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Article

Assembly and Functional Analysis of an S/MAR Based Episome with the Cystic Fibrosis Transmembrane Conductance Regulator Gene

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Abstract: Improving the efficacy of gene therapy vectors is still an important goal toward the development of safe and efficient gene therapy treatments. S/MAR (scaffold/matrix attached region)-based vectors are maintained extra-chromosomally in numerous cell types, which is similar to viral-based vectors. Additionally, when established as an episome, they show a very high mitotic stability. In the present study we tested the idea that addition of an S/MAR element to a CFTR (cystic fibrosis transmembrane conductance regulator) expression vector, may allow the establishment of a CFTR episome in bronchial epithelial cells. Starting from the observation that the S/MAR vector pEPI-EGFP (enhanced green fluorescence protein) is maintained as an episome in human bronchial epithelial cells, we assembled the CFTR vector pBQ-S/MAR. This vector, transfected in bronchial epithelial cells with mutated *CFTR*, supported long term wt *CFTR* expression and activity, which in turn positively impacted on the assembly of tight junctions in polarized epithelial cells. Additionally, the recovery of intact pBQ-S/MAR, but not the parental vector lacking the S/MAR element, from transfected cells after extensive proliferation, strongly suggested that pBQ-S/MAR was established as an episome. These results add a new element, the S/MAR, that can be considered to improve the persistence and safety of gene therapy vectors for cystic fibrosis pulmonary disease.

Keywords: episome; S/MAR; cystic fibrosis; CFTR; gene therapy

Vector assembly

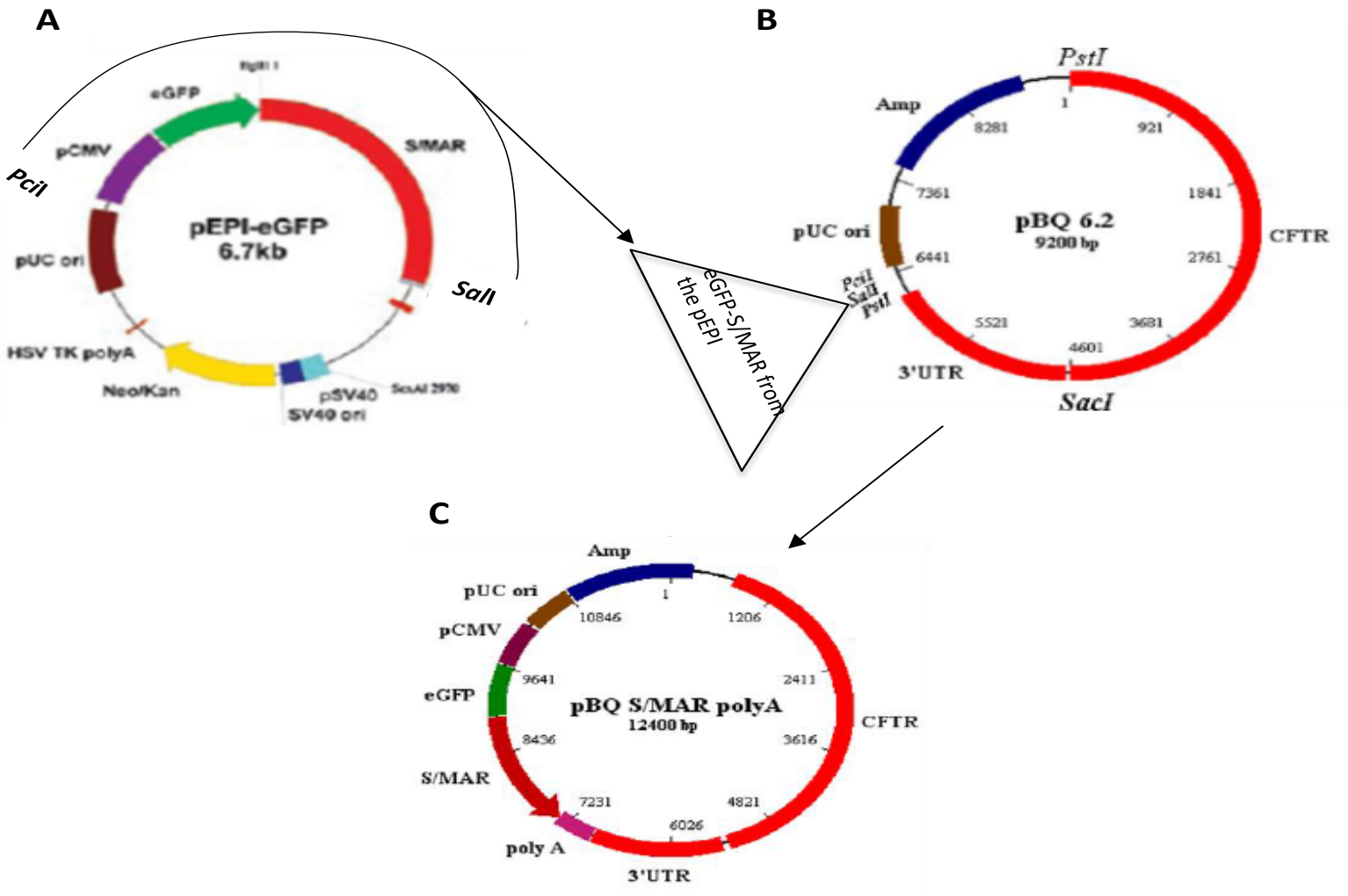
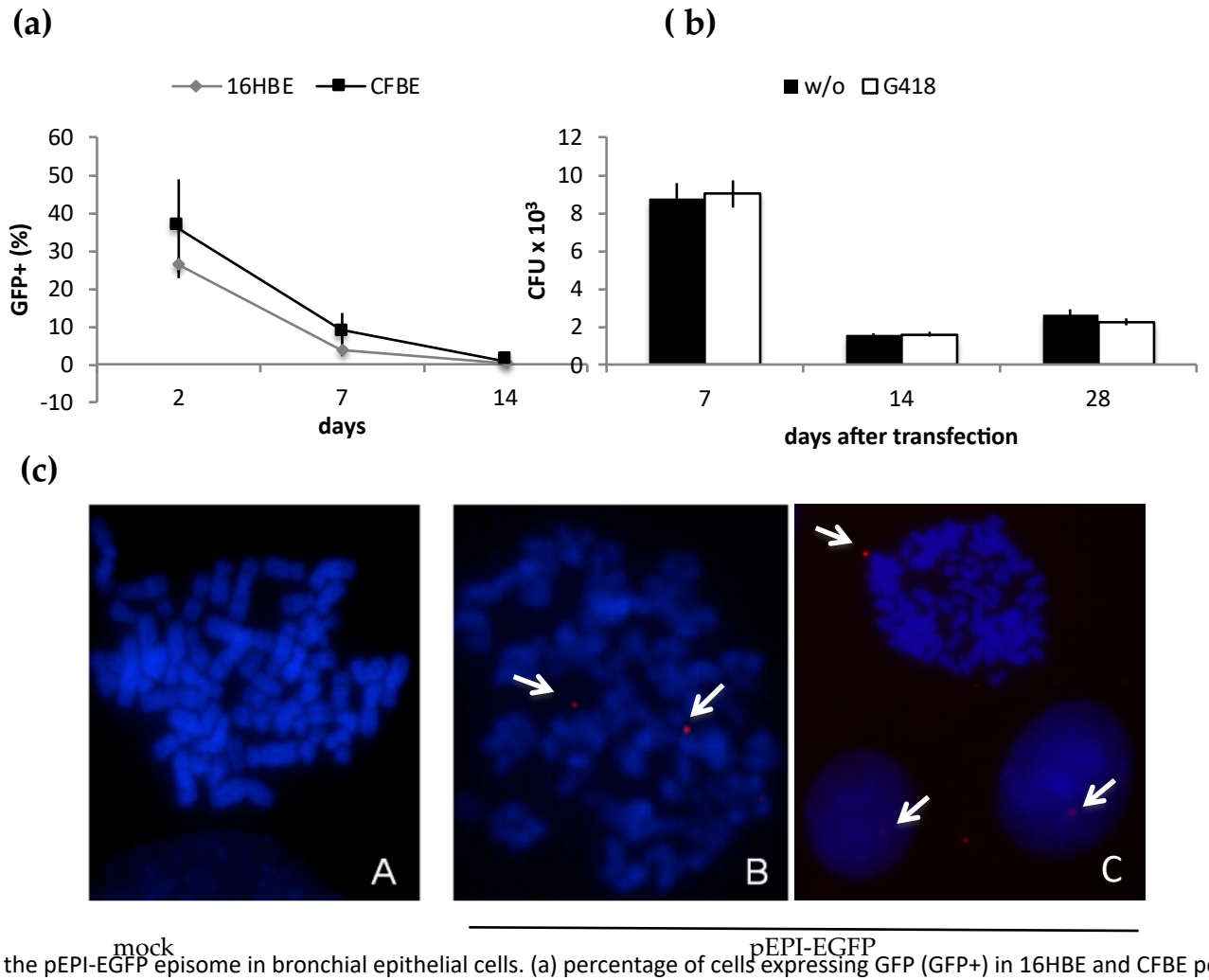


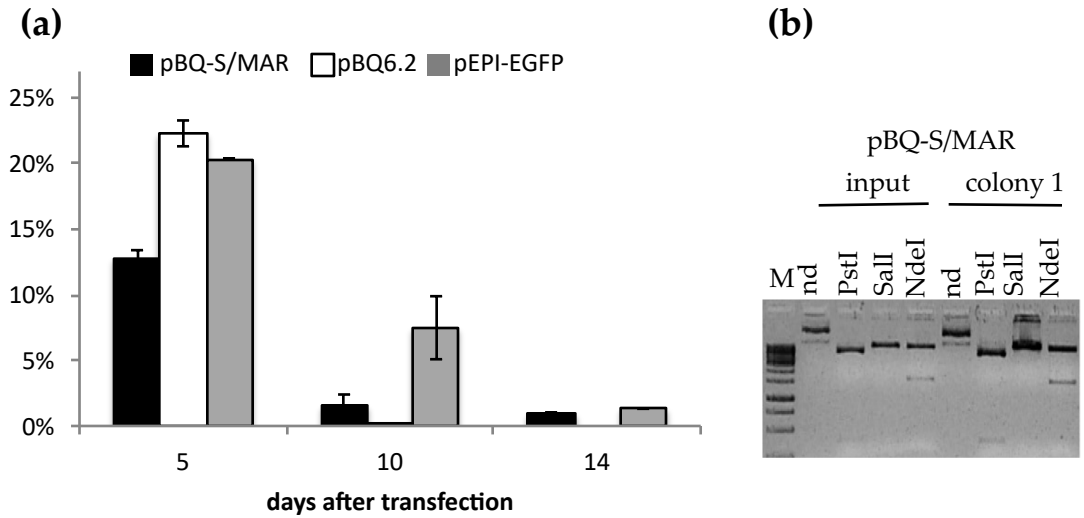
Figure 1: pEPI stability in bronchial epithelial cells



Long-term maintenance of the pEPI-EGFP episome in bronchial epithelial cells. (a) percentage of cells expressing GFP (GFP+) in 16HBE and CFBE populations transfected with the pEPI-EGFP by nucleofection, and propagated in non-selective conditions. Samples were analyzed by flow cytometry at the indicated times after transfection (mean ± SD, n = 2). (b) E. coli rescue from transfected CFBE cells. Hirt extracts were prepared from transfected cells propagated for 7, 14 and 28 days, with (G418) or without (w/o) selection; the pEPI-EGFP was then recovered in E. coli. Data (mean ± SD) refer to the total number of CFU recovered in DH10B electrocompetent cells using 2 µL extracts; control electroporation yielded 5 × 103 ± 50 CFU/10 pg plasmid DNA (pMAX). (c) Metaphase chromosomes hybridized to the pEPI-EGFP probe (red). A, un-transfected cells (mock); B and C representative images of cell transfected with pEPI-EGFP and analyzed 14 days after transfection; episomes are highlighted by white arrows; dots outside the nuclei were not count; images were taken at 630 x magnification.

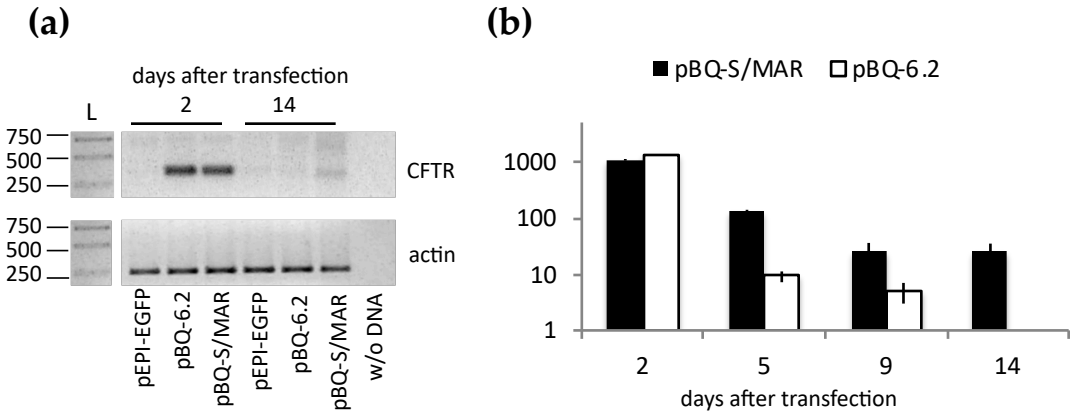
I do not have the scale bar for the images in panel (b), so I indicate the magnification of the microscope.

Figure 2: Recovery in E.coli



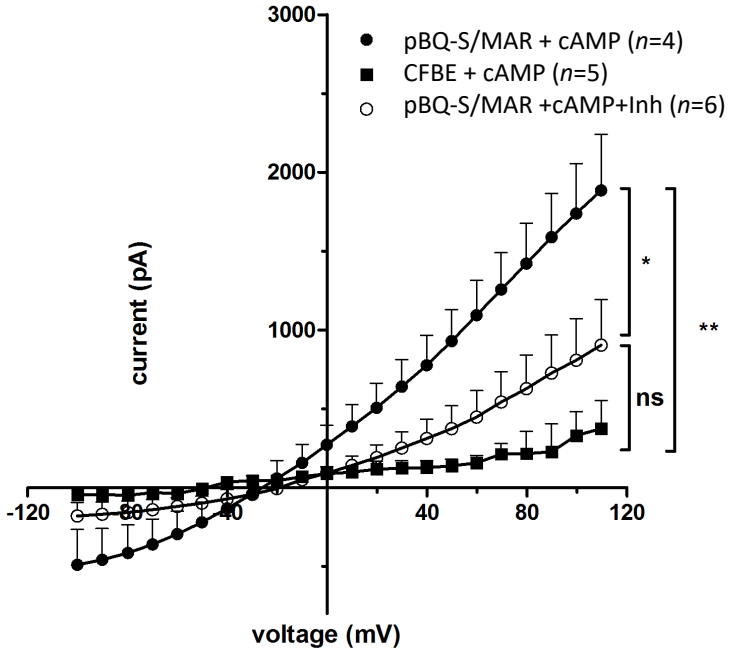
The pBQ-S/MAR vector is maintained as an episome in dividing epithelial cells. (**a**) Percentage variation (mean ± SD, $n = 3$) of *E. coli* transformants at days 5, 10 and 14 with respect to day 2. (**b**) Restriction profile of pBQ-S/MAR (input) and plasmid recovered from CFBE cells at day 14 (colony 1). M, 1 kb molecular weight marker; nd, not digested.

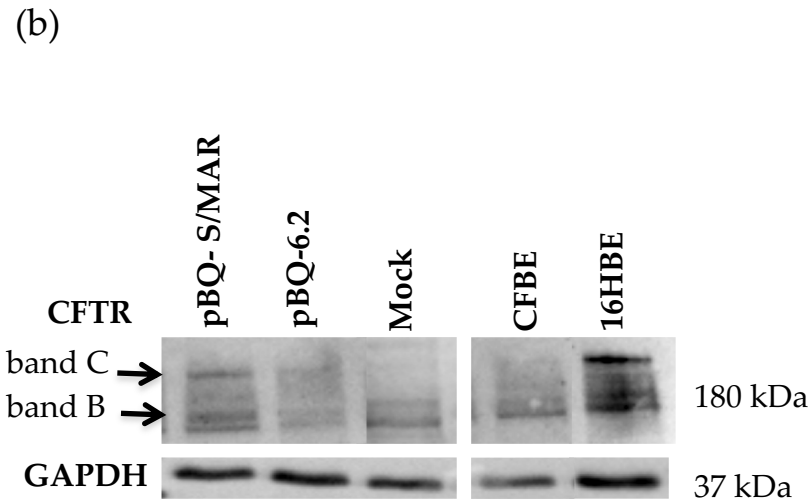
Figure 3



CFTR expression in CFBE cells. (**a**) Reverse transcription-PCR for wt CFTR (upper panel) and β-actin (lower panel) of CFBE cells transfected with the indicated vector propagated for 2 and 14 days after transfection. Samples were separated by agarose gel electrophoresis in 1.5% agarose gels; L, ladder (band size is in bp). (**b**) CFTR mRNA relative quantification (RQ) in the indicated samples. RQ (mean ± SD, n = 2, each in duplicate) was determined by the $\Delta\Delta C_t$ method using parental CFBE cells as calibrator.

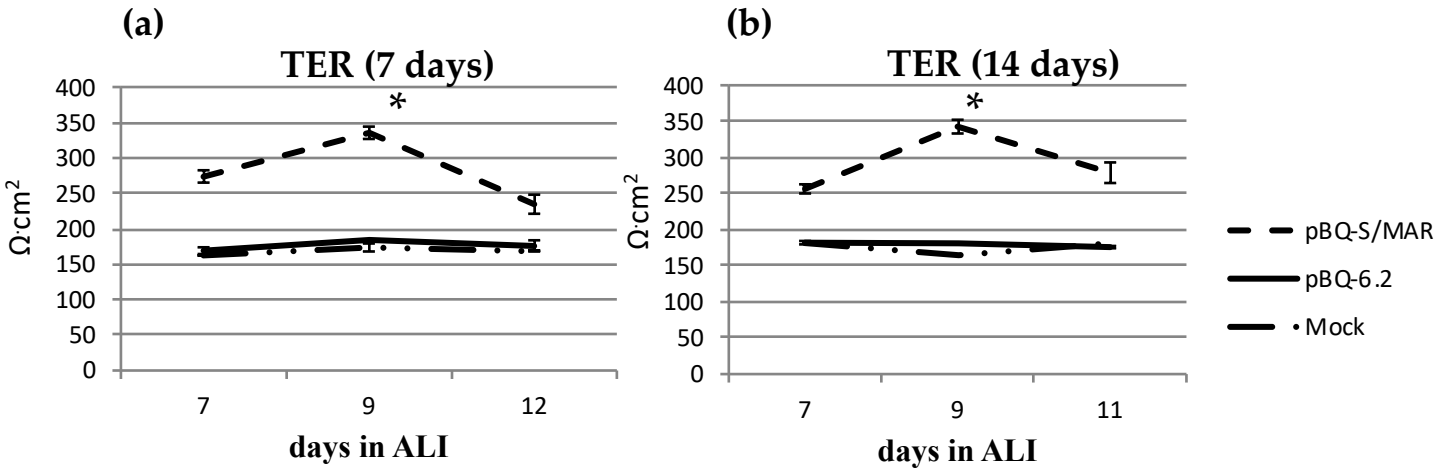
Figure 4





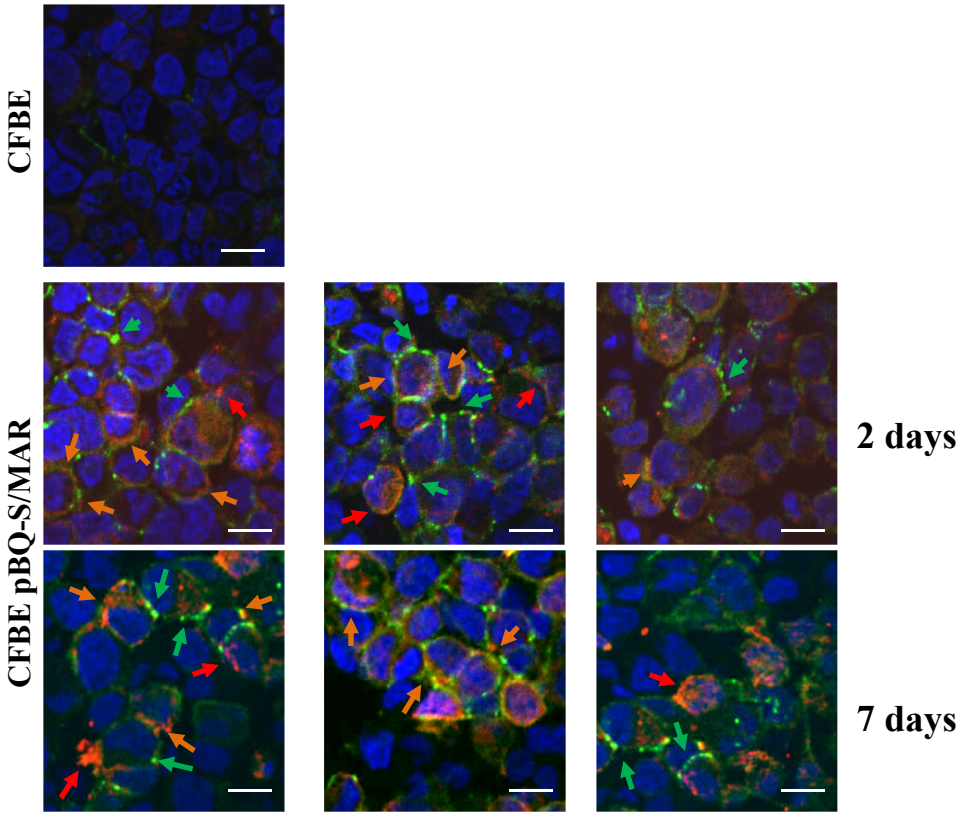
Functional CFTR production in transfected CFBE cells. (**a**) CFTR channel encoded by pBQ-S/MAR increases chloride currents in CFTR deficient cells. The graph shows the average current/voltage relationships in the presence of a cAMP-containing cocktail (+cAMP) of pBQ-S/MAR transfected (pBQ-S/MAR n = 4) and un-transfected CFBE cells (CFBE, n = 5) or plus 10 μM $CFTR_{inh}$-172 (pBQ-S/MAR+ Inh) (n = 6). Data are means ± SEM; * p <0.05; ** p <0.01; Student's t-tests. (**b**) Representative western blots of proteins extracted from cells transfected with pBQ-S/MAR or pBQ6.2 grown for 14 days after transfection, and polarized for 9 more days. Controls: mock, CFBE treated with transfection reagent only; CFBE and 16HBE un-transfected cells. Band B, immature form of CFTR; band C, mature CFTR.

Figure 5



CFTR expression by pBQ-S/MAR increases TER of CFBE cells. TER was measured in CFBE cells, mock or transfected with the indicated plasmids at day 7 (**a**) or 14 (b) after transfection. Data ($\Omega \cdot cm^2$) are shown as the mean ± SD of three independent experiments. *, $p < 0.05$. Statistical analysis unpaired *t*-test pBQ-S/MAR versus pBQ6.2.

Figure 6



CFTR localizes to the apical membrane in pBQ-S/MAR transfected cells. Cells propagated for 2 or 7 days after transfection were seeded on filters, maintained in ALI conditions for 9 days and probed with anti-CFTR and anti-ZO1 antibodies. At that time the average TER values were 280 $\Omega \cdot cm^2$ with no significant variations among filters. Secondary antibodies were: green for ZO1, red for CFTR. Nuclei were counterstained with DAPI (blue). Red and green arrows point to CFTR and ZO1 signals, respectively; orange arrows indicate co-localization of red and green spots. Scale bar, 10 μm.