

GGR PROTOCOL: Cell culture and harvesting

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Primary human keratinocytes were isolated from fresh surgically discarded neonatal foreskin and cultured in Keratinocyte-SFM (Life Technologies 17005-142) and Medium 154 (Life Technologies M-154-500). Pen/Strep (Life Technologies 15140-122) and Anti-mycotic (Life Technologies 15240-062) were also added to the culture. Keratinocytes were induced to differentiate by addition of 1.2 mM calcium (added 12 hours after seeding at confluence) for 6 days in full confluence. Cells were harvested every 12 hours for a total of 13 timepoints and banked into cell pellets, viable batches (10% DMSO in media), or cross-linked (see cross-linking protocol) and frozen down at -80 deg C.