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Transfection of BOSC 23 cells and infection of 3T3s

Source: Warren S. Pear (07/17/93) v1.4
Reference: W.S. Pear, G.P. Nolan, M.L. Scott, & D. Baltimore. Proc. Natl. Acad. Sci. USA (1993), in the press.

All conditions are for 60 mm plates. I have not tried to scale up.

1. The cells are carried in gpt selective media. See recipe on the accompanying page. It is best to pass the cells at 1:3-1:4 to prevent cell clumping which occurs when the cells are passed at low density or when they are allowed to become overconfluent. I pass the cells by rinsing once with PBS and then trypsinizing for about 30 seconds. Note: Plating the cells may be the most important step in obtaining high titers. It is extremely important that the cells are not clumped and are at the correct density. Unlike most adherent cell lines, the BOSC 23 cells do not form nice monolayers. Instead they tend to clump before confluence (at which time the media will become acidic). To overcome the clumping, I usually split the cells 1:1 one or two days before I split them for transfection. This may need to be repeated if the cells do not spread well. After the 1:1 splits, it is best to pass the cells 1:2 for 1 or 2 passages, and then at 1:3 or 1:4. The cells grow much slower than 293 cells, and the 1:3 split should take about 3-4 days to reach confluence. Transfections are better if you plate the cells for transfection before the plate becomes confluent.

2. Plate 2 x 10^6 cells/plate approx 18-24 hrs prior to transfection in 4 mls of 10% FCS without selection. Your transfection efficiency will be higher if the cells appear as single cells rather than clumps (see #1 on how to prevent this). Note: 2 x 10^6 cells/plate is not a typo. The dish should be about 80% confluent prior to transfection. It is also important to count the cells rather than estimating the split. The above cell number is optimized for MFG-lacZ. Other inserts may slow the growth of the cells and it may be necessary to plate more cells prior to transfection. I try to plate at a density so that the cells are 95-100% confluent at 24 hours after transfection

3. Just prior to transfection, change media to 4 mls of 10 FCS containing 25 uM chloroquine.

4. Transfect by adding 6-10 ug DNA to $CaCl_2$/H2O in a volume of 500 ul. Add 500 ul 2X HBS (pH 7.05) by bubbling. Immediately (within 1-2 minutes) add this solution to the cells. Note: It is also fine to halve all of the above reagants. Recipes for HBS and $CaCl_2$ are on the attached pages. (It is probably fine to add the reagents without bubbling in the order suggested above. Add this solution within 1-2 minutes to the cells.)

5. At 10 hours, suck off the media and replace with 4 mls 10% FCS without election. It is important that you do not leave the chloroquine longer than 12 hours. This will cause a large decrease in titer. The range for chloroquine treatment is 7-11 hours.

To infect:

8. Plate 5 x 10^5 3T3s the night prior to infection on a 100 mm plate.

9. Suck off supe from TRANSFECTED PLATES and spin 5 min at 1500 to remove cells (temp is unimportant).

10. Add whatever volume of viral supe you want to DME containing 10% CS such that the final volume is 3 mls AND CONTAINS POLYBRENE AT A CONCENTRATION OF 4 μg/ml. You can freeze the rest of the viral supe at -80. I do not experience more than a 2-fold loss of titer when freezing the supes. 1000x stock

11. Suck off media from 3T3s and pour on the 3 mls containing virus and polybrene. Leave on cells from 3-5 hours (although the cells can survive even longer without loss of titer). After this time, suck off supe and replace with 10 mls DME with 10% CS.

12. Harvest (stain, neo select, etc) these cells at 48 hours.

Notes: It appears that the BOSC 23 cells must be maintained in gpt selection media. In this media, the cell line has been able to consistently produce retroviral stocks with titers in excess of 2 x 10^6 for at least 30 passages. We use MFG-lacZ as our standard for transfection and infection. You should obtain the above titer with this vector. You may have to play with the initial BOSC 23 densities to optimize the titer for your particular construct. Judging from the experience of people in this lab, it will take a little time to get used to working with the BOSC 23 cells. As mentioned above, cell densities are critical and it will take a little practice to get his right. I suggest that you initially concentrate on the transfections and stain the cells for lacZ 48 hours after transfection. Once you are able to transfect at least 30% of the cells, everything else should work fine.

FREEZING THE CELLS:

1. We freeze the cells in a solution containing 90% FCS, 10% DMSO.

2. When thawing the cells, grow them initially in DME with 10% FCS. Once the cells are ready to split, place them in gpt selection media.

Note: The cells which are sent are in DME with 10% fetal calf serum, pen/strep, and glutamine. I suggest growing these cells in this medium until they reach confluence and then freezing several vials. If you plan to grow them for several passages, change to the gpt selection medium.

Reagents*

Reagent	Final []	grams dry
1) 2x HBS		
HEPES, pH 7.05	50mM	5.0
KCl	10 mM	0.37
dextrose	12 mM	1.0
NaCl	280 mM	8.0
Na_2HPO_4 (FW 141.96)	1.5 mM	0.1065 g
		500 ml total, pH to 7.05
2) $CaCl_2$		
$CaCl_2$	2 M	29.4 g/ 100 ml

*stock solutions may be prepared and frozen at -20 C. I am not sure why, but the HBS goes bad after 6 months-1 year, even when stored in the freezer.

V_{total} (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
2xHBS (ml)	0.25	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.25
$CaCl_2$ (µl)	31	62	93	124	155	186	217	248	279
H_2O + DNA (µl)	219	438	657	876	1095	1314	1533	1752	1971

GPT SELECTION:

Reference: Mulligan, RC & P Berg, PNAS (1981) 78, pp 2072-76.

Media per 500 ml bottle:

DME	403 mls (remove 35 mls)
10% Dialyzed FCS	50 mls
PCN/Strp	5 mls (Gibco mix)
25X Xanthine	20 mls
100X Glutamine	5 mls
50X HAT	10 mls
MPA	5 mls
Aminopterin	1 ml
Thymidine	1 ml

Gent 10 mg/l

Note: for initial 293 selection, use double MPA (50 µg/ml), after initial selection and for 3T3 selection 25 µg/ml is fine.

T25 Flask

REAGENTS:

Dialyzed fetal bovine serum: JRH R-10578, $210
Xanthine: Sigma X-2001, 5 g, $20.95
Mycophenolic Acid: Gibco 860-1814IH, 500 mg, $147
Aminopterin: Sigma A3411, 25 mg, $43
Thymidine: Sigma T1895, 1 g, $10.20
50X HAT Supplement; Sigma H-0262, 50 vials, $129

312.5 mg / 50 mls NaOH
0.5 ml 10 M +
49.5 ml milli Q

STOCKS:

0.1 N HCl
100 mL conc + 10 mls
milli Q

0.1 N NaOH
100 mL 10 N + 10 mls
milli Q

25X XANTHINE: 6.25 mg/ml; add 1.56 g to 250 ml 0.1N NaOH; filter and store at 4 degrees.

100X MYCOPHENOLIC ACID: 2.5 mg/ml; add 0.25 g to 100 mls 0.1 N NaOH, neutralize with 0.1 N HCl (check with pH paper), filter and freeze in 5 ml aliquots.

25 mg/10 mls

50X HAT: dissolve in 10 mls H2O, filter and use immediately or freeze.

+ 10 mls H_2O

500X Aminopterin: 1 mg/ml; add 0.05 g to 50 mls DME, filter and freeze.

10 mg/10 ml DMF

500X Thymidine: 3 mg/ml; add 0.15 g to 50 mls DME, filter and freeze.

30 mg/10 ml DMF