

INTRODUCTION

As stem cell scientists, the quality of our research is directly related to the quality of the cell materials used. Poor quality cells can impact reproducibility, jeopardize results, waste time, and drain resources. In screening materials submitted to the WiCell Stem Cell Bank, **we have identified a substantial and concerning variability in hPSC quality, highlighting the need for improved testing strategies and standards.**

As of June 1, 2019, 1732 hPSC lines have been submitted to WiCell for banking and characterization by 31 providing laboratories. The vast majority of these cell lines were generated through grant-funded projects as a resource for the larger scientific community, and reportedly screened prior to submission. Lines were believed to be of good quality by the depositing laboratories. To date, 839 of these lines have been independently tested by WiCell to assure minimum cell quality standards prior to distribution to the larger stem cell community. Results of this screening are reported here.

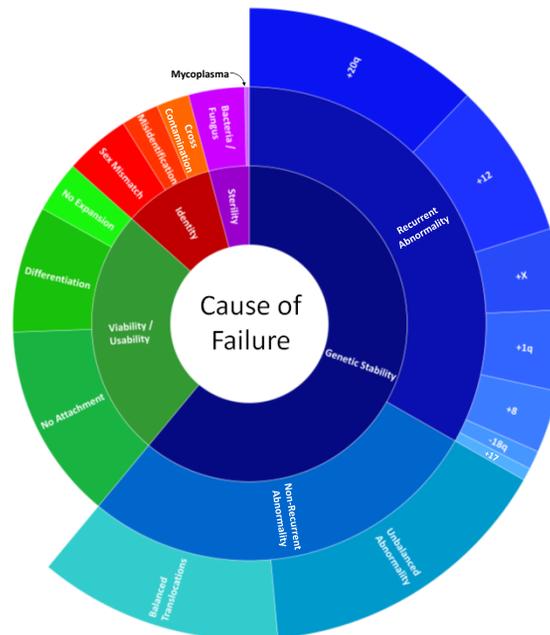
METHODS

Cell lines were tested following submission with little to no continuing culture. Only original deposited lots are included in the data presented. Lines were tested as follows:

- Thaw recovery and assessment of spontaneous differentiation in culture by photomicrograph analysis (WiCell)
- Karyotype by G-Band following clinical guidelines (WiCell)
- Identity via Short Tandem Repeat (STR) analysis using the Promega PowerPlex 16 HS System (UW-Madison Hospitals and Clinics)
- Sterility (Bacteriostasis / Fungistasis) by Direct Transfer method and 14 day culture (Steris).
- Mycoplasma testing by either Lonza Mycoalert or Biological Industries EZ PCR Mycoplasma Kit (WiCell).

RESULTS

Overall, more than one-third of submitted hPSC cell lines failed routine quality testing.



- Of the 839 hPSC lines examined, 285 did not meet minimum quality standards (312 separate instances, due to some lines failing more than one test).
- Primary reason for failure was unexpected abnormal karyotypes (61% of all failures)
- More than half of karyotype failures are due to recurrent abnormalities (54%), while balanced translocations account for 20% of karyotype failures.
- STR anomalies, including cross-contaminated (mixed) cell lines, identity mismatch, and sex mismatch were noted in 10% of failures. This may be an underestimation of actual issues, as misidentifications of unique lines of the same sex may not be identified.
- Eighty cell lines (more than 25% of failures) were unrecoverable at thaw, exhibiting either no attachment or expansion, or excessive differentiation preventing establishment of the culture.
- Twelve (12; 4%) were not sterile, and 1 line was mycoplasma positive.

DISCUSSION

The materials examined in this study were submitted to WiCell for distribution, and believed by the submitting investigators to be of good quality, suitable for use in ongoing research. In many cases, these are the lowest passage materials being used in the laboratory, and the cells being distributed to requesting investigators prior to deposit into the WiCell Bank. Based on this data, we can assume that a substantial percentage of hPSCs used in and shared between investigator laboratories have unidentified quality issues that can impact research - from affecting rigor and reproducibility to invalidating research and necessitating retraction of published results. These results demonstrate that current ad hoc screening strategies are variable and largely insufficient. This underscores the need for routine testing prior to initiating and following studies. Furthermore, **it highlights the need for, and value of, centralized repositories with established quality standards that ensure distribution materials are routinely and appropriately screened.**

RECOMMENDATIONS

Based on these results, to safeguard research and ensure ongoing material quality, we recommend the following workflow within the lab:

