels	Round bottom 12 ml tissue culture tubes	P10, P200 and P1000 tips
Sharps container	Ice box with ice	Pipettor

Thawing of liquid nitrogen cryopreserved B95-8 cell line.

6. Refer to the BSL-3 liquid nitrogen (LN_2) tank contents log for cryovial position.

7. Request access to the BSL-3 LN_2 tank biosecurity key.

8. Request help from another lab member, ask him to don the cryoprotective splash guard, gloves, and face shield.

9. Don nitrile gloves and have a cryovial rack ready for vial transport into BSL-3 suite.

10. Unlock the BSL-3 LN_2 tank.

11. Ask helper to remove the required cane, box, and vials from the LN_2 tank (typically 1 or 2 only). Place vials in cryovial rack, do not transport by hand due to risk of thermal shock explosion.

12. Ask and supervise helper to place box and LN_2 tank cane back.

13. Lock the BSL-3 LN_2 tank and deliver key to helper for immediate referral to lab supervisor.

14. Document removal of cryovials in BSL-3 liquid nitrogen (LN_2) tank contents log.

15. Move into BSL-3 suite with cryovials in rack.

16. Once in BSL-3 suite, document cryovial label in the "B95-8 Cell line expansion log" (http://www.genomica.uaslp.mx/Admin/Lab/B95-8_Expansion_Log.pdf).

17. Remove warmed 20%TCM bottles from water bath, decontaminate with 70% ethanol and place in

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central working area of biological safety cabinet.

18. Partially immerse the B95-8 cryovial(s) in the water bath and gently swirl until contents are completely thawed. Do not submerge completely.

19. Remove cryovial(s) from water bath and decontaminate with 70% ethanol spray bottle. Place in ice box rack located inside the biological safety cabinet.

20. Use Sharpie pen to label each T25 flask accordingly.

21. Don new nitrile gloves, tissue culture robe, N95 respirator before starting tissue culture work.

22. Add 4 ml of 20%TCM to each of the 12 mL round bottom sterile tissue culture tubes using a 5 ml serological pipette (Column A in Table 1).

23. Decontaminate exterior of cryovial once more with 70% ethanol wash bottle.

24. Flick cryovial to spin contents to bottom before opening.

25. Open cryovial and retrieve contents using a 2 ml serological pipette, transfer to corresponding round bottom tube previously loaded with 4 ml of 20%TCM.

26. Rinse serological pipette in these 4 mL of 20%TCM by repeat pipetting. Rinse cryovial twice with this same media.

27. Swirl gently to dilute DMSO present in round bottom tube cell suspension.

28. Spin round bottom tube at 500 g for 5 minutes at 4°C.

29. Using a 5 ml serological pipette, discard as much supernatant as possible without disrupting cell pellet.

30. Using a new 5 ml serological pipette, add 4 ml of 20%TCM to the round bottom tube with pellet and flick or swirl gently to resuspend cells (Column B in Table 1).

31. Using the previous 5 ml serological pipette, transfer these 4 ml of 20% TCM to a sterile 50 ml conical tube.

32. Using a new serological pipette add the corresponding amount of 20%TCM (Column C shown in red highlight in Table 1) to the 50 ml conical tube, slowly and drop by drop.

33. Gently homogenise B95-8 cell suspension by repeated slow pipetting or swirling.

34. Using a 10 ml serological pipette, slowly transfer the corresponding amount of cell suspension (Column G in Table 1) to the bottom corner each of each T25 flask (Column F in Table 1).

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35. Decontaminate interior and exterior of B95-8 cryovial with 0.1% $NaOCl_2$ and place in decontamination bottle previously loaded with 100 ml of 0.1% $NaOCl_2$.

36. Cap each T25 tissue culture flasks tightly and place horizontally on inverted phase-contrast microscope.

37. Document baseline status of cells in each flask with microphotographs at 10x and 25x.

38. Place labelled T25 flasks in CO_2 incubator vertically and untighten capo to allow CO_2 to diffuse. Incubate for 3 to 4 days at 37°C, 5% CO_2 and 95% relative humidity.

39. Document microscopic examination of each T25 flask in the corresponding "B95-8 Cell line expansion log" (http://www.genomica.uaslp.mx/Admin/Lab/B95-8_Expansion_Log.pdf). See below.

B95-8 Cell line expansion log
Laboratorio de Genómica Viral y Humana
Facultad de Medicina UASLP

Seed date: dd/mmm/yyyy — Source:
Cryo date: dd/mmm/yyyy — Source cells/mL:
Base flask: — T-25 ☐ T-75 ☐ T-175 ☐ — Source cryo date: dd/mmm/yyyy — Source viability:

Date	D+	P	Cluster Ø 1 2 3	Spindles Ø 1 2 3	Colour ROYV	Turbidity Ø 1 2 3	Microbes Ø B Y	👁	📷	10%M ⬇	20%M ⬇	⚗	❄	☠	Vini	Vfin	User
dd/mmm/yyyy																	
dd/mmm/yyyy																	
dd/mmm/yyyy																	

Tissue culture expansion of B95-8 cell line.

40. Document CO2 cylinder pressures and incubator and BSL-3 lab parameters daily in the "Tissue culture CO_2 incubator log" (http://www.genomica.uaslp.mx/Admin/Lab/CO2_Incubator_Log.pdf).

Tissue culture CO_2 incubator log
Laboratorio de Genómica Viral y Humana

Date (dd/mmm/yyyy)	Low (psi)	High (psi)	Incub $\%CO_2$	Incub °C	BSL-3 Lab °C	User

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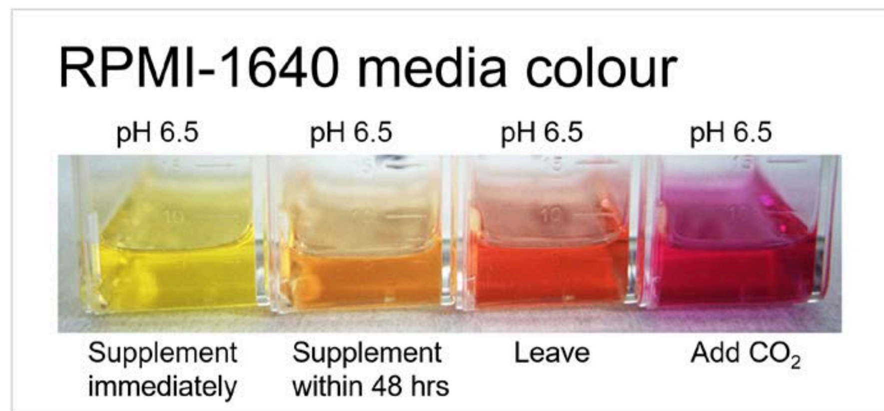
41. On a daily basis, visually inspect tissue culture flask media colour, turbidity and verify caps are loose. Document findings in "B95-8 Cell line expansion log".

42. Every third day microscopically inspect each T25 flask to assess presence of clonal clustering, spindle cells or microbial presence. Document findings in "B95-8 Cell line expansion log".

43. Every week compile a microphotographic documentation of each T25 flask evolution at 10x and 25x under the inverter phase-contrast microscope. Document findings in "B95-8 Cell line expansion log".

44. The B95-8 cell line has a doubling time between 41 and 72 hours.

45. Tissue culture media changes colour as pH varies based on cell growth, the image below helps guide actions according to these changes.



http://www.genomica.uaslp.mx/Admin/Lab/RPMI_Colours.png

46. If media has turned yellowish in colour, prepare biological safety cabinet for use as indicated in step #5.

47. Warm an appropriate amount of 20%TCM in the water bath at 37°C for 5 minutes. Calculate the current volume present in all flasks under incubations which require media addition (those having a yellowish media colour). This is the amount of 20%TCM that will be required.

48. Use a new and sterile 5 ml serological pipette to slowly (drop by drop) add a volume of warm 20%TCM which is equivalent to that currently present in the flasks (i.e., equivalent to the volume shown in Column G on Table 1).

NOTE: The cultured B95-8 cell line concentration should be kept between 0.3 to 0.5 $x10^6$ cells/ml. However, the DSMZ suggests an optimal tissue culture concentration between 0.5 and 1 $x10^6$ cells/ml, a minimum of 0.2 $x10^6$ cells/ml, a seed-out concentration of 0.5 $x10^6$ cells/ml and a maximum density of 3 $x10^6$ cells/ml.

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49. The final T25 volume after this first TCM addition should vary between 8 and 13 ml.

50. Continue visually assessing tissue culture on a daily basis, inspect for colour, turbidity and verify caps are loose. Document findings in “B95-8 Cell line expansion log”.

51. After three to four days, microscopically inspect each T25 flask to assess presence of clonal clustering, spindle cells or microbial presence. Document findings in “B95-8 Cell line expansion log”.

52. If TCM appears yellowish once more, add an equal volume of warm 20%TCM as that present in each of the T25 flasks. This is the second TCM addition step (i.e., between 16 and 26 ml).

53. Continue visually assessing tissue culture on a daily basis, inspect for colour, turbidity and verify caps are loose. Document findings in “B95-8 Cell line expansion log”.

54. Three to four days after the second TCM addition step, prepare biological safety cabinet for use as indicated in step #5.

55. Retrieve a single sample flask from incubator representative of each of the seeded cryovials (base flasks), decontaminate flask exterior with 70% ethanol and place inside biological safety cabinet.

56. Gently homogenize cell suspension by swirling. Then immediately follow by retrieving a 10 ul aliquot of the cell suspension into a 0.6 ml microcentrifuge tube previously loaded with 90 ul of 0.4% trypan blue stain for cell counting and viability assessment following the “Cell count and viability assay” protocol provided in the following link http://www.genomica.uaslp.mx/Protocolos/Cell_counts_ENG.pdf.

57. Assess cell concentration and viability.

58. Correct TCM of flask if cell concentration exceeds or is below the recommended B95-8 cell concentration of 0.3 to 0.5 $x10^6$ cells/ml.

59. If cells are below the 0.3 to 1 $x10^6$ cells/ml, concentrate cell suspension by transferring entire contents to 50 ml conical flask using a sterile 10 ml serological pipette, spin at 500 G for 5 minutes at room temperature, and carefully transfer supernatant to a new 50 ml conical tube without disturbing the cell pellet. Resuspend cells to optimal volume for 0.3 to 0.5 $x10^6$ cells/ml concentration using a 50% to 50% mix of warm fresh 20%TCM and the retrieved supernatant.

60. If cell concentration exceeds the 0.3 to 1 $x10^6$ cells/ml, assess whether cell concentration would be corrected for by the addition of 20%TCM and splitting flasks to seed 5 ml of the diluted cell suspension into individual T25 flasks. Use as many T25 flasks as needed to seed the diluted cell suspension for further expansion.

61. If an appropriate volume of B95-8 cells has been expanded by this point, calculate the required

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volume of 20%TCM required to take concentration of cells to between 5 and 8 x10^6 cells/ml, concentrate and prepare cells for cryopreservation. This is rare and usually not done, it is preferable to continue culture while decreasing FBS supplement by using 10%TCM further on.

62. If the B95-8 cell production protocol warrants further expansion of cell line, consider partitioning (splitting) flask into new subculture flasks after the 20 ml max volume has been reached.

63. Warm an equal volume of 10%TCM as the volume currently present in flasks to be partitioned.

64. Calculate total flask volume and amount of new T25 flask needed by dividing total flask volume by 5. Label new T25 flasks according to protocol (i.e., Base flask 1 turns into subculture flasks 11 and 12, these then turn into subculture flasks 111, 112, 113 and 114 as well as 121, 122, 123, and 124. etc.).

65. To partition or split a T25 flask, homogenize flasks contents by gentle swirling for 15 seconds.

66. Transfer 5 ml of the homogenized cell suspension to each pre-labelled flask and add 5 ml of 10%TCM slowly, drop by drop using a 5 ml serological pipette.

67. Gently swirl flasks to homogenize, document partitioning in the corresponding "B95-8 Cell line expansion log" (http://www.genomica.uaslp.mx/Admin/Lab/B95-8_Expansion_Log.pdf).

NOTE: The B95-8 cell line morphology is predominantly a suspension of single or clustered, spherical or oval lymphoblasts along with roughly 10% adherent cells exhibiting an elongated spindle of fibroblast morphology which do not required trypsin for detachment.

Procedimiento (criopreservación de la línea celular B95-8)

1. Descongele el FBS a temperatura ambiente. ¡No colocar en baño de agua ni en microondas!

2. Rotúlese una cantidad apropiada de crioviales (estimando un total de 1.6 mL de Solución de Criopreservación por criovial) con los siguientes datos: nombre de cepa y # de pase, fecha, número de células totales en el criovial expresadas en millones y porcentaje de viabilidad. Por Ejemplo: B95-8 P1, 20AGO08, 3.5, 90%. Coloque los crioviales en hielo (ver nota # 6).

3. Dispense a cada criovial 500 µL de HI-FBS y 100 µL de DMSO (Solución de criopreservación, ver nota #8).

4. Retire los frascos de cultivo T25 de la incubadora y homogeneice la suspensión celular por agitación gentil. Transfiera dicha suspensión a un tubo de fondo redondo de 15 mL o a uno cónico de 50 mL y centrifuge a 400 G durante 15 minutos.

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5. Rescate el medio de cultivo sobrenadante para procesarlo (refiérase al *Procesamiento del Sobrenadante de EBV*).

6. Resuspenda el pellet celular en 1 mL de RPMI-1640 y realice el conteo de células y la evaluación de su viabilidad de acuerdo al *"Protocolo de Conteo y Viabilidad de CMN"*.

7. Ajuste la suspensión celular con RPMI-1640 para lograr una concentración celular de $5x10^6$ células/mL. Agregue 1 mL de esta suspensión celular lentamente a cada criovial previamente cargado con solución de criopreservación.

8. Homogeneice por agitación gentil (ver nota #7) y mantenga los crioviales en hielo.

9. Coloque los crioviales en el congelador de –20ºC durante al menos 2 horas.

10. Transfiera los crioviales al ultracongelador (-80ºC) durante al menos 8 horas (NOTA: pero no más de 24 hrs).

11. Transfiera los crioviales al tanque #2 de LN_2 en fase líquida (-196°C).

Procedimiento (procesamiento del sobrenadante de EBV)

Under normal tissue culture conditions (35°C, 5% CO_2 and 20% fetal bovine serum (FBS)) the B95-8 cell line proliferates vigorously but does not produce optimum quantities of EBV. However, reducing tissue culture temperature to 32°C and lowering FBS to 10% in growth medium results in highly efficient EBV production in supernatant.

1. Centrifugue el sobrenadante recolectado en el psao #4 del *Procedimiento de Criopreservación de la Línea Celular B95-8* en un tubo cónico de 15 o 50 mL a 2,500 G durante 15 minutos. Este paso permite sedimentar las células o detritus celular presente en el sobrenadante.

2. Recupere el sobrenadante centrifugado sin perturbar al sedimento y esterilícelo por filtración a través de una membrana de Acetato de Celulosa o PVDF con poros de 0.22 µm.

3. Congele el sobrenadante en crioviales de 1.5 o de 2 mL a -80°C para uso mediato (dentro de los siguientes 3 meses) o a -196°C en el Tanque de LN_2 #2 para el largo plazo.

Notes

1. The 20%M tissue culture media is composed of RPMI-1640 + 2 mM Glutamine + 20% fetal bovine serum (FBS).

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2. The doubling time of B95-8 cell lines is of 41 hours

3. For cryopreservation, the quantity of cells should be corrected to a minimum between 5 and 8 x10^6 cells/ml to ensure proper survivability and future resuscitation of cell line.

4.
5. El Medio Básico al 20% (MB20%) consta de RPMI-1640 + 20% HI-FBS (Suero Bovino Fetal Inactivado por Calor) + 1% Pen-Strep (10,000 UI/mL de Penicilina Sódica + 10mg/ml de Estreptomicina). El HI-FBS se obtiene calentando el FBS a 56°C durante 30 minutos en baño de agua. Este medio debe esterilizarse por filtración a través de una membrana de Acetato de Celulosa o PVDF con poros de 0.22 µm. Manténgase refrigerado entre 4 y 8°C.

6. Todos los implementos, desechables o reutilizables, que entren en contacto con alícuotas de EBV o líneas celulares deberán ser descontaminados por inmersión en NaOCl al 0.5% antes de salir del CCC. Para preparar 1 litro de la solución de NaOCl al 0.5% se aforan 100 ml de NaOCl al 5% (concentración doméstica) hasta 1 litro con agua del grifo. Para producir una solución menos concentrada (al 0.1%) se aforan 20 ml de NaOCl al 5% hasta 1 litro.

7. La nomenclatura para las imágenes fotomicrográficas es la siguiente: **00001a2-d3-4x** donde 00001=#ID de la Colección Genómica Mexicana, a2=pozo A2 de la placa P24 en la cual se cultiva (para monocultivos en frascos T25 no es necesario especificar este parámetro), d3 = días de cultivo y 4x = objetivo del microscopio que fue empleado.

8. Cuando existen datos de pobre crecimiento, se puede transferir el cultivo a una placa de 24 pozos a razón de 1 ml por pozo y se continua con la incubación.

9. Por norma general 1 mL del sobrenadante permite transformar 1-2 x10^6 células.

10. Todos los pasos del procedimiento de criopreservación deberán realizarse bajo hielo.

11. Las células que sobren del procedimiento de criopreservación pueden sembrarse nuevamente en un frasco de cultivo T25 a razón de 0.5 millones/mL (5x10^6 de células en 10 mL de MB10%).

12. The liquid nitrogen cryopreservation media should be composed of 20% FBS, 70% RPMI-1640 and 10% DMSO.

13. Los frascos T25 y placas P24 deberán rotularse con el número de identificador único y la fecha de inicio del cultivo de tal modo en que el rótulo no interfiera con las ventanas ópticas.

Referencias

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1. Mayor N, Marsh SGE. EBV Supernatant Production, Protocol for BLCL Generation. Anthony Nolan Research Institute. University College and Roya Free Hospital School of Medicine, London, United Kingdom.

2. Genomic Medicine Biorepository. Growth of B95.8 Cell Line and Production of Human EBV Virus. Protocol GMB008rA:031104REW.

3. Shaw JE, Petit RG, Leung K. Growth of B95-8 cells and expression of Epstein-Barr virus lytic phase in serum-free medium. J Virol. 1987 Dec;61(12):4033-7.

4. Miller G, Robinson J, Heston L, Lipman M. Differences between laboratory strains of Epstein-Barr virus based on immortalization, abortive infection, and interference. Proc Natl Acad Sci U S A. 1974 Oct;71(10):4006-10.

5. Miller G, Shope T, Lisco H, Stitt D, Lipman M. Epstein-Barr virus: transformation, cytopathic changes, and viral antigens in squirrel monkey and marmoset leukocytes. Proc Natl Acad Sci U S A. 1972 Feb;69(2):383-7.

6. Miller G, Lipman M. Release of infectious Epstein-Barr virus by transformed marmoset leukocytes. Proc Natl Acad Sci U S A
7. . 1973 Jan;70(1):190-4.

Revision history

1.0 Original document.

2.0 Optimized protocol.

3.0 Changes to document format only.

4.0 English version prepared, protocol optimized.

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