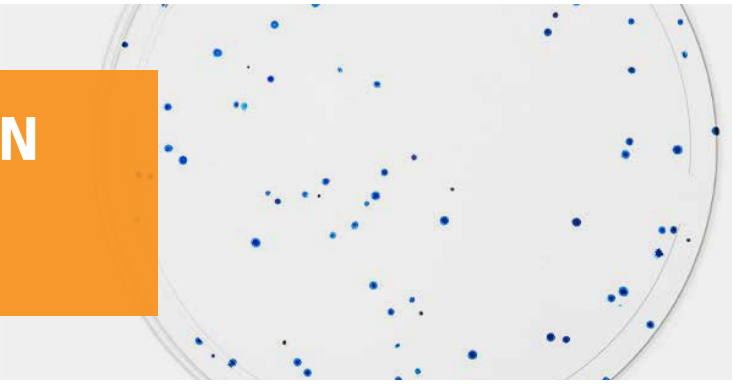
IMPROVE hPSC SURVIVAL IN SINGLE-CELL WORKFLOWS

With CloneR™2



CloneR™2: Enhanced Cloning Efficiency After High-Stress Events

Generate clonal human pluripotent stem cell (hPSC) lines that maintain their genomic integrity and downstream differentiation potential with this defined, serum-free supplement. By using CloneR™2, you can increase the cloning efficiency and survival of human embryonic stem (ES) and induced pluripotent stem (iPS) cells under high-stress conditions, including seeding at low or high densities, post-thaw recovery, and when creating monolayers ahead of downstream differentiation. For your gene-editing workflows, add CloneR™2 to improve ES and iPS cell survival following electroporation and during clonal deposition (see data below).

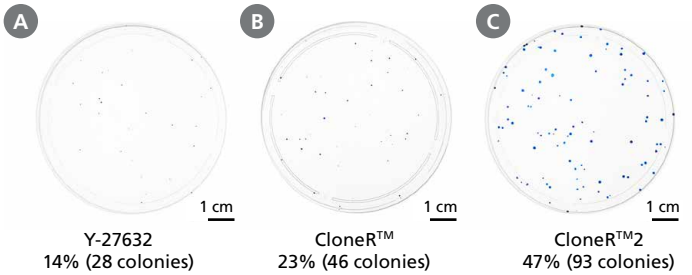


Figure 1. CloneR™ and CloneR™2 Supplements Improve Cloning Efficiency and Colony Size

hPSCs display a considerable increase in cloning efficiency when cloned using (B) CloneR™ compared to using (A) Y-27632 compound. (C) CloneR™2 further improves cloning efficiency and increases colony size when compared to either Y-27632 compound or CloneR™. Shown are examples of H9 hESCs in 10-cm dishes, plated at 200 cells per dish (~4 cells/cm²) in mTeSR™ Plus on Vitronectin XF™.

Why Use CloneR™2?

MORE COLONIES, READY SOONER. Improved cloning efficiencies with clones ready for selection days sooner.

ROBUST AND CONSISTENT CLONING. Similar high performance across culture systems and cell lines.

ENHANCED SURVIVAL. Increased plating efficiency at all densities and after high-stress events such as electroporation or thawing.

STRAIGHT TO SINGLE CELLS. No single-cell passage adaptation phase required.

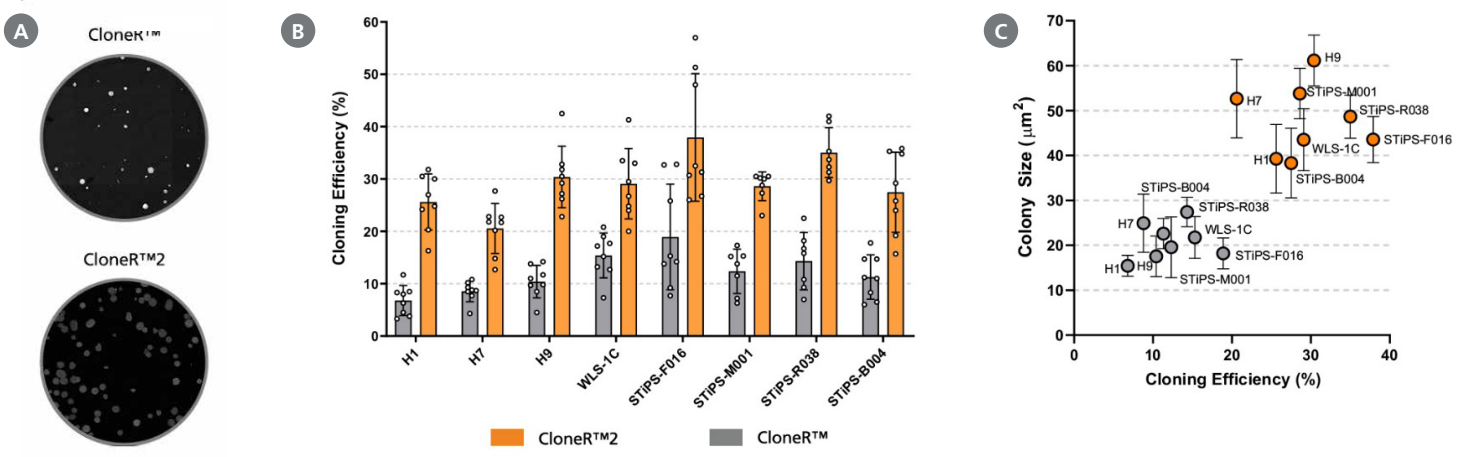


Figure 2. CloneR™2 Enables Improved Cloning Efficiency and Larger Colonies When Compared to CloneR™

(A) Representative images of 200 cells (H9 cell line) in 12-well plates grown in mTeSR™1 on Vitronectin XF™ at day 8 after seeding. Three hES (H1, H7, and H9) and 5 hiPS (WLS-1C, STiPS-F016, STiPS-M001, STiPS-R038, and STiPS-B004) cell lines were seeded at clonal density (50 cells/cm²) on Vitronectin XF™, in mTeSR™1 supplemented with CloneR™ or CloneR™2. mTeSR™1 supplemented with CloneR™2 increases (B) cloning efficiency and (C) colony size of hPSCs when compared with mTeSR™1 supplemented with CloneR™. Each data point in (B) represents an average of 3 technical replicates, with at least 7 biological replicates (n) per cell line.

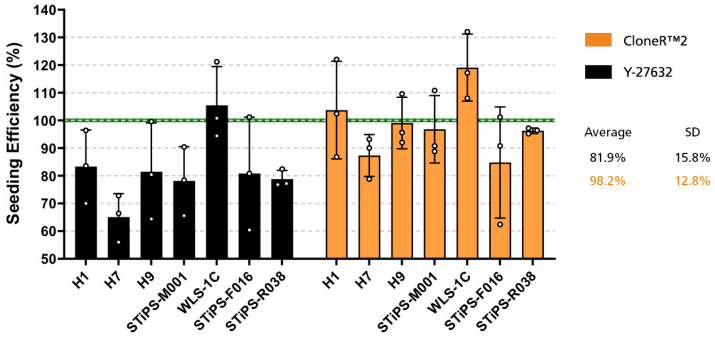


Figure 3. CloneR™2 Improves Seeding Efficiency at High Density

CloneR™2 improves single-cell seeding efficiency when used as a supplement in media for the first 24 hours of culture, compared to using Y-27632 as a supplement. 5.0x10^5 cells were seeded in 12-well plates coated with Corning® Matrigel® in mTeSR™ Plus supplemented with CloneR™2 or Y-27632. Cultures were analyzed 24 hours post seeding. The use of CloneR™2 resulted in an average seeding efficiency of 98.2 ± 12.8% compared to the use of Y-27632, which resulted in an average seeding efficiency of 81.9 ± 15.8%, across all cell lines (n = 3 replicates per line).

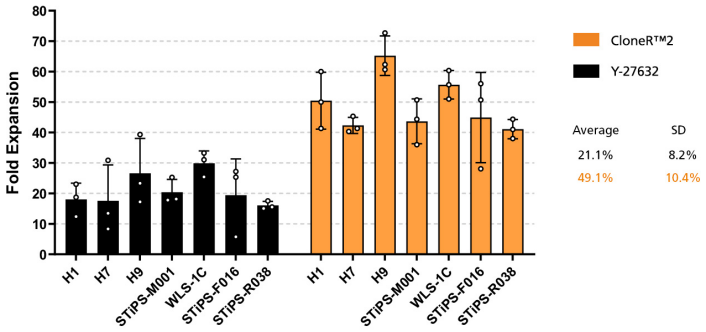


Figure 4. hPSCs Plated in CloneR™2 Show Increased Expansion

When used as a seeding supplement during single-cell passaging, CloneR™2 improves cell expansion when compared to using Y-27632. 3.0x10^4 cells were seeded in 12-well plates coated with Corning® Matrigel® in mTeSR™ Plus supplemented with CloneR™2 or Y-27632. After 24 hours, the cultures were maintained in complete media (without a cloning supplement) and analyzed on day 5. CloneR™2 resulted in an average expansion of 49.1 ± 10.4 compared to Y-27632, which resulted in a lower average expansion of 21.1 ± 8.2, across all cell lines (n = 3 replicates per line).

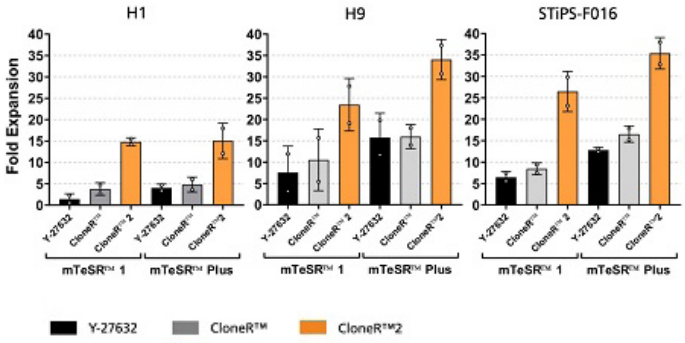


Figure 5. CloneR™2 Improves Recovery of hPSCs Following Electroporation

CloneR™2 can also be used as a survival supplement in gene-editing workflows that require electroporation. Three cell lines were electroporated, then plated in mTeSR™1 and mTeSR™ Plus containing Y-27632, CloneR™, or CloneR™2. After 24 hours, cultures were maintained in complete TeSR™ media (without cloning supplement) and analyzed after 5 days. When compared to both Y-27632 and CloneR™, CloneR™2 dramatically improved cell survival and expansion in all three cell lines when used as a supplement in the first 24 hours immediately following electroporation (n = 2 replicates per cell line).

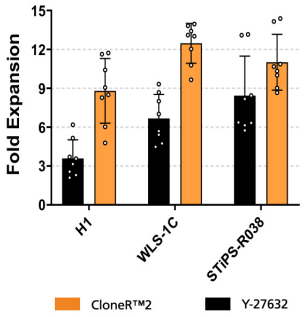


Figure 6. CloneR™2 Improves Post-Thaw Recovery of hPSCs

Thawing cryopreserved cells can result in low expansion or loss of the culture within the first passage. Using CloneR™2 as a seeding supplement within the first 24 hours of thawing cells ameliorates this effect, improving post-thaw recovery of hPSCs. Three cell lines were frozen as single cells, then thawed into mTeSR™ Plus containing Y-27632 or CloneR™2 on Matrigel®. Cultures were maintained in complete mTeSR™ Plus (without cloning supplement) after 24 hours, and analyzed on day 4 or day 5. CloneR™2 improves the fold expansion across all cell lines tested when compared to Y-27632, with at least 7 replicates (n) per cell line.

Also Consider: CloneR™

Genome editing of hPSCs relies heavily on the survival of single cells to establish clonal lines. CloneR™ is the original serum-free supplement formulated for enhancing the cloning efficiency and single-cell survival of hPSCs, especially under clonal and low-density seeding conditions. Designed for use in feeder-free culture systems, this flexible supplement is compatible with TeSR™ maintenance media and a range of cell culture matrices and cell lines. CloneR™ enables the robust generation of clonal hPSC lines without single-cell adaptation, thus minimizing the risk of acquiring genetic abnormalities.