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Effective Lonza 4D Nucleofection with Inexpensive Homemade Buffers

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We use this protocol and it's working

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Abstract

This protocol uses the Lonza 4D nucleofection system. Here I present protocols that reduce the price dramatically of using Lonza 4D equipment.

1. The 16 well strip kits are expensive. These can be washed and re-used. This allows a single strip for hundreds of nucleofection experiments.

2. The nucleofection buffers are limited (P1, P2, etc). However we have used home made buffers that effectivly target many primary cell types. We have used this Cas9 RNPs (protein/RNA) and plasmid delivery. We have used this in T and B cells, cell lines, and more.

The homemade buffer used here was originally used and published in: https://cancerres.aacrjournals.org/content/71/23/7291. It was called Amaxa V. The actual recipe used in that paper was published in the thesis here: https://opus4.kobv.de/opus4-fau/frontdoor/index/index/docId/2189

Here we show a simple nucleofection of plasmid into K562.

Guidelines

There are many options for buffers to try for the nucleofection:
1. You can use the purchased buffers from Lonza
2. Use our AmaxaV buffer published here.
3. For some cells Opti-MEM works well and should be tried.
4.The Porteus Lab also has a homemade buffer that they have published:

- Solution I: Dissolve 2g ATP•Disodium Salt and 1.2g $MgCl_2$•$6H_2O$ in 10mL nuclease-free UltraPure water, filter sterilize, make 20µL aliquots, and store at -20°C for up to two months.
- Solution II: Dissolve 6g KH_2PO_4, 0.6g $NaHCO_3$, and 0.2g glucose in 500mL nuclease-free UltraPure water, adjust pH to 7.4, filter-sterilize, make 1mL aliquots, and store at 4°C for up to 3 months.
- Mix 20µL solution I with 1mL solution II (see Reagent Setup). This makes for 10 electroporations (100µL each) b

Benifit::
The benefit of homemade buffers/ strip re-washing is it becomes inexpensive to scale up experiments. This includes initial optimization of the Lonza program chosen. The published programs are not always the best. Do trials to look at cell death and nucleation efficiency.

Materials

MATERIALS

- Sodium phosphate monobasic monohydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9638
- HEPES **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**H6147
- Sodium succinate, dibasic, hexahydrate **Bio Basic Inc. Catalog #**SB0889.SIZE.500g
- Magnesium chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M2670
- Gibco Penicillin-Streptomycin (10,000 U/mL) (Pen/Strep) **Fisher Scientific Catalog #** 15-140-122
- FBS **Invitrogen - Thermo Fisher**
- Potassium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9333
- Disodium phosphate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S7907
- IMDM **Gibco - Thermo Fisher Scientific Catalog #**12440053
- 4D-Nucleofector System with X Unit **Lonza Catalog #**AAF-1002X
- 4D-Nucleofector® X Kit S (32 RCT) specific for cell type **Lonza Catalog #**V4XC-1032

Troubleshooting

Prepare Reagents

1 **Make Nucleofection Buffer (AmaxaV):**

Stocks can be stored RT for a long time (we use ours longer than 6 months). Making working solutoin from stock solution, vacuum filter through 0.45um filter. This should be stored at 4C. Discard after 3 months or when you see white precipitate.

	Stock Solution	Preparation of Stock Solution	Vol of stock to add to 15ml falcon tube				
	H2O		6 ml				
	Sodium phosphate buffer pH 7.2, 0.5 M	Mix 7 parts of 1 M NaH2PO4 with 18 parts of 1 M Na2HPO4 and 25 parts of water. Check pH. If pH needs to be adjusted, use the two phosphate stock solutions and not HCl or NaOH. (7ml + 18ml + 25ml = 50 ml)	1.8 ml				
	KCl 1 M		50 µl				
	MgCl2 1 M		100 µl				
	HEPES 0.2 M	Just dissolve in water, no pH adjustment necessary.	1 ml				
	Sodium succinate 0.24 M		1 ml				
	filter the solution		Total volume ~10 ml				

2 **Prepare for Nucleofection:**

This nucleofection conditions us 2 ug of plasmid (Cas9 plasmid is about 9kb). Smaller plasmid nucleofect easier. If the plasmid has a fluorescence marker or some other signal, do a titration experiment to see your ideal amount (0.2- 4ug).

Add the plasmid for each condition to a well in the pcr strip. Ideally the volume is less than 5 ul for the Londza 4D 16-well strips, but I have added up to 10 ul of plasmid

solution successfully (final reaction condition would then be 20 ul of cells + 10ul plasmid solution). Plasmids should be in TE or water.

3 **Count and pellet K562 Cells**
cells should be in log phase of growth

Use 500k K562 cells/ reaction well. (1 million also works fine)
Take the total cells needed for all reactions. Pellet at 300 rcf for 4 min.

4 **Re-suspend in AmaxaV:**

Remove supernatant from cells.
Add 20ul AmaxaV buffer for each 500k cells and re-suspend.

i.e. for 6 reactions take 3 million cells, re-suspend in 120ul LonzaBuf1 (**AmaxaV** should be room temp).

5 **Nucleofect**

Add 20ul AmaxaV/Cell solution onto the side of each of the PCR tubes. After adding cells to the side of the PCR tube. Tap the PCR tube on the hood bench until cells fall into the bottom and mix with the pre pipetted plasmid.

Using multi-channel pipette mix 2-4x. Transfer the cells/DNA to the 4D 16-well strip. Tap strip so that there is no air bubbles at the bottom.

Nucleofect with FF-120

This should be done quickly to remove the cells from the electroporation medium.

6 **Move Cells into Well**

As quickly as possible after nucleofection add 70-80ul IMDM/10%FBS/1%PenStrep to each well of the strip (nucleofected cells/DNA/buffer solution). Or opti-mem if using other cell types. Then transfer the entire 100ul solution to a well in a 12-well plate containing 1ml of IMDM/FBS/PenStrep media.

Selection media can be added in 16 hr (next morning)

7 **Wash 16-Well Strip for Re-Use**

Take Lonza 16-well strip and rinse with DI water throughly. Place in 50ml falcon tube. Fill falcon and strip with 70% ethanol. Leave for 5 min. Then put strip on bench in hood to dry. Can also leave it in 70% ethanol and dry directly before using.

16 well strips can then be used 40x or more times.
We have seen no issue with cross contaminations of plasmid from one experiment to another, though in principle that could happen.