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Protocol for 3D drug sensitivity and resistance testing of patient-derived cancer cells in 384-well plates

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ABSTRACT

Establishment of drug testing of patient-derived cancer cells (PDCs) in physiologically relevant 3-dimensional (3D) culture is central for drug discovery and cancer research, as well as for functional precision medicine. Here, we describe the detailed protocol allowing the 3D drug testing of PDCs – or any type of cells of interest – in Matrigel in 384-well plate format using automation. We also provide an alternative protocol, which does not require supporting matrices. The cancer tissue is obtained directly from clinics (after surgery or biopsy) and processed into single cell suspension. Systematic drug sensitivity and resistance testing (DSRT) is carried out on the PDCs directly after cancer cell isolation from tissue or on cells expanded for a few passages. In the 3D-DSRT assay, the PDCs are plated in 384-well plates in Matrigel, grown as spheroids, and treated with compounds of interest for 72 h. The cell viability is directly measured using a luminescence-based assay. Alternatively, prior to the cell viability measurement, drug-treated cells can be directly subjected to automated high-content bright field imaging or stained for fluorescence (live) cell microscopy for further image analysis. This is followed by the quality control and data analysis. The 3D-DSRT can be performed within a 1–3-week timeframe of the clinical sampling of cancer tissue, depending on the amount of the obtained tissue, growth rate of cancer cells, and the number of drugs being tested. The 3D-DSRT method can be flexibly modified, e.g., to be carried out with or without supporting matrices with U-bottom 384-well plates when appropriate for the PDCs or other cell models used.

1. Introduction

Patient-derived cells (PDCs) have, in recent years, revealed a great potential for basic and translational research, including e.g., drug discovery and functional precision medicine [1]. The understanding of the importance of the cell–cell and cell–extracellular matrix interactions for maintaining the tissue-like properties of cell cultures have led to the development of different types of 3-dimensional (3D) cell cultures, including spheroids, and organoids, which are considered to be physiologically more relevant than conventional 2D cell cultures on plastic [2,3]. Various 3D mimicking *in vitro* systems include matrices such as Matrigel, laminin, vitronectin, gelatin, collagen, and several animal-free matrices or scaffolds, as well as mechanistic systems (such as U-bottom plates), forcing the cells to form 3D spheroids. In particular, organoids, derived from self-organizing stem cells, can recapitulate the *in vivo* architecture, functionality, and genetic signature of original tissues [4,5]. On the other hand, co-culture systems have been established, such as those with immune cells like chimeric antigen receptor (CAR) T-cells [6] and natural killer (NK) cells [7], fibroblasts and epithelial cells, to reconstitute the stroma–cancer cell interactions and intestinal

Abbreviations: DSRT, Drug sensitivity and resistance testing; 3D, 3-dimensional; 2D, 2-dimensional; CTG, CellTiter-Glow; PDC, patient-derived (cancer) cell; PDX, patient-derived xenograft; DSS, drug sensitivity score; SSMD, strictly standardized mean difference; QC, quality control.

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organoid co-cultures with microbes to study host–microbe interactions [8,9].

Traditionally, 2D cell cultures have been utilized in the initial phase of drug discovery [10]. There is a lot of existing data from 2D cell line screens that have served as initial findings for drugs nowadays approved for disease treatment. 2D drug testing methods have also been utilized for drug sensitivity screening of PDCs. Unfortunately, 2D screening conditions are not representative of the complex original tissue from where the PDCs are derived [3,11]. In addition, several drugs have been reported to be either less active in 2D (such as kinase inhibitors) or more active in 2D [12,13] (such as cell growth–affecting compounds), than in *ex vivo* conditions mimicking the tumor microenvironment [14]. Moreover, comparison of different models for predicting drug response rates in ovarian cancer have shown 3D *ex vivo* models to be more relevant than those of *in vivo* mice models for predicting Carboplatin responses [15].

Therefore, the selection of cell models to be used in drug testing is highly important depending on 1) the nature of the follow-up studies (whether it is basic cell biology, precision medicine, long-term/short-term studies, patient-derived xenograft (PDX)- or further cell line development) and 2) the throughput needed for such assays. Recent advances in organ-on-a-chip, microwell chips and microfluidic technologies that increase the throughput, robustness and variety of possible readouts will likely advance future drug testing.

While organoid models are physiologically well-representative of certain cell types in the original tissue and can greatly increase our understanding of the biology [16], they also have certain limitations. Their growth is slow, ingredients such as specific growth factors needed for cell culture are fairly expensive, in-depth characterization is required to reveal their physiological representativeness, and the throughput (e.g., cell amounts obtained), may not be sufficient for extensive omics or drug testing. In addition, new methods are required for robust quantification of complex 3D cultures. In functional precision medicine of cancer, the compromise to obtain higher throughput in a shorter time (e.g., in the clinical scope of the treatment decisions) and still provide a more tissue-like environment than 2D cultures can be obtained by utilizing cells isolated from fresh cell suspension of the cancer tissue or early passage 2D cultures [17]. Drug testing can be performed on such cells within a matrix or on U-bottom multi-well plates to allow spheroid formation.

Here, we describe our protocols for 3D drug testing of PDCs, originally established for renal cell carcinoma samples [18], in a patient-consented study. The use of freshly isolated cancer cells or establishment of PDC cultures (2D/3D) and 3D drug testing can be performed within a 1–3-week time frame, depending on the amount of the obtained tissue, cell growth rate, and the number of drugs being tested. The cancer tissue is obtained directly from the clinics (after surgery or biopsy) and processed into a single cell suspension. Systematic drug sensitivity and resistance testing (DSRT) is carried out on the representative PDC models in very low passages or directly after isolation of cancer cells from tissue (Fig. 1). Earlier, we have described e.g., the basic 2DDSRT, including the preparation of the drug plates in detail [19]. In the 3D-DSRT assay, the PDCs are plated in 384-well plates in Matrigel with robotics, grown as spheroids, and treated with compounds of interest for 3 days. The cell viability is measured using luminescence-based readout. Prior to the cell viability readout, drug-treated cells can be directly subjected to automated high-content bright field imaging or stained for fluorescence (live) cell microscopy for an additional source of data. The method can be flexibly modified to, e.g., be carried out with/without supporting matrices on U-bottom 384-well plates, if appropriate for the cell models to be used.

All patient samples used for the development of this protocol have been collected with signed patient consents. Sample collection was performed as part of clinical studies in accordance with international ethical regulations.

2. Materials

a. Reagents

Table 1
Reagents used in this protocol are listed together with catalogue number, provider and storage condition, when applicable.

Product	Catalogue number	Provider	Storage condition
Tissue dissociation kit	130-095-929	Miltenyi	+4°C
Red blood cell lysis buffer	130-094-183	Miltenyi	+4°C
TrypLE Express	12605036	Gibco	Room temperature
Matrigel Basement Membrane Matrix	354234	Corning	-20°C
CellTiter-Glo® 2.0 reagent (CTG)	G9241	Promega	-20°C
Cell culture medium	Cell type dependent	Cell type dependent	+4°C
Cells of interest	Cell type dependent	Cell type dependent	-

Note: Any drugs of choice can be used. For our basic protocol for compound storage, drug plate preparation and design for 2D DSRT, please see (Kulesskiy E et al. 2016).

b. Equipment

Table 2
Equipment needed for the protocol is listed. Catalogue number/Model and Provider/Manufacturer is provided when specific equipment is used. Otherwise, general capabilities and examples of equipment and instruments used here are provided.

Product	Catalogue number/Model	Provider/Manufacturer
gentleMACS Dissociator	130-093-235	Miltenyi
gentleMACS Tube C	130-093-237	Miltenyi
Falcon Cell Strainers 70 μm	352350	Corning
Scalpels	-	-
Sterile forceps	-	-
Bottles/dishes for cell culture	-	Sarstedt
Ultra Low Attachment (ULA) 384-well plates	3827 or 4588*	Corning
Tissue culture (TC)-treated 384-well plates for 2D	3764	Corning
Biomek 384-well multichannel tips	AP384 P30XL	Beckman Coulter
Echo 550 Acoustic Dispenser	-	Beckman Coulter
PHERAstar FS multimode plate reader	-	BMG Labtech
MultiFlo FX with RAD	-	BioTek
MultiFlo FX RAD 5 µl dispensing cassette	1260016	Agilent BioTek

*4588 is the suggested replacement for 3827 by the manufacturer.

c. Software

The DSRT data is analyzed using the Breeze web application (https://breeze.fimm.fi). Breeze is an open source, publicly available drug screening data analysis pipeline, which helps users with running data through the Quality Control (QC) statistics, dose–response curve fitting, and calculating compound quantification metrics such as IC50/EC50/DSS along with interactive visualizations. Other openly available tools for drug screening data analysis include e.g. GRmetric [20], cellHTS [21] and PharmacoGX [22].

Breeze is built with the help of R, PHP, JavaScript and MySQL. Breeze accepts the data in the tabular format which can hold data from multiple screens in a single file. The details of the input formats, tutorial and technical documentation is available on the Breeze website: https://breeze.fimm.fi/DSRT_documentation/docs.html.

3. Procedure

1. Experimental instructions

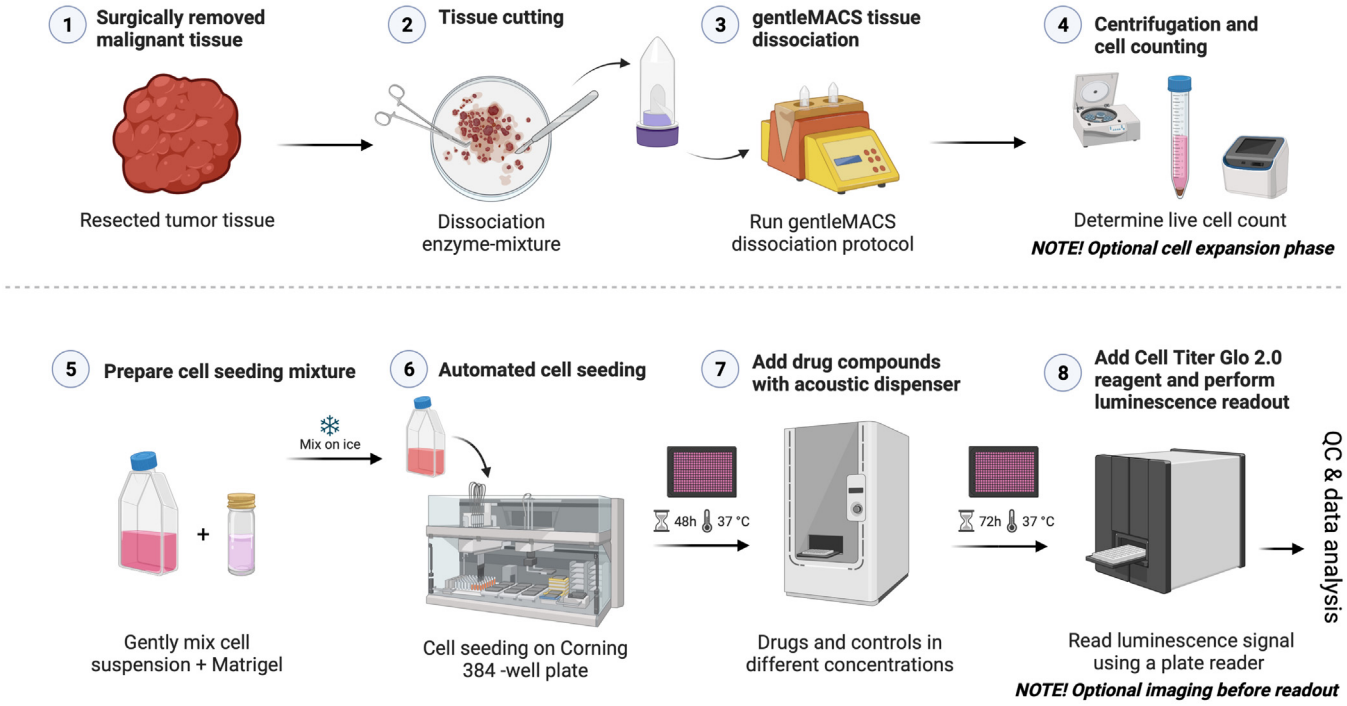

Fig. 1. Stepwise demonstration of protocol workflow for 3D DSRT for PDCs in functional precision medicine. In short, the malignant tissue is processed by manual and enzymatic dissociation into single cells, mixed with Matrigel and seeded on 384-well plates to allow the formation of the spheroids. Drug compounds are then added to the spheroids at different concentrations, followed by a 3-day incubation, cell viability readout with luminescence, quality control and data analysis.

Completion of entire DSRT screening protocol: 3 days

Tissue processing

Timing: 90–120 min

Tissue processing should be initiated as soon as possible after surgical removal, preferably within 2 h after the blood circulation is cut off. For the soft tissue processing, follow the steps of Miltenyi's Tumor Dissociation kit protocol below (NOTE 1).

1. Prepare a dissociation mixture by adding 200 uL of Enzyme H, 100 uL of Enzyme R and 25 uL of Enzyme A to 4.7 mL cell culture medium, e.g., DMEM with no additives. Refer to Table 3 for a tabular description of the component mixture. These volumes are suggested for sample sizes 0.2–1 g.
   Pipet the dissociation mixture to 10 cm culture dishes and cut the tissue into small pieces using scalpel and forceps (NOTE 2).

Transfer to gentleMACS Tube C. Use gentleMACS™ Dissociator to process the tissue according to the following procedure:

a Run gentleMACS Dissociator protocol named h_tumor_01.
b Place the tube at 37°C on a rotator for 30 min.
c Run gentleMACS Dissociator protocol named h_tumor_02 and repeat the incubation step (b.).
d Run gentleMACS Dissociator protocol named h_tumor_03.
e Transfer dissociated cell suspension to microcentrifuge tubes and wash gentleMACS Tube C once with pure medium.
f Centrifuge 5 min at 300 xg. Depending on how the dissociated sample looks, follow the steps below:
   i. Homogenous sample: Proceed to the next step (g).
   ii. Tissue clumps present in solution: Transfer the resuspended sample through a cell strainer. Pellet cells by centrifugation at 300 xg for 5 min. Proceed to the next step (g).
   iii. Homogenous sample, containing a lot of red blood cells: Resuspend pellet in 10 ml 1X red blood cell lysis buffer. Vortex for 5 sec and incubate 2 min at room temperature (RT). Centrifuge for 10 min at 300 xg. Wash cell suspension 2x with appropriate buffer. Proceed to the next step (g).
   iv. Both tissue clumps and red blood cells are present: Proceed with step f. ii. and then f. iii., remove supernatant and continue then with step 7.
g Resuspend the cell pellet with a complete growth medium and plate on culture dishes as explained below (NOTE 3). The suspension can be divided to several plates depending on the pellet size. (*Please see NOTE 4 to proceed directly with seeding of the cells to the drug plates*).

1% Matrigel coating of culture plates (if the cells are cultured in 2D prior to the screening using Matrigel coating protocol; see also "Subculturing PDCs prior to DSRT")

Timing: 30–60 min

1. Defrost Corning® Matrigel Basement Membrane Matrix on ice overnight and mix with cold high glucose DMEM without any additional supplements to a final 1% Matrigel coating solution. Avoid bubbles when handling Matrigel.
2. Add prepared coating solution to the culture dish and spread evenly to cover the plate surface (as reference; 7–8 mL/10 cm dish).
3. Prepare coating by incubating the dish for 15–30 min at 37°C or at RT for 1 h.
4. Remove excess coating solution just before plating the cells to avoid plates from drying. Refer to NOTE 5 for culture initiation of defrosted cells prior to the DSRT.
5. If dishes are to be used later, plates can be sealed and covered with parafilm and stored for about 1 week at +4°C.

Subculturing PDCs prior to DSRT

Timing: 30–60 min

Certain cell types may have specific requirements for successful *in vitro* cell growth. We have previously established PDC cultures using a protocol originally described in [23], e.g., irradiated mouse 3T3 feeder

cells and medium supplemented with Rho-kinase (ROCK) inhibitor, for prostate, renal and ovarian cancer PDCs [18,24]. Additionally, Renal and bladder cancer PDCs have been successfully cultured on 1% Matrigel-coated plates in the presence of ROCK inhibitor–supplemented culture media [18].

Subculture cells every 2–10 days or when 80% confluency is reached. Change culture media every 3–5 days according to sample-specific growth requirements. Cell culture incubators are set at 37°C, 5% CO_2. Follow the steps below for dissociating adherent PDCs:

1. Remove culture media and wash once with phosphate-buffered saline (PBS).
2. Add TrypLE Express and incubate in a cell incubator (37°C, 5% CO_2) until the cells have detached.
3. Ensure single cell dissociation by gentle up and down pipetting.
4. Pellet cells by centrifugation at 300 xg for 5 min at RT.
5. Remove the supernatant and resuspend cells in complete cell culture media.
6. Use an automated cell counter to count cells and
   a Transfer back on coated culture plates if cells are subcultured or
   b Proceed with preparing cell suspension if cells are prepared for DSRT.

Drug sensitivity and resistance testing in 3D Matrigel in 384-well plates

Timing: 120–150 min (incubation steps (48 h + 72 h) not included)

1. Gently mix the optimized cell amount with Matrigel on ice. This is typically 500–4000 cells/well depending on cell type; e.g., 3000 cells/25 μl of growth media in 10% Matrigel per well of a 384-well plate (NOTE 4).
2. Place the reservoir containing cell–Matrigel suspension on top of a cold block to prevent the solution from solidifying (NOTE 6). Pipet 25 μl of the mixture/well to Corning black 384 Ultra Low Attachment plate #3827/4588 using automated 384 multichannel head (here Biomek FX (Beckman Coulter)). Refer to Supplemental file 1 for detailed instructions on instrument setup and cell seeding with MultiFlo FX RAD (Random Access Dispensing).
3. Remove possible air bubbles from the wells with slow centrifugation, 300 xg 15–20 sec.
4. To allow the spheroids to form, pre-culture the cells on the plates for 48 h at 37°C in a 5% CO_2 cell incubator. (NOTE 7, the length of inhibition may vary for cell type. Optimization may be required.)
5. Use a cell culture microscope to visually inspect the formation of spheroids in the wells of the 384-well plate. Alternatively, wells can be imaged with bright field, using a high-content imaging microscope.
6. Add compounds in 5 different concentrations to the plates (here acoustic dispenser Echo 550, (Beckman Coulter) in 2.5–25 nl volumes of DMSO. In this protocol 5 concentration doses are used for each compound, with 10,000-fold concentration range in 25 µl final volume. Each compound has an individually optimized concentration range; thus the stocks have different starting concentrations in DMSO. For negative and positive controls 0.1% DMSO and 100 μM Benzethonium Chloride are used, respectively. See [19] for more detailed information on compound handling.
7. Incubate the plates with cells and compounds for 72 h in an incubator at 37°C and 5% CO2.
8. Take an aliquot of CellTiter-Glo 2.0 reagent (CTG) from -20°C to thaw for 30 min at RT prior to the assay.
9. Let the plates cool to RT for 30 min before adding the CTG. For optional microscopic imaging prior to Step 8, please see NOTE 8 and Supplemental File 1.
10. Perform metabolic luminescence readout by adding 25 μl of CTG to each individual well using automated liquid dispenser (refer to NOTE 9 for how to perform this with MultiFlo FX RAD). Keep CTG reagent protected from light at all times.
11. Incubate plates for 5 min on a plate shaker (covered with aluminum foil), spin plates for 3 min at 300 xg and incubate 20 min (in the dark).
12. Read luminescence using a plate reader (here PHERAstar FS (alternatively e.g. Varioskan LUX, Thermo Fisher Scientific) multimode plate reader) to obtain the signal for the compound-treated spheroids as well as for positive and negative controls. Refer to Supplemental file 1 for detailed instructions on instrument setup and metabolic readout with MultiFlo FX RAD.
13. Perform QC and data analysis according to Section 6 Instructions for Data Analysis.

Tabular description of component mixtures

Table 3
Dissociation mixture preparation is shown in a tabular form. Indicated volumes (given in microliter (µL)) are mixed prior to tissue dissociation of small-sized samples. Refer to Tissue processing step 1 for details.

	Volume (μL)
Culture medium	4700
Enzyme H	200
Enzyme R	100
Enzyme A	25

Critical steps

NOTE 1. Tissue samples should be kept on ice prior to processing. Tissue dissociation protocol should be optimized for each tissue type. This protocol has been utilized for tissue samples derived from kidney, ovarian, prostate, and bladder. Depending on tissue origin, solutions of DNase I [25] or collagenase [18] may be suitable as alternatives for tissue digestion.

NOTE 2. Alternatively, the tissue can be cut in cold PBS or saline solution and then added to the gentleMACS Tube C containing the dissociation mixture.

NOTE 3. Tissue processing of solid non-soft tissues may result in tissue pieces being present after the last incubation step. However, we have observed that when the dissociated tumor suspension is added to be cultured on pre-coated dishes, cell growth may be initiated from remaining tissue pieces.

NOTE 4. In the original protocol, the PDCs are pre-cultured in 2D to allow their expansion prior to the drug testing in 3D. Alternatively, 500–2000 cells can be directly plated after tissue dissociation and red blood cell lysis (Miltenyi, 130-094-183) (if red blood cells are present). To ensure homogenous distribution of cells among all wells, it is recommended to apply the cells through a cell strainer, such as Falcon Cell Strainer (70 microns), prior to cell seeding. Ensure that cell suspension is homogenous to avoid clogging of the dispensing tip.

Here, different types of DSRT assays can be performed:

a) “Direct 3D matrix DSRT”: fresh cell suspension instead of pre-culture steps is mixed with Matrigel as in the protocol described above.
b) “Direct 2D DSRT”: fresh cell suspension is seeded on the pre-administered compounds in 384-well plates (#3764) (for clear cell renal cancer PDCs [18], or for acute myeloid leukemia PDCs [26]: see also [27] for protocol).
c) “Direct 3D single spheroid DSRT”: fresh cell suspension is seeded on the pre-administered compounds in U-bottom 384-well plates (#3827/4588) (see NOTE 9 and [17]).

NOTE 5. Gently defrost a cryovial of PDCs in a 37°C degree water bath. Make sure to keep the O-ring above the water level to reduce the chance of contamination. When almost everything is defrosted, transfer the cells in 9 ml of complete culture media and centrifuge for 5 min at 300 xg. Remove supernatant and resuspend the pellet in complete

culture media. Count cells using a cell counter and plate the cells on a pre-coated culture dish. It is preferable to culture defrosted cells for 1–2 passages prior to the DSRT.

NOTE 6. Cell–Matrigel suspension is also kept on ice during the automated pipetting, here with Biomek FX. Place, e.g., a cold metal block (stored -20°C) under the reservoir to prevent the Matrigel from polymerizing.

NOTE 7. PDCs should be used for DSRT from as early passages as possible since cell proliferation may slow down and original cell composition may change. Ideally, always seed cells for DSRT one or two days after the previous subculture procedure. PDCs and original tumor tissues must always be characterized by genetic profiling of somatic mutations and copy number variations, and by image-based phenotyping to ensure that the PDCs are matching the original tumor tissue by genotype and phenotype.

NOTE 8. An optional high-content imaging step may be performed prior to metabolic readout. Shortly, the image-compatible 384-well plates (Corning #4588) can be imaged using the OperaPhenix high-content confocal microscope (or other similar e.g. ImageXpress Micro Confocal (Molecular Devices, San Jose, CA)), using bright field and 5x–20x objective. The fluorescent staining of live cells can also be performed prior to cell viability measurement, see e.g. [17].

NOTE 9. A similar procedure can be applied for drug screening of PDCs in U-bottom plates, allowing the formation of one spheroid due to the U-shape of the well, as described [17]. Shortly, seed a total of 500–5000 cells on U-bottom 384-well plate (Corning, #3830) with pre-administered compounds in a final volume of 25 μl using an automated liquid dispenser. Centrifuge for 5 min at 300 xg to allow the cells to settle to the bottom of the wells. Incubate plates for 72 h at 37°C, 5% CO_2 prior to the microscopy [17] and the measurement of cell viability, as described in this protocol.

Troubleshooting

Table 4
Listing of potential problems that may arise while performing the protocols. Each of the problem has been indicated with the occurring step (DSRT refers to the step of "Drug sensitivity and resistance testing in 3D Matrigel in 384-well plates") where it may occur, and the corrective action needed to resolve the problem.

Problem	Step of occurrence	Corrective action
Not sufficient or too many cells	Subculturing PDCs prior to DSRT	Passage cells until enough, without exceeding maximum passage number. Adjust cell number using automated cell counter.
Matrigel is not well mixed with the cell culture suspension	DSRT Step 1	Make sure Matrigel is ice-cold. Warm and solidified Matrigel does not mix well.
Clogging of the automated liquid dispenser tube	DSRT Step 2 and/or Step 10	Perform excess washing and backflush of the tubing system with sterile MQ and PBS until the fluid flows normally.
No formation of spheroids in 10% Matrigel	DSRT Step 4	Pre-culture time may be adjusted to fit the cell type characteristics. Ensure cells are not quiescent.
QC Z_prime < 0.5	DSRT Step 13	Careful consideration of the reliability of the results. DSRT screen is recommended to be repeated to obtain Z_prime > 0.5. Ensure single cell suspension during seeding step to avoid unequal amount of spheroids/well.

Flow diagram

Instructions for Data Analysis

The analysis pipeline should consist of QC steps, dose–response fitting and drug quantification metrics such as drug sensitivity score (DSS) [28] and quantitative scoring of drug responses [29]. Breeze [28] accepts the plate reader output in a table format file in CSV/TXT/XLSX format. The table should include columns representing well identifier, plate number in the set, drug name, concentration, well signal and screen/sample identifier. (Demo data can be found in the supplementary files and on the Breeze website). Go to Breeze website (https://breeze.fimm.fi), upload this file and click "Start". Once the process is finished, the user can view or download the results to the computer. The results folder contains QC statistics and plots in the form of interactive visualizations such as scatterplots and plate heatmaps in standalone HTML format. Dose–response fits for each screen/sample with drug quantification metrics are provided in Excel file format and in the form of interactive heatmaps and bar plots.

4. Anticipated results

Here, we provide a protocol for testing drug responses of PDCs in the 3D matrix, as well as for QC and data analysis using our open-source software, Breeze (See Fig. 1 for the stepwise demonstration of protocol workflow). The protocol has been utilized, e.g., to compare how the different supporting matrices and 2D conditions affect drug responses of PDCs from ovarian cancer patients (Feodoroff et al., submitted manuscript). Additionally, the modified matrix-free version of the protocol has been used for 3D DSRT of cell lines and pediatric solid tumor PDCs on U-bottom plates [17]. As such, the protocol is robust and can be modified for different liquid handling systems. It can also be performed manually if the pre-drugged plates are available, however, the quality may not be as good as with the automated cell seeding. Essential for the protocol is to ensure that the cells are seeded in a single cell suspension, and that they are well mixed with Matrigel. Higher concentrations of matrix (here 10% Matrigel) may be needed for PDCs from some cancer types to allow the spheroid formation, however, those can be hard to mix well to obtain a homogenous solution with cells and to deliver with an automated system. Potential challenges in the workflow and the corrective actions are listed in Table 4´.

Essential to any high-throughput drug testing assay is the measurement of the assay quality. This is ensured by including the positive and negative controls to the drug plates and analyzing their performance by looking at the QC metrics such as Z'/SSMD or Signal vs Background. Visual inspection helps in identifying, e.g., technical or systematic errors, patterns, or signal window between controls. Analytical tools, such as Breeze, help quantify drug responses with DSS, describing the drug sensitivity of PDC spheroids on tested drugs. This also allows us to compare the results when DSRT is performed using the same drug concentrations, in 2D conditions, with different supporting matrices or in matrix-free conditions. In addition, the DSS of healthy controls (e.g from a DSRT assay with cells from benign tissue of the same patient) can be used to calculate the selective DSS [26], revealing the PDC-specific drug responses from generally toxic agents.

Web references

Breeze web application [28] https://breeze.fimm.fi/41420_mc41otqxmjgwmcaxnju3mdeynda0/index.php; [accessed 5 July 2022].

Author contributions

VP, PM, and MF designed the laboratory experiments and PM carried them out. VP, PM, MF, and SP analyzed the data. SP prepared the QC. OK, MA and AM provided the clinical samples, and MA and AM prepared the PDCs. LT and PM designed and performed the compound testing pipeline including the automation. VP originally supervised and led the project. VP, MF, LT, SP and PM wrote the manuscript. VP, MF, and PM prepared the figures, tables and data files included in the paper. All authors read and approved the final manuscript.

Declaration of Competing Interest

Piia Mikkonen reports a relationship with UPM that includes: employment and funding grants. Vilja Pietiäinen reports a relationship with UPM that includes: funding grants. Olli Kallioniemi reports a relationship with Medisapiens that includes: board membership. Olli Kallioniemi reports a relationship with Sartar Therapeutics that includes: board membership. Olli Kallioniemi reports a relationship with Sweden's Innovation Agency that includes: funding grants. Piia Mikkonen was performing the experiments of the study when working at FIMM-UH, and is now working at UPM. FIMM-UH and UPM had a research collaboration project in 2016–2018.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.slasd.2022.11.003.

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