Proceedings of the National Academy of Sciences of the United States of America 2017 vol.114 N10, pages 2550-2555

Microfluidic droplet platform for ultrahigh-throughput single-cell screening of biodiversity

Terekhov S., Smirnov I., Stepanova A., Bobik T., Mokrushina Y., Ponomarenko N., Belogurov A., Rubtsova M., Kartseva O., Gomzikova M., Moskovtsev A., Bukatin A., Dubina M., Kostryukova E., Babenko V., Vakhitova M., Manolov A., Malakhova M., Kornienko M., Tyakht A., Vanyushkina A., Ilina E., Masson P., Gabibov A., Altman S.

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2017, National Academy of Sciences. All rights reserved. Ultrahigh-throughput screening (uHTS) techniques can identify unique functionality from millions of variants. To mimic the natural selection mechanisms that occur by compartmentalization in vivo, we developed a technique based on single-cell encapsulation in droplets of a monodisperse microfluidic double water-in-oil-in-water emulsion (MDE). Biocompatible MDE enables in-droplet cultivation of different living species. The combination of droplet-generating machinery with FACS followed by next-generation sequencing and liquid chromatography-mass spectrometry analysis of the secretomes of encapsulated organisms yielded detailed genotype/phenotype descriptions. This platform was probed with uHTS for biocatalysts anchored to yeast with enrichment close to the theoretically calculated limit and cell-to-cell interactions. MDE-FACS allowed the identification of human butyrylcholinesterase mutants that undergo self-reactivation after inhibition by the organophosphorus agent paraoxon. The versatility of the platform allowed the identification of bacteria, including slow-growing oral microbiota species that s uppress the growth of a common pathogen, Staphylococcus aureus, and predicted which genera were associated with inhibitory activity.

http://dx.doi.org/10.1073/pnas.1621226114

Keywords

Butyrylcholinesterase, Cell-cell interactions, Microfluidic encapsulation, Staphylococcus aureus, Ultrahigh-throughput screening

References

[1] Agresti JJ, et al. (2010) Ultrahigh-throughput screening in drop-based microfluidics for directed evolution. Proc Natl Acad Sci USA 107(9):4004-4009.

[2] Zhang H, et al. (2016) Autocrine-based selection of drugs that target ion channels from combinatorial venom peptide libraries. Angewandte Chemie International Edition 55(32):9306-9310.

[3] Gupta RD, et al. (2011) Directed evolution of hydrolases for prevention of G-type nerve agent intoxication. Nat Chem Biol 7(2):120-125.

[4] Aharoni A, Amitai G, Bernath K, Magdassi S, Tawfik DS (2005) High-throughput screening of enzyme libraries: Thiolactonases evolved by fluorescence-activated sorting of single cells in emulsion compartments. Chem Biol 12(12):1281-1289.

[5] Tawfik DS, Griffiths AD (1998) Man-made cell-like compartments for molecular evolution. Nat Biotechnol 16(7):652-656.

[6] Miller OJ, et al. (2006) Directed evolution by in vitro compartmentalization. Nat Methods 3(7):561-570.

[7] Najah M, et al. (2014) Droplet-based microfluidics platform for ultra-high-throughput bioprospecting of cellulolytic microorganisms. Chem Biol 21(12):1722-1732.

[8] Yan J, et al. (2013) Monodisperse water-in-oil-in-water (W/O/W) double emulsion droplets as uniform compartments for high-throughput analysis via flow cytometry. Micromachines (Basel) 4(4):402-413.

[9] Zinchenko A, et al. (2014) One in a million: Flow cytometric sorting of single cell-lysate assays in monodisperse picolitre double emulsion droplets for directed evolution. Anal Chem 86(5):2526-2533.

[10] Zhang Y, et al. (2013) A programmable microenvironment for cellular studies via microfluidics-generated double emulsions. Biomaterials 34(19):4564-4572.

[11] Masson P, Lockridge O (2010) Butyrylcholinesterase for protection from organophosphorus poisons: Catalytic complexities and hysteretic behavior. Arch Biochem Biophys 494(2):107-120.

[12] Geyer BC, et al. (2010) Plant-derived human butyrylcholinesterase, but not an organophosphorous-compound hydrolyzing variant thereof, protects rodents against nerve agents. Proc Natl Acad Sci USA 107(47):20251-20256.

[13] Ilyushin DG, et al. (2013) Chemical polysialylation of human recombinant butyrylcholinesterase delivers a long-acting bioscavenger for nerve agents in vivo. Proc Natl Acad Sci USA 110(4):1243-1248.

[14] Kelsic ED, Zhao J, Vetsigian K, Kishony R (2015) Counteraction of antibiotic production and degradation stabilizes microbial communities. Nature 521(7553):516-519.

[15] Tenconi E, Guichard P, Motte P, Matagne A, Rigali S (2013) Use of red autofluorescence for monitoring prodiginine biosynthesis. J Microbiol Methods 93(2):138-143.

[16] Lopez-Garcia MT, Rioseras B, Yague P, Alvarez JR, Manteca A (2014) Cell immobilization of Streptomyces coelicolor: Effect on differentiation and actinorhodin production. Int Microbiol 17(2):75-80.

[17] Luker KE, et al. (2015) Comparative study reveals better far-red fluorescent protein for whole body imaging. Sci Rep 5:10332.

[18] Sawyer AJ, et al. (2013) A peptide-morpholino oligomer conjugate targeting Staphylococcus aureus gyrA mRNA improves healing in an infected mouse cutaneous wound model. Int J Pharm 453(2):651-655.

[19] Smith AJ, Jackson MS, Bagg J (2001) The ecology of Staphylococcus species in the oral cavity. J Med Microbiol 50(11):940-946.

[20] Shim JU, et al. (2013) Ultrarapid generation of femtoliter microfluidic droplets for single-molecule-counting immunoassays. ACS Nano 7(7):5955-5964.

[21] McDonald JC, et al. (2000) Fabrication of microfluidic systems in poly(dimethylsiloxane). Electrophoresis 21(1):27-40.

[22] Mazutis L, et al. (2013) Single-cell analysis and sorting using droplet-based microfluidics. Nat Protoc 8(5):870-891.

[23] Teh S-Y, Khnouf R, Fan H, Lee AP (2011) Stable, biocompatible lipid vesicle generation by solvent extraction-based droplet microfluidics. Biomicrofluidics 5(4):044113.

[24] Terekhov S, et al. (2015) A novel expression cassette delivers efficient production of exclusively tetrameric human butyrylcholinesterase with improved pharmacokinetics for protection against organophosphate poisoning. Biochimie 118:51-59.

[25] Gasparian ME, et al. (2013) Heterogeneous catalysis on the phage surface: Display of active human enteropeptidase. Biochimie 95(11):2076-2081.

[26] Wu S, Letchworth GJ (2004) High efficiency transformation by electroporation of Pichia pastoris pretreated with lithium acetate and dithiothreitol. Biotechniques 36(1):152-154.

[27] Ralph EC, Xiang L, Cashman JR, Zhang J (2011) His-tag truncated butyrylcholinesterase as a useful construct for in vitro characterization of wild-type and variant butyrylcholinesterases. Protein Expr Purif 80(1):22-27.

[28] Lõoke M, Kristjuhan K, Kristjuhan A (2011) Extraction of genomic DNA from yeasts for PCR-based applications. Biotechniques 50(5):325-328.

[29] Kitz R, Wilson IB (1962) Esters of methanesulfonic acid as irreversible inhibitors of acetylcholinesterase. J Biol Chem 237(10):3245-3249.

[30] Ellman GL, Courtney KD, Andres V, Jr, Feather-Stone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7(2):88-95.