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Multimodal detection of protein isoforms and nucleic acids from mouse pre-implantation embryos

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Abstract

Although mammalian embryo development depends on critical protein isoforms that arise from embryo-specific nucleic acid modifications, the role of these isoforms is not yet clear. Challenges arise in measuring protein isoforms and nucleic acids from the same single embryos and blastomeres. Here we present a multimodal technique for performing same-embryo nucleic acid and protein isoform profiling (single-embryo nucleic acid and protein profiling immunoblot, or snapBlot). The method integrates protein isoform measurement by fractionation polyacrylamide gel electrophoresis (fPAGE) with off-chip analysis of nucleic acids from the nuclei. Once embryos are harvested and cultured to the desired stage, they are sampled into the snapBlot device and subjected to fPAGE. After fPAGE, ‘gel pallets’ containing nuclei are excised from the snapBlot device for off-chip nucleic acid analyses. fPAGE and nuclei analyses are indexed to each starting sample, yielding same-embryo multimodal measurements. The entire protocol, including processing of samples and data analysis, takes 2–3 d. snapBlot is designed to help reveal the mechanisms by which embryo-specific nucleic acid modifications to both genomic DNA and messenger RNA orchestrate the growth and development of mammalian embryos.

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Author contributions

E.R.-C. conceived the idea for the snapBlot assay. E.R.-C. and A.G. performed immunoblotting experiments and analyzed immunoblotting data. A.J.M. collected, cultured and handled mouse embryos, performed RT–qPCR experiments and analyzed RT–qPCR data. All authors wrote the manuscript.

Code availability

MATLAB analysis scripts are available in Supplementary Data 1. These analysis scripts (particularly intProf.m, fitPeaks.m and goodProfiles.m) have been adapted from those posted as part of the *summit* code for single-cell western blot analysis, which can be found at https://github.com/herrlabucb/summit.

Competing interests

The authors are inventors on pending patents related to snapBlot assays.

Supplementary information is available for this paper at https://doi.org/10.1038/s41596-020-00449-2.

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Reporting Summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Introduction

Embryo-specific nucleic acid modifications, including retrotransposon activity-derived genomic modifications and alternative splicing of messenger RNA (mRNA), are crucial for the development of mammalian embryos[1]. The importance of alternative splicing as a developmental regulatory mechanism, however, has been established by monitoring mRNA isoform levels and not protein levels[2,3]. Determining if all genomic modifications and mRNA isoforms translate to protein variations remains an intriguing question that requires simultaneously measuring (i) nucleic acids (DNA variations and mRNA isoforms) and (ii) protein isoforms in early-stage embryos.

Recently introduced technologies allow interrogation of the genome, epigenome, transcriptome, metabolome and proteome at single-cell resolution[4-7]. In addition, various approaches can now be combined to measure proteins and DNA and/or RNA from the same single cell[4], allowing researchers to link genome, transcriptome and proteome in challenging cell types with low availability, such as rare cell populations or cells that cannot be expanded by culture. However, the specificity of the protein measurement in such assays typically relies on antibody probes alone, leading to nonspecific cross-reactivity and making selective detection of specific protein isoforms problematic when isoform-specific antibody probes are not available[8]. As a result, identifying different proteoforms arising from modifications to DNA or mRNA remains extremely challenging.

To overcome this challenge, we recently developed a technique for performing same-embryo nucleic acid and protein isoform profiling[9]. We designed a polyacrylamide (PA) gel-based device, called snapBlot, consisting of a 200-μm-thick PA gel covalently grafted to a polymer substrate. After sampling embryos into microwells patterned on the PA gel, we perform fractionation polyacrylamide gel electrophoresis (fPAGE), where the cytoplasmic membrane of the embryos, but not the nuclear envelope, is lysed, and the cytoplasmic fraction is subjected to polyacrylamide gel electrophoresis (PAGE) in the PA gel abutting each microwell, while each nucleus remains intact in the microwell. The polymer substrate allows for laser excision of specific areas of the gel, or gel pallets, containing the microwells with the fractionated nuclei. The nuclei-containing gel pallets are then collected to perform transcriptomic measurements.

Overview of the procedure

We use microfluidic design to integrate fPAGE with off-chip analysis of mRNA and DNA for paired nucleic acid and protein isoform measurements on the same embryos (Fig. 1). The technique starts with fabrication of the snapBlot device, consisting of a 200-μm-thick PA gel grafted onto a clear, polyester substrate (GelBond PAG film) (Steps 1–19). The PA is patterned with an array of microwells that are 200 μm in diameter and depth. Next, once embryos are harvested and cultured until the desired stage is reached, embryos are treated

for removal of the zona pellucida and sampled into the microwells of the snapBlot device using a mouth-controlled pipette assembly[10] (Steps 20–35). Embryos are then subjected to a fractionation lysis buffer that lyses the cytoplasmic compartment of the cells but not the nuclei (Steps 36–40). An electric field is applied to drive the cytoplasmic proteins into the PA layer, and proteins are resolved by molecular mass, granting an additional degree of specificity to the protein measurement (Step 41). Proteins are then immobilized into the PA layer by a process of photo-blotting, where ultraviolet (UV) light activates benzophenone moieties in the gel and covalently binds the proteins to the gel (Steps 42–46). The nuclei, which are retained in the microwells, are excised from the device in sections termed ‘gel pallets’ (Steps 47–58). These gel pallets are then removed from the device and processed for analysis of DNA or mRNA or both (Steps 59–62). Gel pallets are treated with proteinase K to remove remaining proteins, and DNA and mRNA are extracted using single-cell nuclear extraction kits (Steps 63–68). Although any type of nucleic acid analysis can then be performed, we describe here analysis of the mRNA by reverse transcriptase quantitative polymerase chain reaction (RT–qPCR; Step 68). Proteins in the remaining device are probed with fluorescently labeled antibodies and imaged for immunoblot analysis (Steps 69–98). Area under the curve (AUC) analysis is performed on immunoblots to quantify expression of proteins (Steps 99–109).

Comparison with other methods

Current same-cell mRNA and protein analysis methods can be categorized into three main assay strategies: imaging, division of lysate and conversion to common molecular format (Table 1)[11]. Imaging-based assays combine fluorescence-activated cell sorting (FACS)[12], mass cytometry[13] or imaging mass cytometry[14] to measure protein targets, with fluorescence in situ hybridization[12,14,15] or proximity ligation assay for RNA (PLAYR)[16] to quantify mRNA. Methods that divide single-cell lysates into fractions for protein or mRNA analysis apply RT–qPCR to the mRNA fraction and proximity extension assay (PEA)[17] or digital proximity ligation assay (dPLA)[18] to the protein fraction. Finally, a third category of techniques map protein information to nucleic acid information using oligonucleotide-labeled antibodies[19,20] or PEA[21]. Thus, the assay converts both DNA/RNA and protein measurements to a common molecular format: nucleic acids that can be amplified and read out using RT–qPCR[21] or single-cell RNA sequencing[19,20]. Although techniques combining single-cell RNA sequencing and oligo-labeled antibody staining can be very powerful in terms of multiplexing (10,000s of RNA targets and 10s–100s of protein targets)[19,20], these methods are predominantly designed for the detection of surface proteins[19,20]. These methods can only be extended to intracellular targets using reversible chemical cross-linking[22], which was found to reduce the number of genes detected by ~10%[22], and can lead to common fixation artifacts, such as epitope masking[23-25].

Advantages

Although all of the methods described in the ‘Comparison with other methods’ section have been developed for single cells, not all are easily applicable to preimplantation embryos. Methods that require large starting sample sizes (100s–1,000s of cells), such as FACS-based methods, are difficult to implement when studying embryos. An average of ~20 embryos are collected from a single superovulated C57BL/6J female mouse. Therefore, obtaining a lower

estimate of 100s–1,000s of embryos would require sacrificing dozens of mice for a single experiment, which is unrealistic for the average laboratory.

The specificity of the protein measurement for most methods relies solely on detection by antibodies. PLA, dPLA and PEA-based methods[17,18,21] increase specificity by requiring two antibodies to bind to the same target for amplification to occur. However, it might be difficult to identify two isoform-specific antibodies that can bind a certain isoform at the same time (i.e., antibodies that bind to different epitopes)[18]. Our technique adds specificity by resolving proteins by molecular mass before immunoprobing, allowing protein isoforms of different molecular mass to be resolved using a single antibody probe.

This protocol has been designed agnostic to the downstream nucleic acid analysis. Thus, any single-cell genomic or transcriptomic assay can be applied at the off-chip analysis stage once nucleic acids are extracted from the gel pallets. Because snapBlot captures intact nuclei in gel pallets, it has the added benefit of minimizing nucleic acid loss. This is in contrast to strategies that divide the cell lysate or permeabilize and fix the cell, which might reduce the number of transcript copies available to detect.

Limitations

The major limitations of this protocol relate to the fractionation of cells into cytoplasmic and nuclear fractions to isolate the nuclei for downstream nucleic acid extraction. Because only cytoplasmic proteins are subjected to fPAGE for subsequent protein isoform detection, this might constitute a limitation when proteins of interest are present in the nucleus or when studying the subcellular localization of proteins. If information on subcellular localization is desired, performing a whole-embryo immunoblotting experiment[9] is recommended instead.

Because only the nuclei are harvested for mRNA analysis, only the transcripts present in the nucleus will be analyzed. Although gene expression measured from nuclei correlates well with whole-cell measurements for most cell types[26], the same might not be true of pre-implantation embryos, given that total mRNA of pre-implantation embryos comprises both maternally inherited mRNA and zygotically active mRNA[27]. After zygotic genome activation and maternal clearance, transcripts found in the nuclei should largely represent the active transcriptome[28]. However, given that the kinetics of this transition are not yet clear, care must be taken when interpreting mRNA analysis results, as total embryonic mRNA might differ from nuclear mRNA.

Another limitation of the current protocol is that it is not suitable for fixed tissues. To directly compare embryo samples, running them on the same gel is recommended. Thus, being able to fix samples, collect, store and assay them together is often desirable. Because the protocol is not designed for fixed samples, it might be difficult to simultaneously obtain different stages and phenotypes at the same time. However, we have demonstrated analysis of multiple different types of stages by staggering timed matings so that, at the moment of processing, the desired development stages can be collected simultaneously[9].

Applications of the method

Although we originally developed this approach for pre-implantation embryo studies, the multimodal approach is anticipated to be relevant for analyses of a wide range of cellular specimens, from single cells to ~100s of cells. We are currently working to expand the scope of the snapBlot to a broader range of cell types, including patient-derived cells (circulating tumor cells[29] and tumor tissue sections[30]) and cultured mammalian cell lines (e.g., glioblastoma cell lines[31], such as U373 and U251, and breast cancer cell lines[29,32], such as MCF-7, SKBR-3 and BT-20).

Furthermore, with a nuclei collection function that is agnostic to downstream analysis methods, we anticipate a myriad of nucleic acid analysis methods to be suitable to the nuclear fraction, including DNA sequencing, RNA sequencing and whole-genome sequencing[26,33].

Experimental design

Superovulation and mating—Embryo production can be guided by various methods; two of the more accessible methods are timed mating and superovulation. For timed mating, male and female mice are housed one to one and checked each morning for evidence of mating by the presence of a copulatory plug[10]. As this event typically occurs around midnight, the presence of a plug has been accepted to denote 0.5 days post-coitum (0.5 dpc), and, as such, embryo collection can be matched to this timeline. This method typically generates 8–10 embryos. The alternative method of superovulation has the benefit of increased number of embryos (10–25) as well as temporal control of the timing of mating, helping to more precisely coordinate each experiment. The hormones pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) act as synthetic analogs of gonadotropins that are naturally produced in the anterior pituitary glands during sexual development and reproduction[34]. The hormones are introduced via injection ~46–48 h apart (PMSG then hCG) to mimic their natural counterpart cycle. At the same time as the hCG injection, females are housed with males to produce zygotes (0.5-dpc embryos).

The number of females and embryos required for each snapBlot experiment depends on the specific experimental design. In general, an individual successful mating provides enough material for a measurement to be statistically meaningful. As an example, when the goal of the experiment is to test conditions where the researcher does not require a direct comparison, then one or two females housed individually with males for timed mating would generate approximately eight embryos from each mating. However, if a direct comparison is to be made between ‘wild-type’ and ‘mutant’ animals, or a developmental time course is needed, then a more complex strategy involving superovulation is required. For a direct comparison, two or three females of each genetic background would be needed to compensate for the occasional failures associated with superovulation. If only one of each cohort responds well to the hormone regimen, the researcher can expect ~15 embryos of each condition. If a developmental time course is needed, then the hormone regimen could be staggered so that each collection date overlaps with one another. For additional information and guidance for such an experiment, we refer researchers to *Manipulating the Mouse Embryo,* if more experimental details are desired for this procedure[35].

Embryo collection and processing—Various protocols exist to help guide the reader in collecting embryos at the desired stage[10]. Once collected, embryos must first be stripped of the zona pellucida: a glycoprotein layer that has an essential role in spermatozoa binding, acrosome reaction and fertilization[36] (Fig. 1; Steps 20–29). Although this structure is important for fertilization, its presence provides challenges during fPAGE. The zona pellucida almost totally consists of the immensely abundant proteins ZP2 and ZP3, which might interfere with downstream measurements and interpretations. As a technical consideration, the zona pellucida is resistant to lysis and might 'trap' proteins of interest, preventing their migration through the PA. To prevent these issues, the zona is fully eroded by transient exposure to acidic Tyrode's (AT) solution. This dissociates the zona protein constituents while leaving the cell membrane intact. The timing of exposure to AT is empirically determined, as batch-to-batch variation is common. Typically, however, 60–90 s with gentle agitation is sufficient. If the AT solution is unable to fully dissolve the zona pellucida, quick exposure to a second droplet of AT solution is effective.

Expertise needed to implement the protocol—Collection and culture of embryos requires basic mouse embryo manipulation skills, including the ability to perform surgeries to collect pronuclear-stage embryos from superovulated females and the ability to handle embryos. To successfully perform the assay, knowledge and handling of the following equipment is required: a CO_2 laser cutter, a high-voltage power supply and a high-power UV light source (350–360 nm). To process and analyze DNA/RNA, researchers require molecular biology training. Our immunoblot image analysis scripts are written in MATLAB and available as Supplementary Data 1.

Materials

Animals

- 3- to 6-month-old male mice and 3- to 5-week-old female mice (C57BL/6J (Jax 000664) or C57B/6N (Jax 005304) **! CAUTION** All appropriate authorizations and compliance for using live mice must be acquired from institutional regulatory bodies before attempting the procedures described hereafter. All animal use, including, but not limited to, housing, breeding, production, sample collection for electrophoresis and euthanasia, was performed in accordance with the Animal Welfare Act and American Veterinary Medical Association Guidelines for the Euthanasia of Animals, in compliance with the Institute for Laboratory Animal Research *Guide for Care and Use of Laboratory Animals* and University of California, Berkeley Institutional Animal Care and Use Committee (IACUC) guidelines and policies. Our animal care and use protocol was reviewed and approved by our IACUC for this project.

Reagents

Superovulation and mating

- Pregnant mare serum gonadotropin, lyophilized (PMSG; ProspecBio, cat. no. HOR-272)

- Human chorionic gonadotropin, lyophilized (hCG; Millipore, cat. no. 230734)
- Dulbecco's phosphate-buffered saline (DPBS), calcium and magnesium free (Gibco, cat. no. 14190-144)

Embryo collection, culture and simple preparation

- M2 medium (Zenith Biotech, cat. no. ZFM2-050)
- Bovine serum albumin (BSA; Sigma-Aldrich, cat. no. A3311, embryo culture grade)
- Hyaluronidase/M2 (Millipore, cat. no. MR-051-F)
- Acidic Tyrode's (AT) solution (Sigma-Aldrich, cat. no. T1788, embryo culture grade)
- KSOM+AA medium (potassium-supplemented simplex optimized medium plus amino acids; Zenith Biotech, cat. no. ZEKS-050)
- Mineral oil (Millipore, cat. no. ES-005C, embryo grade)

Reagents for buffer preparation

- β-Mercaptoethanol (Sigma-Aldrich, cat. no. M3148) **! CAUTION** β-Mercaptoethanol is toxic on inhalation, on contact with skin and if swallowed, and it is hazardous to the aquatic environment. Avoid contact with skin, eyes and clothing, and handle it with gloves in a chemical fume hood.
- Triton X-100 (Thermo Fisher Scientific, cat. no. BP-151)
- Sodium dodecyl sulfate (SDS) (BioReagent, suitable for electrophoresis, for molecular biology, ≥98.5% (wt/vol); Sigma-Aldrich, cat. no. L3771) **! CAUTION** SDS is a flammable solid and is harmful if swallowed or inhaled. Avoid contact with skin and eyes, and handle it with gloves.
- Sodium deoxycholate (≥97% (wt/vol), Sigma-Aldrich, cat. no. D6750)
- 0.5 M Tris-HCl, pH 6.8 (Teknova, cat. no. T1568)
- 1.5 M Tris-HCl, pH 8.8 (Teknova, cat. no. T1588)
- Pre-mixed 10× Tris-glycine electrophoresis buffer (when diluted to 1×, contains 25 mM Tris, pH 8.3, 192 mM glycine; Bio-Rad, cat. no. 161-0734)
- Deionized water (ddH_2O, 18.2 MΩ, obtained using an ultrapure water system from, for example, Millipore)
- Tris-buffered saline with Tween-20 (20× TBST, Santa Cruz Biotechnology, cat. no. sc-281695)
- Digitonin (Sigma-Aldrich, cat. no. D141) **! CAUTION** Toxic if swallowed. Might cause damage to organs through prolonged or repeated exposure. Avoid contact with skin and eyes, and handle it with gloves.
- Sucrose (Sigma-Aldrich, cat. no. S0389-500G)

- Magnesium chloride ($MgCl_2$) (Sigma-Aldrich, cat. no. M8266)
- HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid); Sigma-Aldrich, cat. no. 90909C)
- Bovine serum albumin (BSA) (heat-shock fraction, protease free, fatty acid free, essentially globulin free, pH 7, ≥98% (wt/vol); Sigma-Aldrich, cat. no. A7030)
- Ethanol (VWR International, cat. no. 71001-866) **! CAUTION** Ethanol alcohol is flammable, and it might cause skin and eye irritation. Avoid contact with skin, eyes and clothing, and handle it with gloves in a chemical fume hood.
- **! CAUTION** Isopropyl alcohol is flammable, and it might cause skin and eye irritation. Avoid contact with skin, eyes and clothing, and handle it with gloves in a chemical fume hood.
- Primary antibodies against protein targets. Example unlabeled primary antibodies include the following: rabbit anti-β-tubulin (Abcam, cat. no. ab6046, RRID: AB_2210370), mouse anti-CDX-2 (Abcam, cat. no. ab157524, RRID: AB_2721036), rabbit anti-SOX-2 (Millipore cat. no. AB5603, RRID: AB_2286686) and goat anti-green fluorescent protein (GFP) (Abcam, cat. no. ab5449, RRID: AB_304896). Example labeled primary antibodies include rhodamine anti-actin primary antibody (Bio-Rad, cat. no. 12004163, RRID: AB_2861334)
- Secondary antibodies. Example antibodies include the following: Alexa Fluor 594 donkey anti-mouse IgG (Thermo Fisher Scientific cat. no. A-21203, RRID: AB_2535789), Alexa Fluor 488 donkey antirabbit (Thermo Fisher Scientific cat. no. A-21206, RRID: AB_2535792) and Alexa Fluor 555 donkey anti-goat (Thermo Fisher Scientific cat. no. A32816, RRID: AB_2762839)
- Phosphate-buffered saline (PBS, pH 7.4, Gibco, cat. no. 10010-023)
- Hoechst 33342 Solution (20 mM, Thermo Fisher Scientific cat. no. 62249)

snapBlot device fabrication

- Tetramethylethylenediamine (TEMED; Sigma-Aldrich, cat. no. T9281) **! CAUTION** TEMED is highly flammable, is corrosive and is toxic on inhalation, on contact with skin and if swallowed. Avoid contact with skin, eyes and clothing, and handle it with gloves in a chemical fume hood.
- Ammonium persulfate (APS; Sigma-Aldrich, cat. no. A3678) **! CAUTION** Harmful if swallowed; might cause irritation to skin and eyes and cause respiratory irritation.
- Acrylamide/bis-acrylamide, 30% (wt/wt) solution (BioReagent, suitable for electrophoresis, 37.5:1; Sigma-Aldrich, cat. no. A3699) **! CAUTION** This material is highly toxic, carcinogenic and teratogenic. Avoid direct contact, and review and understand all Material Safety Data Sheet information.

- BPMAC, N-(3-((3-benzoylphenyl)formamido)propyl) methacrylamide can be custom synthesized by PharmAgra Labs (cat. no. PAL0603)[31,37,38] or a positional isomer (para-form, *N*-(3-((4-benzoylphenyl) formamido)propyl) methacrylamide) of the BPMAC was synthesized in-house[37].
- GelSlick solution (Lonza, cat. no. 50640) ▲ **CRITICAL** The protocol has been optimized using this reagent to render SU-8/silicon molds hydrophobic. Using other materials might yield different results.
- Lonza GelBond PAG Film for acrylamide gels (Lonza, cat. No. BMA54746) ▲ **CRITICAL** The protocol has been optimized using this material as the snapBlot device substrate. Using other materials might yield different results.

Nucleic acid extraction and analysis

- SYBR Gold (Thermo Fisher Scientific, cat. no. S11494)
- Agarose (Thermo Fisher Scientific, cat. no. BP-1356-500)
- TRIzol (Thermo Fisher Scientific, cat. no. 15596026)
- Proteinase K (New England Biosciences, cat. no. P8107S)
- DNA/RNA Shield buffer (Zymo Research, cat. no. R1100-50)
- Quick-DNA/RNA, Microprep Plus (Zymo Research, cat. no. D7005)
- SuperScript4 (Thermo Fisher Scientific, cat. no. 18091050)
- PerfeCTa PreAmp SuperMix (QuantaBio, cat. no. 95146)
- SSO Universal SYBR Green SuperMix, (Bio-Rad, cat. no. 1725275)
- StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, cat. no. 437660)
- Taq PCR kit (New England Biosciences, cat. no. E5000S)
- LE agarose (BioExpress, cat. no. E-3120-500, analytical grade)
- Tris Base (Thermo Fisher Scientific, cat. no. 77-68-1, molecular biology grade)
- Acetic acid (Thermo Fisher Scientific, cat. no. 64-19-7, Glacial Certified ACS)
- 1 Kb Plus DNA Ladder (Thermo Fisher Scientific, cat. no.10787018)
- EtBr (ethidium bromide, 10 mg/ml; Thermo Fisher Scientific, cat. no. 15585011, Ultrapure) **! CAUTION** EtBr is considered a potent mutagen. Avoid skin, eye, mouth and upper respiratory exposure by wearing appropriate personal protective equipment. Nontoxic alternatives can be used, such as Sybr-Safe (Thermo Fisher Scientific, cat. no. S33102)
- Gel loading dye, 6× (Thermo Fisher Scientific, cat. no. R0611, 6×)
- qPCR forward and reverse primers (designed using the NIH tool Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), ordered from Integrated DNA Technologies and diluted to 5 nM. Store at −20 °C for up to 1 year). Example primer sequences include for β-actin (F: GGC

TGTATTCCCCTCCATCG, R: CCAGTTGGTAACAATGCCATGT) and GFP (F: AAGTTCATCTGC ACCACCG R: TCCTTGAAGAAGATGGTGCG)

Equipment

Laboratory materials

- Petroleum jelly (Cumberland Swan Petroleum Jelly, cat. no. 18-999-1829)
- 100-mm tissue culture plates (e.g., Corning, cat. no. 353003)
- SU-8 wafer with an array of microposts with the following specifications: micro-posts of 200 μm in diameter, 200 μm in height and horizontal and vertical spacing between microposts of 5 mm (Fig. 2a). See Supplementary Fig. 1 for 1:1 scaled SU-8 wafer design and Supplementary Data 2 for the original editable design files. The SU-8 wafer can be fabricated by following the protocol described by Kang et al.[37].
- Four-well rectangular slide plate (Thermo Fisher Scientific Nunc, cat. no. 267061)
- Vacuum line
- Spray gun connected to a nitrogen line
- Syringe needle (e.g., PrecisionGlide Needle, 19G × 1 1/2”, BD Biosciences, cat. no. 305187) **! CAUTION** Syringe needles are sharp. Handle them with care, and do not recap.
- Razor blades (VWR International, cat. no. 55411-050) **! CAUTION** Razor blades are sharp. Handle them with care.
- Standard plain glass microscope slide (25 mm × 75 mm) (e.g., Thermo Fisher Scientific, cat. no. 3011-002)
- Large microscope glass plate (152.4 mm × 152.4 mm (6” × 6”), 1.5 mm (1/16”), Chemglass, cat. no. CG-1904-39)
- Large glass coverslip (0.17 ± 0.005-mm-thick #1.5 H glass coverslip, e.g., Ibidi, cat. no. 10812)
- Kapton tape (Uline, cat. no. S-11730)
- Parafilm (Sigma-Aldrich, cat. no. P6543)
- Slide mailers (Heathrow Scientific, cat. no. HS159836)
- Curved tip stainless steel tweezers (e.g., Cole-Parmer precision stainless steel tweezers with fine, curved tips, cat. no. UX-07387-12)

Equipment for embryo culture

- Standard cell culture equipment, including laminar flow hood (e.g., SterilGARD III; The Baker Company, cat. no. SG-603), humidified tissue culture incubators at 37 °C and 5% CO_2 (e.g., Heracell 150i; Thermo Fisher Scientific), water bath

at 37 °C (e.g., Isotemp; Thermo Fisher Scientific), refrigerator (e.g., Sanyo Labcool), −20 °C freezer (e.g., StableTemp; Cole-Parmer) and −80 °C freezer (e.g., RLE60086A, Thermo Fisher Scientific)

- Standard tissue culture disposables, including sterile tissue culture plates (e.g., 40-mm Corning Falcon Easy-Grip Tissue Culture Dishes; Thermo Fisher Scientific) and sterile serological pipettes (e.g., 5-ml serological pipettes; Falcon, cat. no. 356543) for culturing embryos
- Mouth pipette assembly for embryo manipulation. Embryos are manipulated using a mouth-controlled assembly composed of a 15” aspirator tube (Sigma-Aldrich, cat. no. A5177) attached to a glass transfer pipette pulled from glass capillary tubes (Sigma-Aldrich, cat. no. P0674) over an open flame. An ideal glass transfer pipette has a 4–5-cm tip of near uniform diameter, with an opening of ~5× the diameter of an oocyte. To make a mouth pipette, first cut the 15” rubber aspirator tube in half and connect both new ends to a 0.45-μm PVDF syringe filter to prevent contamination from the researcher. Next, insert a glass transfer pipette into the clear pipette holder.

Equipment for snapBlot assay

- Electrophoresis (EP) chamber. The EP chamber can be fabricated out of acrylonitrile butadiene styrene with a fused deposition molding 3D printer (e.g., MakerBot Replicator 2×) as previously described in Kang et al.[37].
- Safety cover (for covering EP chamber during electrophoresis (e.g., 18 cm × 13 cm × 4.5 cm clear, plastic cover))
- Cutting grid. An array of 5-mm × 5-mm squares engraved on a clear acrylic sheet (acrylic sheet McMaster-Carr, cat. no. 8560K615) (see Supplementary Fig. 2 for 1:1 scaled cutting grid design)
- Power supply (Bio-Rad Powerpac Basic) **! CAUTION** The power supply can be the source of a lethal electrical current. Consult manual before use and use proper safety measures. Always use a GFCI plug adapter when plugging the power supply into an outlet.
- 350–360-nm UV illumination system (Hamamatsu Lightning Cure LC5 or similar source, which can irradiate gel with at least ~40 mW/cm^2 of 350–360-nm UV light) **! CAUTION** UV light is hazardous. Appropriate personal protective equipment should be worn while using a UV source.
- CO_2 laser cutter (Full Spectrum Laser, cat. no. HL40-5G-110)
- Bath sonicator (e.g., VWR Scientific Ultrasonic Bath Aquasonic Model 50D)
- Centrifuge (Thermo Fisher Scientific, Sorvall ST 8 Small Benchtop Centrifuge, cat. no. 75007200)
- Centrifuge tubes, 15 ml (Thermo Fisher Scientific Nunc, 15-ml conical sterile polypropylene centrifuge tubes, cat. no. 339651)

- Rotator (Thermo Fisher Scientific, Compact Digital Waving Rotator, cat. no. 88880021)
- Light microscope (MFL-06 Duo-scope Microscope)
- Imaging software (Molecular Devices MetaMorph 7.8.0.0: https://www.moleculardevices.com/products/cellular-imaging-systems/acquisition-and-analysis-software/metamorph-microscopy)
- Fluorescence microarray scanner (e.g., Molecular Device, Genepix 4300A)
- Epifluorescence microscope system, including microscope (Olympus IX71 inverted fluorescence microscope), CCD camera (Andor, iXon+ EMCCD camera) and mercury lamp light source (Lumen Dynamics, X-cite)
- Mini centrifuge (VWR International, Galaxy mini centrifuge)
- Image analysis software (NIH Fiji ImageJ v2.0.0 (https://imagej.net/Fiji) and MathWorks MATLAB R2019 (https://www.mathworks.com/products/matlab.html))
- Immunoblot image analysis MATLAB scripts (Supplementary Data 1)

Reagent setup

100 mM BPMAC—Dissolve 70.048 mg of BPMAC in 2 ml of DMSO. Aliquot 100 μl per 0.65-ml Eppendorf tube and store at −20 °C in the dark for a maximum recommended storage time of 3 months. Avoid freeze and thaw cycles.

1× TBST used as a washing buffer for immunoprobing steps—Add 50 ml of 20× TBST to 950 ml of ddH_2O to make a 1× TBST solution. The final concentration of Tween-20 in 1× TBST is 0.05% (vol/vol). Store the buffer solution at 4 °C for a maximum recommended storage time of 3 months.

Fractionation lysis buffer—Prepare a 0.125 mg/ml digitonin, 0.5% (vol/vol) Triton X-100 and 0.5× tris/glycine solution by combining 15.625 mg of digitonin, 0.625 ml of Triton X-100, 6.25 ml of 10× Tris-glycine electrophoresis buffer and enough ddH_2O for a total volume of 125 ml. Store the buffer solution at 4 °C for a maximum recommended storage time of 3 months.

Nuclei wash buffer—Prepare a 320 mM sucrose, 5 mM $MgCl_2$ and 10 mM HEPES solution by combining 10.9 g of sucrose, 47.5 mg of $MgCl_2$ and 238 mg of HEPES and add enough ddH_2O up to 100 ml. If necessary, adjust the pH to 7.4. Store the buffer solution at 4 °C for a maximum recommended storage time of 3 months.

Antibody probing buffer—Prepare a 2% (wt/vol) BSA in TBST solution by dissolving 2 g of BSA in 100 ml of 1× TBST. Store the solution at 4 °C for a maximum recommended storage time of 3 months.

Stripping buffer—To prepare 100 ml, add 12.5 ml of 0.5 M Tris-HCl pH 6.8 buffer (final concentration of 62.5 mM Tris-HCl), 2 g of SDS (final concentration of 2% (wt/vol)) and 800 µl of β-mercaptoethanol (final concentration, 0.8% (vol/vol)) to 87 ml of ddH_2O in a 100-ml glass container. Mix the buffer well before use. Store at room temperature (20–25 °C) in a chemical fume hood for a maximum recommended storage time of 3 months.

10% (w/v) APS—Dissolve 10 mg of APS in 100 µl of ddH_2O. Store the solution at 4 °C for short-term (<12-h) storage.

▲ **CRITICAL** Freshly prepare the solution before use.

10% (vol/vol) TEMED—Dissolve 10 µl of TEMED in 90 µl of ddH_2O. Store it at 4 °C for short-term (<12-h) storage.

▲ **CRITICAL** Freshly prepare the solution before use.

1 mg/ml Hoechst—Dilute Hoechst 33342 Solution (20 mM) in PBS to a final concentration of 1 mg/ml. Aliquot and store at −20 °C for up to 6 months.

Procedure

snapBlot device fabrication ● Timing ~1 h per device

▲ **CRITICAL** A video of the device fabrication procedure is available as Supplementary Video 1.

1. Prepare the SU-8 wafer mold by pipetting enough GelSlick to cover the entire area of the array and letting it sit for ~1 min before removing the GelSlick and drying the mold (Fig. 2a). This prevents the gel from adhering to the SU-8 mold.
2. To assemble the fabrication setup, first cut a section of GelBond PAG Film into a rectangle with dimensions of a standard microscope slide: 25 mm × 75 mm (Fig. 2c).
3. With the treated side facing up (test the treated side by adding a ~10-µl drop of water and making sure the water spreads across the surface), cut off the top-left corner when the long side of the GelBond is horizontal (Fig. 2b) (this will serve to indicate that the gel side is facing up once the device has been fabricated).
4. Tape the silicon wafer with microposts facing up onto a flat benchtop surface (Fig. 2b).
5. Prepare gel precursor solution in a 1.5-ml Eppendorf tube by adding the reagents listed in the table below in the order listed. Selection of acrylamide %T might require optimization, where a higher % T (e.g., 10%T) will resolve smaller protein targets (e.g., <50 kDa), and a lower %T (e.g., 7%T) might be required for resolving larger protein targets (e.g., >100 kDa).

	7%T/2.7%C Gel Precursor		10%T/2.7%C Gel Precursor	
Reagent	**Volume**	**Final concentration**	**Volume**	**Final concentration**
ddH_2O	336 µl	—	285 µl	—
Acrylamide/bis-acrylamide, 30% (wt/wt), 37.5:1	116 µl	7% (wt/wt)	167 µl	10% (wt/wt)
100 mM BPMAC	15 µl	3 mM	15 µl	3 mM
1.5 M Tris-HCl pH 8.8 buffer	25 µl	75 mM	25 µl	75 mM

6. Use a P200 pipette set to 100 µl and pipette the gel precursor solution up and down at least ten times to thoroughly mix the solution. If needed, spin down at 2,000*g* and room temperature (20–25 °C) for 3 s to remove solution from the cap of the Eppendorf tube.

7. Carefully puncture the cap of the Eppendorf tube with a syringe needle and attach a vacuum line to de-gas the solution in a sonicator for 6 min. If bubbles remain in the tube after sonication, gently tap or flick the tube to dislodge bubbles from the sides of the tube.

   ▲ **CRITICAL STEP** Oxygen must be removed from solution, as it is a polymerization inhibitor.

   ! CAUTION Handle needle carefully and discard in a sharps container after use.

8. Add 4 µl of 10% (wt/vol) APS (0.08% (vol/vol) final concentration) and 4 µl of 10% TEMED (0.08% (vol/vol) final concentration). The total volume of the solution should now be 500 µl.

   ▲ **CRITICAL STEP** Once APS and TEMED are added, polymerization starts. Proceed immediately to the next step.

9. Working quickly but carefully, use a P1000 set to 300 µl to pipette the solution up and down at least three times to mix without introducing bubbles.

10. Load a ~300-µl drop of gel precursor solution immediately over the silicon wafer from Step 4. With the help of forceps, if necessary, gently place the GelBond polymer from Step 3 (with the treated side facing down, touching the precursor) over the drop until the precursor solution spreads across the entire patterned area (Fig. 2c).

11. Place the large glass plate (6” × 6”) over the GelBond strip.

    ▲ **CRITICAL STEP** Take care not to move the GelBond strip around the silicon wafer under the weight of the glass wafer. This could cause shearing the microposts off the wafer (Fig. 2c).

12. Incubate for 30 min to allow gel to polymerize to completion.

13. Carefully lift the glass wafer using an upward movement, so as to not move the GelBond strip around the SU-8 wafer.

14. Pipette ~ 1–2 ml of PBS around the GelBond strip to hydrate the entire gel area. Using forceps, carefully peel the now PA-coated GelBond off the SU-8 wafer (Fig. 2c).

▲ **CRITICAL STEP** Failing to rehydrate the gel might cause tearing of microwells when lifting the gel from the wafer.

15. Place the GelBond-PA gel device in a four-well plate (or equivalent container) filled with PBS, with the gel surface facing up (Fig. 2c).

▲ **CRITICAL STEP** From this step forward, take care to not scratch the gel surface.

16. Inspect the microwells of the gel under a bright-field microscope to make sure that microwells look circular.

? TROUBLESHOOTING

17. Place the plate on a shaker and wash in PBS solution for 30 min.

■ **PAUSE POINT** Gels can be stored in PBS at 4 °C and in the dark for up to 3 d. If longer storage is needed, replace PBS with ddH_2O and place on shaker for 10 min (rinsing the gel in ddH_2O before drying prevents salt crystal formation, which can damage the gel). Then, use a nitrogen stream to dry the gels. Store at 4 °C in the dark for up to 1 week.

Fabrication of 'auxiliary blank' gel ● Timing ~ 45 min per device

▲ **CRITICAL** To facilitate embryo sample preparation, 'blank' gel devices without containing microwells are fabricated. Other non-treated surfaces onto which embryos will not adhere can also be used (Supplementary Fig. 3).

18 Following the same procedure described above for the polymer gel device (Steps 1–17), fabricate an auxiliary 'blank' gel with no microwells and with a 25 mm- × 37.5-mm section (half a microscope slide) of GelBond polymer.

▲ **CRITICAL STEP** Follow Steps 1–17, but, instead of an SU-8 wafer patterned with an array of microposts, use a flat glass surface rendered hydrophobic with GelSlick and spacers. Because the thickness of the auxiliary blank gel is not critical, a variety of spacers can be used, such as tape.

19 Rinse with ddH_20 and dry with a nitrogen stream.

■ **PAUSE POINT** These gels can be stored in the dark at room temperature for up to ~3 months.

Embryo sample preparation ● Timing ~1 h per device (~5 min per embryo)

20 *Removal of zona pellucida (Steps 20–29):* The zona pellucida will clog the PA gel pores when the electric field is applied during fPAGE and must, therefore, be removed before sampling embryos into microwells of the snapBlot device. First, incubate an aliquot of AT solution at 37 °C until equilibrated.

21 Place a dry ‘auxiliary blank’ gel (the PA gel prevents zona-free embryos from adhering to the surface) from Step 19 in a 100-mm tissue culture plate (Fig. 3a).

22 Treat the inside of the capillary of a mouth-controlled pipette assembly (Fig. 3a) by pipetting ~40 µl of a 5% (wt/vol) BSA in PBS solution up and down several times.

▲ **CRITICAL STEP** Embryos will stick to the glass walls of the capillary tube if not treated with BSA.

23 Vortex the AT solution and pipette two drops (~10 µl) of AT solution and three larger drops (~20 µl) of PBS onto the blank gel (Fig. 3a). Make sure that the drops are separated enough that they do not coalesce.

24 Under a stereoscope, collect embryos (~5–20 embryos) from an embryo culture dish and place them into the first drop of PBS to wash embryos of culture medium.

25 Collect the embryos and place them in the first drop of AT solution. Cover the tissue culture plate to prevent the drops from drying and place in the incubator (37 °C).

26 After 2–3 min, check the embryos under the microscope. If the zona pellucida has still not dissolved (Fig. 3b), transfer embryos to the second droplet of AT, cover the tissue culture plate and place in the incubator for another ~2 min.

27 Remove the plate from the incubator and inspect the embryos under the stereoscope. Using the mouth pipette, create gentle agitation of the embryos inside the droplet while monitoring the embryos under the stereoscope for evidence for erosion of the zona pellucida (Fig. 3b).

28 Place embryos into the second drop of PBS to wash any remaining AT solution before proceeding to sampling embryos into the snapBlot device.

▲ **CRITICAL STEP** Keeping embryos in AT solution after removing zona pellucida will harm embryos. Make sure that there is no transfer of AT solution to the snapBlot device.

29 (Optional) If staining nuclei with Hoechst, place a ~10-µl drop of 1 mg/ml of Hoechst in PBS on an auxiliary blank gel. Place embryos in the drop for ~1 min. Wash embryos by placing them into a third drop of PBS to wash any remaining Hoechst solution. If a Hoechst step is not used, the third drop of PBS can be used to further dilute any remaining AT.

30 *Embryo sampling into microwells of the snapBlot device (Steps 30–34):* Treat the inside of the capillary of a mouth-controlled pipette assembly by pipetting 40–50 µl of a 5% (wt/vol) BSA in PBS solution up and down several times.

▲ **CRITICAL STEP** Embryos will stick to the glass walls of the capillary tube if not treated with BSA.

31 In a lab notebook, draw a grid with 3 rows and 15 columns in preparation for annotating the type of sample (e.g., embryo stage and number of cells) added to each microwell (Fig. 3c).

32 Place a snapBlot device from Step 17 in a 100-mm tissue culture plate and rehydrate the snapBlot device by incubating in 10 ml of PBS for at least 2 min.

33 With tweezers, pick up the device, gently dry the back (GelBond side) with a KimWipe, and place it over the lid of a 100-mm tissue culture plate. Pipette ~1 ml of PBS over the gel so a thin layer remains over the gel area. Drain any excess PBS from the lid of the plate using a KimWipe (Fig. 3d).

▲ **CRITICAL STEP** The gel should remain hydrated at all times, but the taller the layer of PBS over the gel the more difficult embryo settling will be.

34 Using the mouth pipette, carefully place each embryo from Step 29 into the microwells of the snapBlot device (Supplementary Video 2) following the suggested layout (Fig. 3c):

- Blank controls: Leave Row A, 1–3 blank. These will be excised as blank microwells and will serve as negative controls.
- Recommendation: Leave Row B empty.
- Embryo loading controls: Load 1–3 embryos in the top row (Row A) as loading controls. These microwells will not be excised from the device and will serve as positive controls for protein bands (i.e., to ensure that the absence of a protein band indicates no protein expression as opposed to the protein being excised from the device).
- Embryos: Start loading embryos in Row C. If needed, load the remaining embryos in Row A, 7–15.

  ▲ **CRITICAL STEP** To prevent embryos from being displaced from the microwells, wait enough time for each embryo to settle to the bottom of the microwell before sampling the next (~30 s to 1 min) (Fig. 3e).

  ▲ **CRITICAL STEP** Take care when moving the device or tissue culture plate lid, as tilting the device to angles >45° or shaking the device will cause embryos to be displaced from the microwells.

  ? TROUBLESHOOTING

35 (Optional) If required, image the sampled embryos in the snapBlot device with a bright-field or fluorescence microscope.

▲ **CRITICAL STEP** Take care when moving the device or tissue culture plate lid, as tilting the device to angles > 45° or shaking the device will cause embryos to be displaced from the microwells.

fPAGE ● Timing ~30 min per device

36 Assemble the fPAGE setup (Fig. 4a). First, turn on the UV light source (Hamamatsu) and allow enough time for the lamp to warm up. Tape the EP chamber to a flat benchtop area. Plug in the power supply using a GFCI outlet adapter. Connect the alligator clips to the appropriate EP chamber electrodes. Set up the power supply to provide constant voltage. The suggested E field is 40 V cm^{-1}, giving a suggested voltage for the given EP chamber dimensions of 240 V.

! CAUTION The power supply can be the source of a lethal electrical current. Consult the manual before use and use proper safety measures. Always use a GFCI plug adapter when plugging the power supply into an outlet. Confirm that the power supply is properly and safely connected to the EP chamber.

37 Prepare a 12.5-ml aliquot of fractionation lysis buffer and equilibrate to room temperature.

▲ **CRITICAL STEP** The volume of lysis buffer must be enough to cover the surface of the snapBlot device and, thus, depends on the dimensions of the EP chamber.

38 Prepare a container with ice and place a ~50-ml aliquot of nuclei wash buffer over ice.

39 Placing the snapBlot device into the EP chamber. Using a swab, place four dabs of petroleum jelly on the EP chamber at the points where the corners of the snapBlot device will touch the chamber (Fig. 4b). Carefully lift the snapBlot device from the tissue culture plate lid and onto the EP chamber, so that the three uncut corners are in contact with the petroleum jelly. Using forceps, press the device down onto the petroleum jelly until the device is resting flat over the EP chamber. Avoid tilting the polymer/gel device, as this might dislodge embryos from the microwells (Fig. 4b).

40 *fPAGE run (Steps 40–42):* Carefully pour the fractionation lysis buffer over in the top-left corner of the EP chamber. Place the safety cover over the assembly (Fig. 4c). Incubate for a suggested time of 60 s. Lysis time will depend on the sample type and might have to be adjusted.

▲ **CRITICAL STEP** Pouring the lysis buffer directly over the area of the gel with microwells can cause displacement of the embryos from the microwells (Fig. 4c).

▲ **CRITICAL STEP** Steps 40–42 must be carried out immediately after one another and must be carried out quickly. Completing a ‘dry run’ with the EP chamber alone (i.e., no snapBlot device, but making sure to add the lysis buffer) will help ensure that all steps (lysis, electrophoresis and photo-blotting) are completed correctly and in a timely manner.

? TROUBLESHOOTING

41 Press the ‘Run’ button on the power supply set to the suggested constant voltage (Fig. 4d). Run electrophoresis for a suggested time of 2 min and 17 s. Then, press the ‘Stop’ button on the power supply and immediately proceed to the next step.

42 Remove the safety cover lid and apply UV light (350–360 nm) to the entire surface of the device at ~1.8 J cm^{-2} to photo-blot the proteins (Fig. 4e).

▲ **CRITICAL STEP** Applying UV light immediately after stopping the electrophoresis will ensure maximum capture of proteins before rapid loss due to diffusion out of the gel and into the surrounding buffer.

! CAUTION Use proper UV personal protective equipment, including UV-blocking goggles and UV-blocking face shield, for the user and any personnel present at the time of UV illumination.

! CAUTION After photo-blotting, ensure that the power supply and UV light source are turned off before proceeding.

43 Remove the safety cover and carefully remove the snapBlot device using tweezers.

44 Place the gel immediately in a well of a four-well plate, gel side facing up, containing 5–10 ml of ice-cold nuclei wash buffer (enough to cover the snapBlot device).

▲ **CRITICAL STEP** From this point on, keep the container holding the device over ice whenever it is not being handled.

45 Incubate for ~10 min and place in a new well of the four-well container with 5–10 ml of fresh ice-cold nuclei wash buffer. Keep the container over ice.

46 Repeat Step 45 to wash once more.

Laser excision of gel pallets ● Timing ~2 h per device (~5 min per microwell)

47 Turn on the CO_2 laser cutter. Focus the laser head by placing the cutting grid on the cutting bed and the spacer puck over the cutting grid. Rest the laser head on the spacer puck and tighten the laser head to fix the laser head in place. Remove the spacer puck and cutting grid.

48 Open the CO_2 laser controller software. Draw a 3-mm × 2-mm rectangular geometry and set the cutting parameters. The recommended settings for an HL40-5G-110, Full Spectrum laser, set to cutting, are the following: Speed = 20, Power = 10, Passes = 1.

▲ **CRITICAL STEP** Each laser cutter will require a different setting to cut through the GelBond PAG film. We recommend performing a polymer/device cutting test using an auxiliary blank gel before proceeding to the snapBlot device containing your samples.

49 Fill an ice bucket with ice. Keep all working solutions, device and samples over ice whenever these are not being handled.

50 Clean the petroleum jelly off the back of the device from Step 46 with an ethanol-soaked KimWipe, making sure not to touch the gel surface. Return the device to the container with ice-cold nuclei wash buffer. If the nuclei wash buffer is contaminated with petroleum jelly, place the snapBlot device in a new well of the four-well container with 5–10 ml of fresh nuclei wash buffer.

51 Label enough PCR tubes as the number of samples you will collect. We suggest the following labeling scheme: Row [letter]-Column [number] (e.g., microwell situated in the first row, first column should be A-1, etc). For blanks, add a 'BLANK' at the end to indicate a blank control (e.g., A-1 BLANK).

52 Fill the PCR tubes with 20 μl of DNA/RNA Shield solution and place the tubes over ice.

53 *Laser excision of gel pallets (Steps 53–58):* Carefully retrieve the snapBlot device with tweezers and set over the cutting grid (with markings facing up) so that the gel is facing down (Fig. 5a). The gel and the cutting grid surface with markings should be in contact. Make sure that there is abundant nuclei wash buffer between the grid surface and the gel to keep the gel hydrated during the laser excision step (Fig. 5a).

▲ **CRITICAL STEP** Test the laser excision procedure on the blank wells on the first row of the snapBlot device (Columns 1–3, left blank as indicated in the suggested layout; Fig. 3c) before moving on to microwells that contained samples. If the microwell area has dried during the cutting, adjust passes/power/speed settings and test again. If the gel pallet cannot be removed from the device, adjust settings (i.e., increasing passes/power/speed) and test again.

54 Under a bright-field microscope, place the device over the grid so that the microwells are aligned over the cutting grid as indicated in Fig. 5b. To ensure that the device does not move and become misaligned during handling, use a KimWipe to drain excess buffer between the device and the cutting grid.

55 Place the assembly (cutting grid and device) on the cutting bed. Tape the cutting grid down to the cutting bed to prevent it from moving (Fig. 5c).

56 Align the laser beam over the top-left corner of the grid of the microwell that you want to excise, as shown in Fig. 5c, and use the 'perimeter tracing' function of the laser cutter to make sure that the cut is performed along the edges of the grid. Realign the grid if necessary.

57 Perform the cut to create the gel pallet. Immediate after cutting, pipette ~20 μl of ice-cold nuclei wash buffer over the area of the gel pallet to hydrate the area, as the laser will have dried the cut edges.

58 Place snapBlot device under a bright-field benchtop microscope, and image the excised area to ensure that the gel pallet contains the unharmed microwell. If embryos have been stained with Hoechst in Step 29, imaging the nuclei under fluorescence microscopy is possible here.

? TROUBLESHOOTING

59 *Gel pallet collection (Steps 59 and 60)* (Fig. 5d): Carefully pick up the gel pallet with fine-tip tweezers and place into a PCR tube containing DNA/RNA Shield solution from Step 52. If the gel pallet is still slightly attached to GelBond film, use tweezers to gently pull the gel pallet upward and away from the snapBlot device to fully detach.

▲ **CRITICAL STEP** Make sure to not scratch the surface of the gel.

60 Carefully close the cap of the PCR tube and spin down using a tabletop microcentrifuge to ensure that the gel pallet reaches the bottom of the tube and is submerged in DNA/RNA Shield solution (Fig. 5d). Place the PCR strip or tubes immediately over ice.

61 Repeat Steps 53–60 for all microwells containing embryos and for controls.

▲ **CRITICAL STEP** Remember to excise a suggested total of 3–6 empty wells left as blanks (Fig. 3c), which will serve as negative controls or contamination controls for the RT–qPCR.

62 Once all gel pallets have been collected in their corresponding PCR tubes, place tubes at −80 °C.

■ **PAUSE POINT** The gel pallets can be stored at −80 °C for up to 1 year.

Gel pallet nucleic acid extraction and RT–qPCR analysis ● Timing ~2–3 h per device (~5–10 min per microwell)

63 Collect the PCR strip or all individual PCR tubes containing gel pallets and thaw over ice.

64 Extract the total RNA and DNA using the Quick-DNA/RNA Microprep Plus Kit according to the manufacturer’s instructions.

65 Elute the samples in 6-µl elution buffer.

■ **PAUSE POINT** The samples can be used immediately or stored at −80 °C for up to 6 months.

66 Convert the entire RNA samples to complementary DNA (cDNA) through reverse transcriptase reaction using SuperScript4, as per the manufacturer’s instructions.

67 Pre-amplify the entire 20-µl sample using the Quantabio Perfecta PreAmp SuperMix as per the manufacturer’s instructions. There are a few important notes to consider. Primers designed for specific gene targets of interest must be included during this step. Failure to include them will result in an inability to later assay via RT–qPCR. Up to 50 primer pairs can be combined. We recommend using the 14-cycle option.

■ **PAUSE POINT** At this point, sample volume will be at 50 µl. Samples can be stored at −20 °C for up to 1 year, but, if used immediately, dilute 1:20 before RT–qPCR analysis in Step 68. Remember to process blank gel pallets as contamination controls.

68 Perform RT–qPCR using SSO Universal SYBR Green SuperMix, as per the manufacturer's instructions. A minus reverse transcription (−RT) control and no template control (NTC) should be included to test for contamination. Instrumentation for running and analyzing each assay (e.g., StepOnePlus Real Time PCR System) is up to the discretion of the researcher.

? TROUBLESHOOTING

Immunoprobing proteins ● Timing ~12 h per probing round

69 Clean a large glass plate with an ethanol-soaked KimWipe and tape onto a flat benchtop area.

70 Prepare 80 μl of antibody dilution in antibody probing solution (2% (wt/vol) BSA in TBST) per device. The antibody dilution will depend on the antibody affinity and might require empirical determination. Typically, a 1:10 or 1:5 dilution of primary antibody is sufficient. The antibody dilution required will be much higher than standard recommendations for slab western blots because thermodynamic partitioning limits the concentration of antibody in the gel in the snapBlot[39].

▲ **CRITICAL STEP** Multiple antibodies can be tested simultaneously if primary antibodies are raised in different species and secondary antibodies are conjugated to different fluorophores (e.g., for primary antibodies goat-anti-β-tubulin and rabbit-anti-β-actin, secondary antibodies could be Alexa Fluor 555 donkey-anti-goat and Alexa Fluor 488 donkey-anti-rabbit). If, however, primary antibodies are raised in the same animal, or secondary antibodies are conjugated to the same fluorophore, the gels can be stripped and re-probed with the new set of antibodies (see Steps 90–98).

71 To prevent antibody solution from evaporating through the holes left in the device from excising the gel pallets, Kapton tape is used to cover the holes (Fig. 6a). Cut a strip of Kapton tape that is about twice the width of the holes. Place the tape strip adhesive side up on the bench (fold the edges over so the edges are stuck to the bench). With the gel side of the device facing up, lay the device over the Kapton tape. Make sure that the tape adheres to the back of the device (on the side opposite to the gel), so that the tape seals the holes. Pick up the device and Kapton tape and gently flatten the tape onto the GelBond using the edge of a pair of tweezers. Cut off the extra tape hanging off the sides of the device using a razor, but leave a small (1-mm) overhang so you can peel the tape off after the probing is completed.

72 After all holes are covered with Kapton tape, rehydrate the gels by placing them gel side down in a 100-mm tissue culture plate with 10 ml of 1× TBST for at least ~ 2 min. Try not to completely submerge the gels such that the back (GelBond side) remains dry, to prevent the antibody solution from spreading across the back and not the gel surface of the device in Step 74 (Fig. 6a).

73 After the gel is rehydrated, pick it up and wipe TBST off the back of the GelBond (otherwise, antibody solution will spread across the back of the GelBond rather than the front of the gel). Leave the gel side hydrated. Place the gel, gel side down, on a glass plate or large glass microscope slide (2” ×3”), tilted so that only one side touches the glass. Prop the other side of the gel up with the curved tips of the tweezers facing up (Fig. 6b).

74 Pipette the antibody solution prepared in Step 70 (use the entire 80-µl diluted antibody solution per device) under the part of the gel where it is touching the glass plate. Make sure that the antibody solution does not wick over the top of the GelBond. Then, slowly lever the tweezers so that the gel slowly lowers and the antibody solution spreads under the gel. If there are bubbles, raise the gel a bit to let them escape and then lower again (Fig. 6b).

75 Soak a KimWipe with ddH_2O and place it near the antibody incubation and create a sealed chamber by placing the lid of a four-well plate on top. Place a weight, such as a tape dispenser (and foil, if needed, to prevent photobleaching of fluorescently labeled antibodies), over the chamber so that it makes contact with the lab bench (or large glass plate) and the chamber is sealed (Fig. 6b).

76 Incubate the gel with antibody solution for 4 h at room temperature. Check at 2 h to make sure that the KimWipe is still hydrated and soak again with ddH_2O if needed.

77 After the 4-h incubation, disassemble the chamber and carefully lift the device from the glass wafer and place in a four-well rectangular plate with fresh TBST solution.

78 Incubate in TBST on a shaker for 1 h.

79 Replace the TBST solution with fresh TBST solution and incubate on a shaker for another 1 h.

■ **PAUSE POINT** If needed, the device can be rinsed with ddH_2O, dried using a nitrogen stream and stored in the dark at 4 °C overnight or up to 1 d. Do not remove the Kapton tape.

(Optional) Immunoprobing with secondary antibodies ● Timing ~6 h

▲ **CRITICAL** This section should be followed only if primary antibodies are not directly labeled with fluorophores and incubation with a fluorophore-labeled secondary antibody is necessary.

80 Rehydrate the device in TBST (if starting from dried, stored device) by soaking in TBST for ~2 min.

81 Prepare 80 µl of secondary antibody probing solution per device. The dilution can be prepared at 1:20 in antibody probing solution (e.g., 4 µl of secondary antibody in 76 µl of antibody probing solution). Spin down the antibody solution for 5 min at 10,000*g* at room temperature using the tabletop centrifuge.

▲ **CRITICAL STEP** Centrifugation removes aggregates that will cause punctate signal during the imaging step.

82 Repeat Steps 71–79 with the solution of secondary antibody, except for Step 76, and incubate the secondary antibody for only 2 h.

83 Place the device in a well of a four-well plate containing 10 ml of ddH_2O for ~2 min to wash. Remove the Kapton tape by peeling gently from the back of the device.

■ **PAUSE POINT** If the user does not want to proceed to imaging directly, devices can be dried and stored in the dark at room temperature for up to 1 d. Dry the gel side of the device using a nitrogen stream. Place in a slide mailer. Place a clean microscope slide behind the back side of the device (not on the gel side) so the device does not curl when completely dry.

Protein fluorescence imaging ● Timing ~30 min per device and per protein

84 Remove devices from slide mailers by using tweezers to gently grab the snapBlot from the corners.

85 (Optional) If starting from a dried device, first rehydrate the device by placing it in 10 ml of ddH_2O for at least ~2 min. Because GelBond film shows autofluorescence patterns, the gel device is imaged hydrated to move the protein bands farther away from the GelBond layer.

86 Prepare the imaging assembly (Fig. 6c). Carefully clean a full-size glass coverslip (~0.2-mm thickness, 25 mm × 75 mm) with an ethanol-soaked KimWipe. Add the coverslip over the gel side of the device; avoid bubbles. Dry the front (glass coverslip side) and back (GelBond) gently with a KimWipe. Carefully dry any excess water from the holes as well. Add a standard microscope glass slide (not coverslip) to the back of the device (GelBond film side). Make sure that there is no water between the glass slide and the gel bond.

! CAUTION Glass coverslips are very thin and break easily. Dispose of broken coverslips in a sharps bin.

87 Place the device, coverslip and glass slide assembly facing coverslip down into the microarray scanner. The standard microscope slide ensures that the sample is thick enough that the pins on the microarray scanner push down on the device (Fig. 6c).

88 Scan the device for protein peaks. Use the embryo loading controls to optimize image settings (gain and power) for each device separately, so that protein peaks are clearly visible but not saturated (Fig. 6d).

▲ **CRITICAL STEP** Given that the area around the gel pallet hole can be saturated, the protein bands can be very faint in comparison and might be difficult to see. Thus, optimize the imaging settings using the protein peaks from the embryo loading controls (Fig. 6d,e) and then check the embryo samples from

which gel pallets were excised. Adjust the brightness and contrast to make sure that the protein peaks from the embryo samples with gel pallet holes are visible.

? TROUBLESHOOTING

89 Once the settings are optimized, scan the entire area of the gel (approximately 25 mm × 75 mm) (Fig. 6f). Inspect the protein peaks from the embryo samples with the gel pallet holes. Protein peaks should be clearly defined (Fig. 6g) and not cut off during the laser excision. If protein peaks are present for embryo loading controls (Fig. 6d), but bands are partially or completely missing from embryo wells with gel pallet holes (Fig. 6h), then the electrophoresis step was not run for long enough. The experiment will have to be repeated with longer electrophoresis times to ensure that bands migrate farther into the gel and away from the gel pallet region.

? TROUBLESHOOTING

(Optional) snapBlot device stripping ● Timing ~2 h

▲ **CRITICAL** If the primary antibodies used in Step 70 were raised in the same animal, or secondary antibodies used in Step 81 are conjugated to the same fluorophore, the gels can be stripped and re-probed with the new set of antibodies according to the steps below.

90 In a fume hood, place the snapBlot device in a slide mailer and fill the slide mailer with 10 ml of stripping buffer or enough to cover the entire snapBlot device. Use Parafilm to seal the slide mailer to avoid any leakage of the stripping buffer during the next steps.

! CAUTION Stripping buffer contains β-mercaptoethanol, which is an acutely toxic chemical after oral, inhalation or dermal exposure. Always handle the solution in a fume hood and use appropriate personal protective equipment.

91 Incubate in a water bath set to 55 °C for 1 h.

92 In a fume hood, cool to room temperature. Open the slide mailer, and, holding the snapBlot device to make sure that it does not slide out of the mailer, carefully discard the stripping buffer in an appropriately labeled liquid waste container.

93 Add 10 ml of ddH_2O to fill the slide mailer and discard in the appropriately labeled liquid waste container. Repeat this step twice.

94 Carefully remove the device from the slide mailers by using tweezers and add to a four-well plate containing 10 ml of TBST.

95 Place on a shaker to wash for 1 h.

96 Replace the TBST with 10 ml of fresh TBST and wash for another 1 h.

97 Prepare the gel for imaging by following Steps 84–89 and scan using the microarray scanner to ensure that most signal has been removed.

? TROUBLESHOOTING

98 Reprobe the gel with new primary (and secondary) antibodies according to Steps 70–83 and perform protein fluorescence imaging as described in Steps 84–89.

Protein image analysis ● Timing ~1 h per device and multiple protein targets

99 *Post-processing in ImageJ (Steps 99 and 100):* Open the scanned image in ImageJ. Using remaining microwells as fiducials and the known distance between microwells, draw a line across each row of excised microwells to indicate the original position of the microwells (Fig. 7a). Delete the pixels in the line and re-save image as a .tiff file. Although the deleted pixel values near the microwell will be converted to zero, this will not affect quantitation as long as nothing is changed below the gel pallets (near the protein band). When re-saving the image with the added line, make sure not to save in a compressed file format, which might alter the quantitative pixel fluorescence. See Source Data 1 for an original pre-processed example scan.

100 Draw a rectangular region of interest (ROI) that encompasses the entire protein band. We recommend a width of 2,500 μm (500 pixels if scanned at 5 μm per pixel) and enough length along the separation lane to fit the entire protein band. Obtain the intensity profile along the width of the lane. Make sure that the protein band is centered and measure the width of the background area to the left and right of the protein band. Calculate the background width as the average between the width of background to the left and to the right of the protein band. (Fig. 7b).

101 *Densitometry analysis (Steps 101–108):* Perform quantitative analysis of the image file at full resolution and bit depth in MATLAB for accurate quantification (e.g., the image produced with the Genepix microarray scanner is a ~80-Mb .tiff file (5 μm per pixel)).

▲ **CRITICAL STEP** Steps 101–108 describe densitometry analysis of immunoblots using MATLAB automated analysis scripts adapted from Kang et al.[37]. See Supplementary Data 1 for all scripts and an example of how to call each function. The immunoblots can also be analyzed using ImageJ or custom code written in MATLAB, R scripts or other analysis platforms.

102 Create ROIs using the roiGenerationSnapBlot.m function. Input the image filename, horizontal and vertical microwell spacing (both 5 mm with the proposed microwell array design) and ROI width (2.5 mm with proposed design) and ROI length (5 mm with proposed design). Lengths should be input in pixel units (Fig. 7c).

103 When prompted by the roiGenerationSnapBlot function to click on the center of the leftmost microwell, click on a point on the line drawn in Step 99 that aligns with the protein band of interest (i.e., the location of the microwell before the gel pallet excision) (Fig. 7c). Next, do the same for the adjacent microwell when prompted to select the rightmost well (Fig. 7c). This should (i) align the image and rotate it so that the separation lane is now completely vertical and (ii) create an ROI of 2.5 mm × 5 mm that comprises the protein band, starting from the

location of the microwell before excision. Note that, while it is recommended to analyze separation lanes one by one, the scripts can also be used to generate and analyze multiple ROIs within one row by selecting the leftmost and rightmost microwells that are several microwell spacings apart.

104 Using the intProf.m function, calculate the intensity profile along the separation axis. To obtain accurate protein quantification results, perform axial background subtraction within each ROI. Set the background width to the calculated background in Step 100 and perform axial background subtraction. If the scripts are unable to detect the protein peaks, the image may be too noisy; increasing the size of the median filter (medfilt2 MATLAB function) used in intProf.m may help. Also, note that the intensity around the gel pallet hole can be much greater than the protein peak intensity. Thus, the protein bands in the intensity profile may be visible only after zooming in on this specific region of the intensity profile.

105 Given the diffusion that the protein band experiences as it migrates down the separation lane during fPAGE, protein peaks typically follow a Gaussian distribution along the axis of the separation lane. To quantify protein expression, use fitPeaks.m (with the signal-to-noise ratio threshold set to 3 or greater) to fit a Gaussian curve and obtain the parameters of peak amplitude, peak center and peak width ($4*\sigma$). These parameters are used to calculate protein expression as the AUC for the peak width ($4*\sigma$). Note that the intensity around the gel pallet hole can be much greater than the protein peak intensity. Thus, when prompted by fitPeaks.m to select the left and right boundaries of the peaks, you may need to use the zoom tools in the figure window to zoom in to see the protein peaks, prior to clicking ‘ok’ on the dialog window.

106 Perform additional quality control by verifying that the intensity profile fits a Gaussian curve well ($R^2 > 0.6$) and by manually inspecting the intensity profiles overlaid with their Gaussian fit (Fig. 7d). This can be done using goodProfiles.m; when each plot appears, if the intensity profile has noise (e.g., a sharp spike or dip within the protein peak) or does not align well with the Gaussian fit, you can choose to exclude the separation lane from analysis by clicking on the blue intensity profile. The intensity profile should turn red, indicating that it will be excluded from analysis. Again, note that you may need to zoom in to see the protein peak, owing to the high intensity near the gel pallet hole.

107 After analyzing all separation lanes, plot AUC values for protein targets (Fig. 7e). If detecting multiple peaks per separation lane, using the peak location and peak width ($4*\sigma$), calculate the separation resolution (R_S) between two peaks to determine if they are fully resolved and can be attributed to different protein isoforms or nonspecific binding events of the antibody probes. Key metrics (e.g., AUC or migration distance) can be extracted from the resulting data structure using results.m.

108 To estimate the molecular mass of protein bands, first probe the snapBlot for loading control proteins of known molecular mass (e.g., β-tubulin, β-actin and GAPDH). Plot the log of molecular mass as a function of the relative migration distance and perform a linear regression fit. Solve for unknown molecular masses using the migration distance and the linear regression parameters.

Troubleshooting

Troubleshooting advice can be found in Table 2.

Timing

Steps 1–17, snapBlot device fabrication: ~1 h per device

Step 18 and 19, fabrication of auxiliary blank gel: ~45 min

Steps 20–35, embryo preparation: 1 h per device (~5 min per embryo)

Steps 36–46, fPAGE: ~30 min per device

Steps 47–62, laser excision of gel pallets: ~2 h per device (~5–10 min per microwell)

Steps 63–68, gel pallet nucleic acid extraction and RT–qPCR analysis: ~2 h per device (~5–10 min per microwell)

Steps 69–79, immunoprobing proteins: ~10 h per probing round

Steps 80–83, immunoprobing with secondary antibodies: ~6 h per device (multiple protein targets)

Steps 84–89, protein fluorescence imaging: ~20 min per device setup + ~10 min per protein target Steps 90–98, snapBlot device stripping: ~2 h per device

Steps 99–108, protein image analysis: ~1 h per device (multiple protein targets)

Anticipated results

The snapBlot protocol first performs fPAGE on single embryos, separating cytoplasmic proteins by molecular mass and immobilizing them in the PA layer of the snapBlot for subsequent immunoprobing. Proteins are then detected by immunoprobing. Characteristics such as migration distance (used to calculate molecular mass), band width and relative abundance can be quantified by analyzing the intensity profiles from each immunoblot. In the case of embryos expressing fluorescent or fluorescently labeled proteins, a positive correlation between the whole-cell fluorescence measured from embryos before lysis (Fig. 8a) and the immunoprobed signal (Fig. 8b and Source Data 1) demonstrates that the snapBlot adequately quantifies protein abundance ($n = 6$ blastocysts, Pearson correlation $R^2 = 0.788$) (Fig. 8c and Source Data 1 and 2). mRNA is extracted from gel pallets (Fig. 8d) and analyzed by RT–qPCR (Source Data 1 and 2). Including the recommended negative controls (blank gel pallets, −RT and NTC) helps verify amplification of mRNA from pallets

and not from contamination (Fig. 8e and Source Data 2). Finally, users can look for correlations between gene expression and protein expression of desired targets (Fig. 8f and Source Data 2). To attribute variation between samples to biological (not technical) variation, coefficient of variation thresholds were previously determined to be 0.77% and 7.4% for the mRNA and protein measurements, respectively[9]. Any variation exceeding these thresholds is expected to constitute biological variation between samples. We recommend running all samples on the same gel, as differences in gel fabrication, lysis/electrophoresis conditions (e.g., temperature) or immunoprobing times could lead to variation in results from gel to gel. The limit of detection (LOD) for proteins was experimentally determined to be 27,000 copies[38], although the LOD is dependent on antibody affinity and, thus, will differ between protein targets. The LOD of mRNA was determined by the manufacturer (Zymo) using HeLa cells (roughly 1/30 the volume of an embryo) to reliably detect transcripts from a single cell, which they estimate to have originated from 0.015 ng of total RNA extracted.

Our protocol enables same-embryo protein and mRNA profiling of up to ~25 embryos per snapBlot device. Embryo stages varying from oocyte to blastocyst can be assayed together on the same snapBlot device. Given that the superovulation protocol typically recovers 10–20 viable embryos per female[10], the throughput allows for studying variation within embryo populations of the same fertilization event as well as across different genotypes or conditions. The photo-active PA gel of the snapBlot has been demonstrated to support detection of up to ~11 protein targets by employing a combination of antibodies raised in different species and rounds of stripping and re-probing[31]. Given the use of conventional RT–qPCR for analysis of mRNA, up 50 mRNA targets can be profiled alongside the protein targets. Given that different protein targets might require different migration times, pore sizes or even lysis times, the troubleshooting table (Table 2) can be used to optimize the snapBlot protocol for the desired protein and mRNA targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Source data are provided with this paper.

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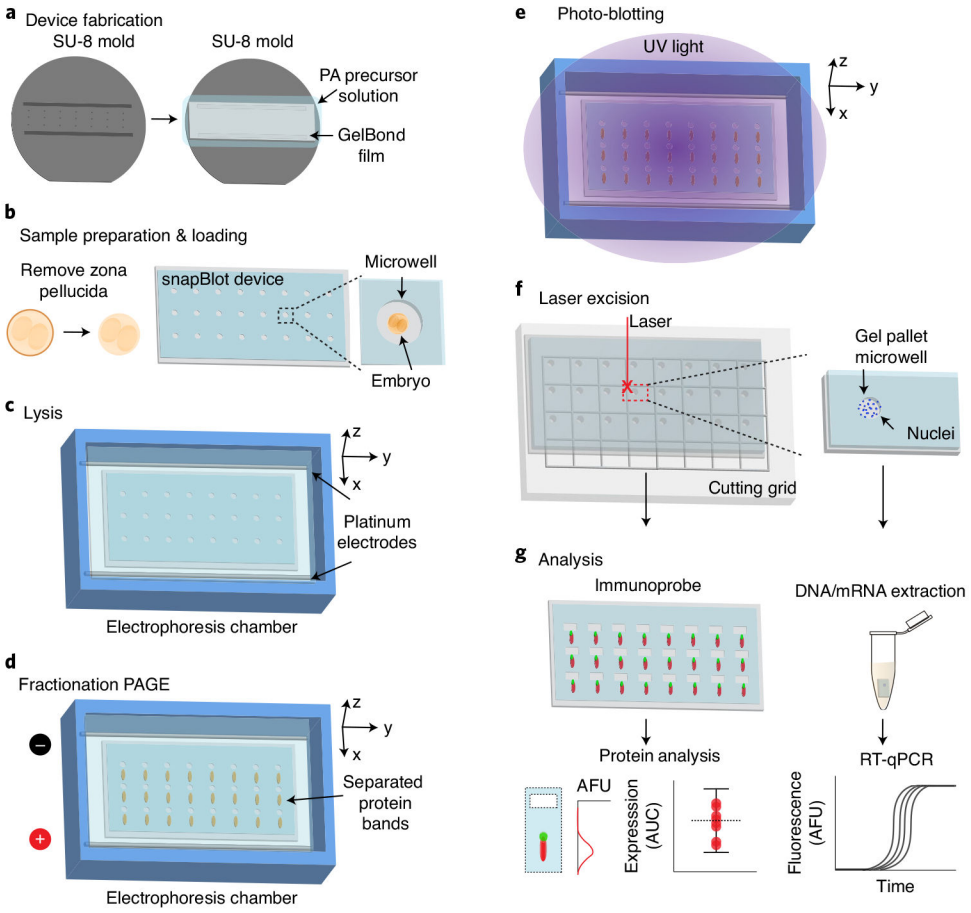

Fig. 1 |. Overview of the snapBlot technique.

a, Device fabrication (Steps 1–19). The snapBlot device is fabricated by polymerizing a 200-μm-thick layer of PA between a treated polyester polymer film (GelBond) and a silicon/SU-8 mold patterned with an array of micro-posts (200 μm in diameter and height, 5-mm × 5-mm spacing) that render microwells of the same dimensions in the PA layer. **b**, Sample preparation (Steps 20–35). The zona pellucida is removed from embryos before loading them into the microwells of the snapBlot device. **c**, Lysis (Steps 36–40). Embryos are lysed with a fractionation lysis buffer that selectively lyses the cytoplasmic membrane but not the nuclear envelope. **d**, fPAGE (Step 41). An electric field is applied to inject cytoplasmic proteins into the PA gel abutting the microwell, which serves as a sieving matrix for resolving proteins by molecular mass. **e**, Photo-blotting (Step 42). Once separated, proteins are covalently bound to the PA matrix by UV-driven activation of benzophenone moieties in the PA gel in a process known as photo-blotting. **f**, Laser excision (Steps 47–68). 2-mm x 3-mm sections containing microwells housing intact nuclei are excised from the snapBlot device. The sections, or gel pallets, are collected for mRNA extraction and analysis. **g**, Analysis (Steps 69–109). The snapBlot device is incubated with antibodies to probe for targets, resulting in immunoblots on which AUC analysis is performed. mRNA extracted from gel pallets is analyzed by RT–qPCR.

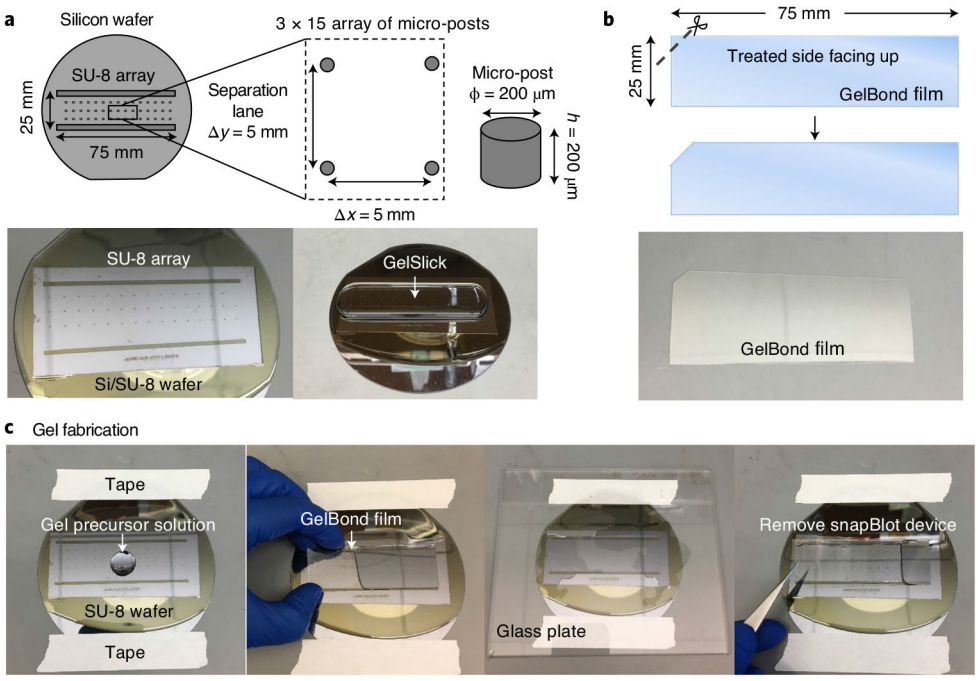

Fig. 2 |. Gel fabrication.

a, Step 1: schematic of silicon/SU-8 wafer used for snapBlot device fabrication; an array of SU-8 micro-posts 200 μm in diameter and height, with 5-mm × 5-mm vertical and horizontal separation. Below, photographs of the silicon/SU-8 wafer being treated with GelSlick solution to render the surface hydrophobic. **b**, Steps 2 and 3: schematic of a 25-mm × 75-mm strip of GelBond film. The top-left corner is trimmed to indicate that the treated side is facing up. Below, photograph of trimmed GelBond film. **c**, Steps 10–14: photographs of device fabrication steps, including pipetting a drop of precursor solution over the taped-down wafer, placing GelBond strip over the drop with the treated side facing down, placing a large glass plate over the GelBond and incubating for 30 min before hydrating the gel with PBS and gently removing the fabricated snapBlot device from the SU-8 wafer.

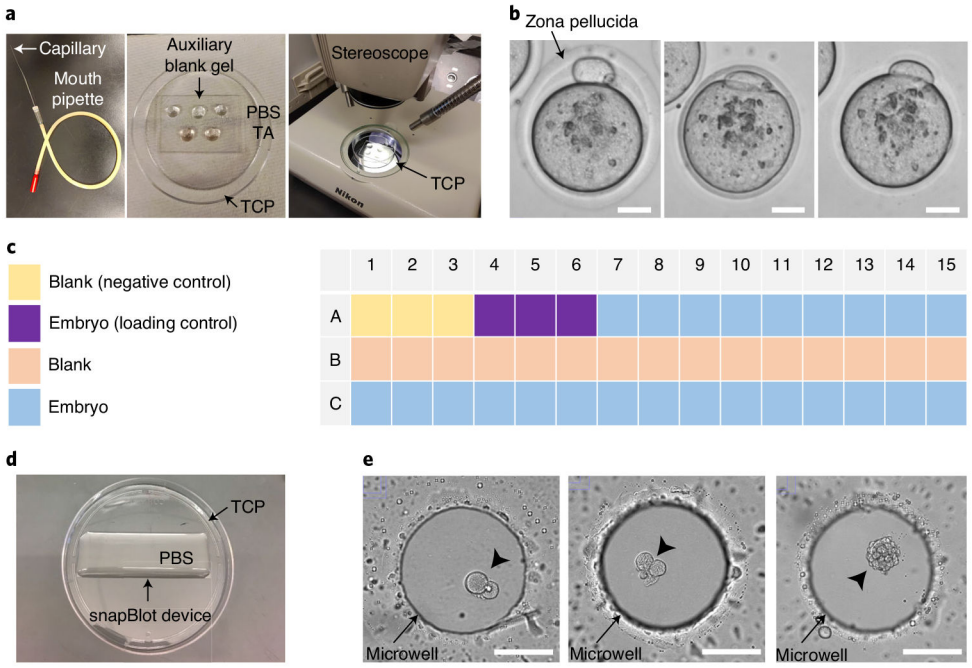

Fig. 3 | Embryo sample preparation and loading.

a, Steps 21–23: Under a stereoscope, a mouth pipette assembly is used to pipette droplets of PBS and AT solution over an auxiliary blank PA gel placed in a tissue culture plate (TCP). **b**, Step 27: removal of zona pellucida. Bright-field micrographs of zona pellucida dissolving as an embryo is incubated in AT solution. Scale bars, 20 μm. **c**, Step 31: suggested layout for loading embryo samples. Yellow boxes indicate empty microwells that will be excised into gel pallets to serve as negative controls; purple boxes indicate microwells loaded with embryos that will not be excised into gel pallets (will serve as protein separation controls); orange boxes indicate microwells left empty; and blue boxes indicate microwells loaded with embryos that will be excised into gel pallets. **d**, Step 33: snapBlot device placed on a tissue culture plate lid and hydrated with a layer of PBS in preparation for embryo loading into the microwells. **e**, Step 34: bright-field micrographs of embryos loaded into microwells. Embryos are indicated with arrowheads. Scale bars, 100 μm. Appropriate institutional regulatory board permission was granted for obtaining and handling of mouse embryos.

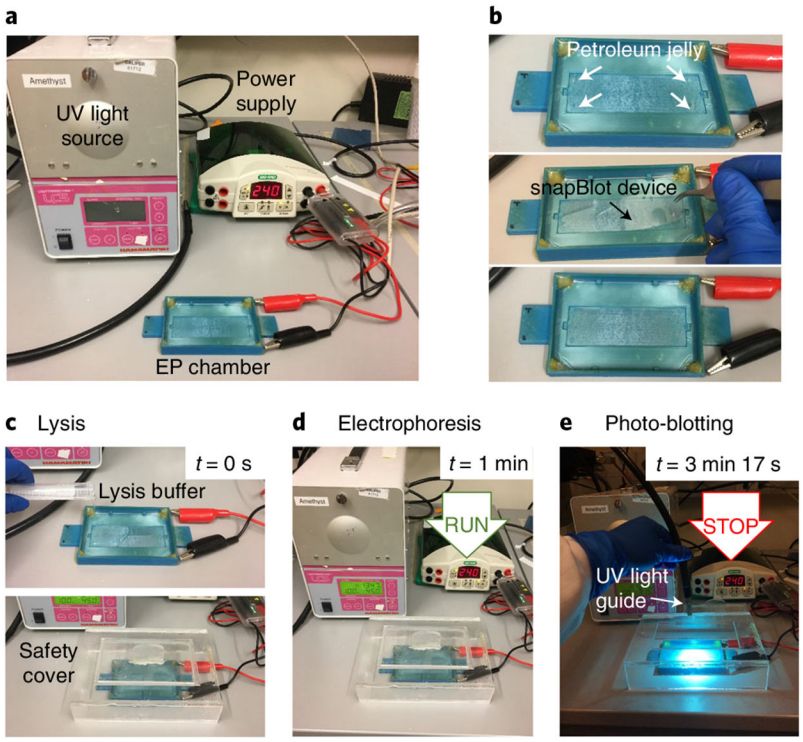

Fig. 4 | fPAGE.

a, Step 36: The fPAGE setup includes the UV light source and a power supply connected to the electrodes of the EP chamber. **b**, Step 39: placing the snapBlot device into the EP chamber. Four spots of petroleum jelly placed on the corners of the EP buffer are used to immobilize the snapBlot device after carefully placing it over the petroleum jelly and carefully pushing down the corners to secure the device in place. **c**, Step 40: Lysis starts when the fractionation buffer is carefully poured over the corner of the device. During the 60-s incubation, the safety plastic cover is placed over the EP chamber. **d**, Step 41: The electric field is applied by pressing the 'Run' button on the power supply. **e**, Step 42: After 2 min and 17 s of electrophoresis, the power supply is turned off by pressing the 'Stop' button on the power supply and immediately applying UV light for 45 s to photo-blot, or capture, the proteins onto the PA.

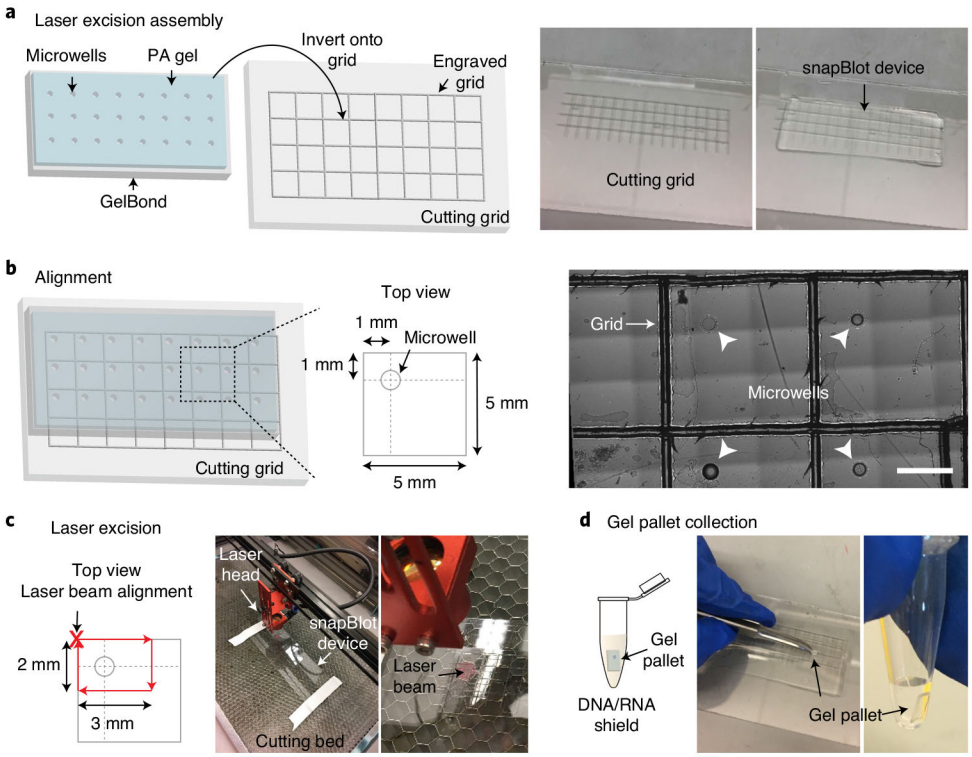

Fig. 5 | Laser excision of gel pallets from the snapBlot device.

a, Step 53: The hydrated snapBlot device is placed (gel facing down) onto the cutting grid (an array of 5-mm × 5-mm squares engraved on a clear acrylic sheet). **b**, Step 54: Under a bright-field microscope, the array of microwells on the snapBlot device is aligned over the cutting grid as shown in the schematic (left) and the bright-field micrograph (right) where the microwells are indicated by white arrowheads (scale bar, 2 mm). **c**, Steps 55 and 56: The cutting grid and the aligned snapBlot are taped down onto the cutting board of the laser cutter, and the laser beam is aligned over the top-left corner of the grid encompassing the target microwell. A 3-mm× 2-mm rectangle is cut into the snapBlot device, yielding a gel pallet. **d**, Steps 59 and 60: The gel pallet is collected with tweezers and placed in a PCR tube containing 20 μl of DNA/RNA Shield solution and kept over ice.

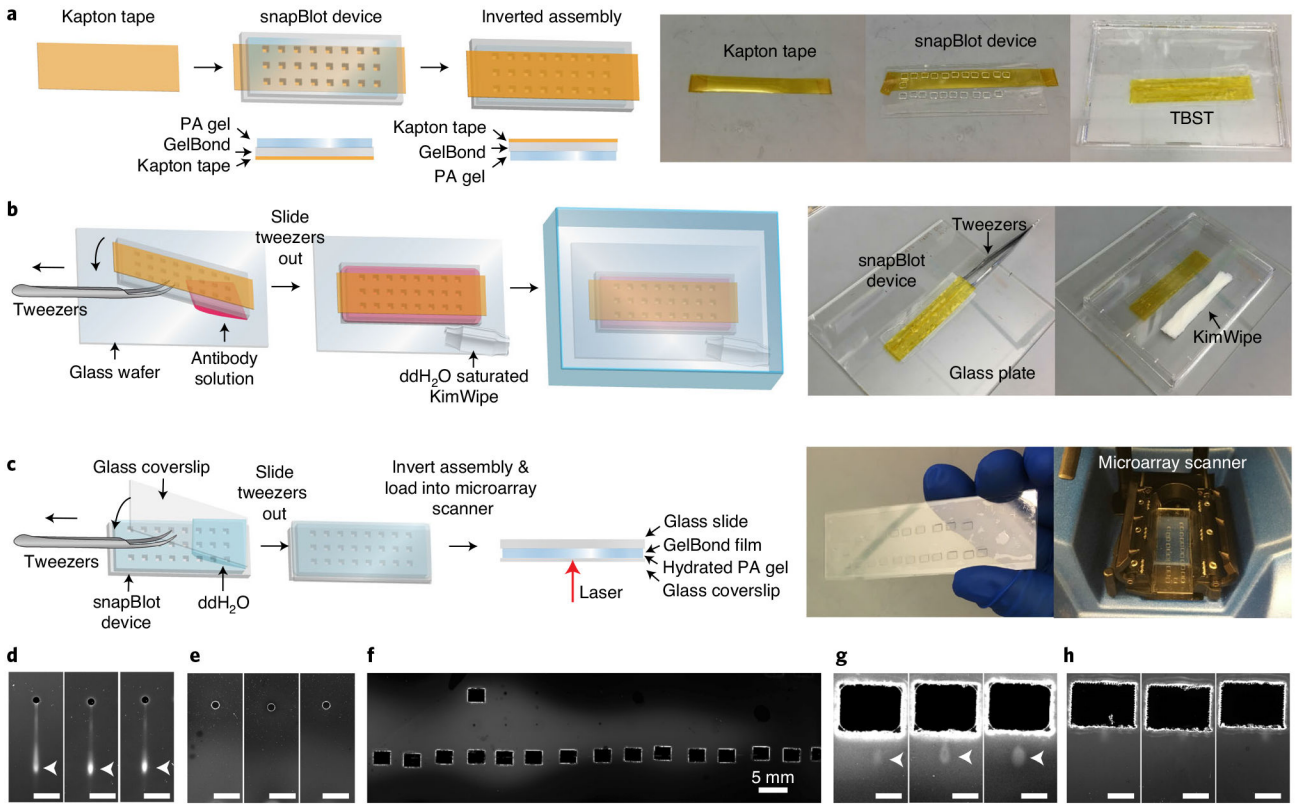

Fig. 6 | Immunoprobing and imaging protein targets.

a, Steps 71 and 72: Kapton tape is used to cover the holes created in the snapBlot device by the gel pallet excision. To hydrate the PA gel, the snapBlot is placed into TBST solution with the gel side facing down. **b**, Steps 73–75: The hydrated snapBlot device is lowered onto an antibody solution (gel facing down) and incubated in an enclosed humidified chamber. Water vapor is created by evaporation of water from a water-soaked KimWipe placed in the enclosed chamber. **c**, Steps 86 and 87: The snapBlot device is hydrated with ddH_2O and assembled for imaging by layering a coverslip over the gel and a microscope slide behind the GelBond film. The assembly is introduced into the microarray scanner with the coverslip facing down. **d**, Step 88: microarray laser scanning micrographs of protein bands from embryo loading controls used to adjust imaging settings. **e**, Step 88: microarray laser scanning micrographs of embryo loading controls displaying no protein bands. **f**, Step 89: microarray laser scanning micrograph of whole immunoprobed snapBlot device. **g**, Step 89: microarray laser scanning micrograph of protein bands from embryo samples, where the dark rectangle corresponds to the section of the device removed with gel pallet. **h**, Step 89: microarray laser scanning micrograph of embryo samples that do not display protein bands. Arrowheads mark the position of protein bands. Scale bars in **d**,**e**,**g** and **h** are 1 mm. Scale bar in **f** is 5 mm. Appropriate institutional regulatory board permission was granted for obtaining and handling of mouse embryos.

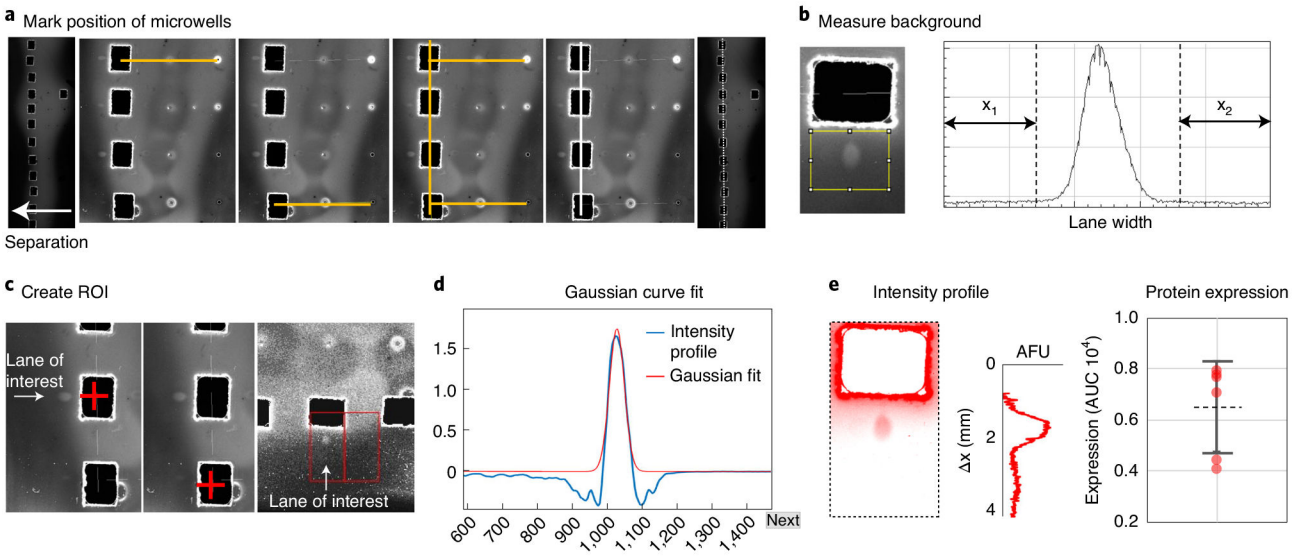

Fig. 7 | snapBlot protein analysis workflow.

a, Step 99: For image post-processing using ImageJ, use the intact microwells as fiducials to draw a line marking the location of the microwells before excision. **b**, Step 100: Measure the axial background to the left (x_1) and right (x_2) of the protein band within an ROI that encompasses the protein band and has a width of 2.5 mm. The micrograph is rotated 90 degrees counterclockwise from the images in **a**. **c**, Step 102: Create an ROI by clicking on the location of the microwell in the lane of interest and the adjacent microwell. **d**, Step 105: Gaussian curve fitting obtains parameters of peak center, peak width and peak height for performing AUC analysis. **e**, Step 107: Results include the background subtracted intensity profile for the given protein immunoblot (shown to the right of the false-colored fluorescence micrograph of the immunoblot) and dot plot of protein expression (AUC) for a given experiment consisting of $n = 6$ immunoblots (blastocysts, $n = 6$, immunoprobed GFP expression; horizontal lines indicate average ± s.d.). Appropriate institutional regulatory board permission was granted for obtaining and handling of mouse embryos.

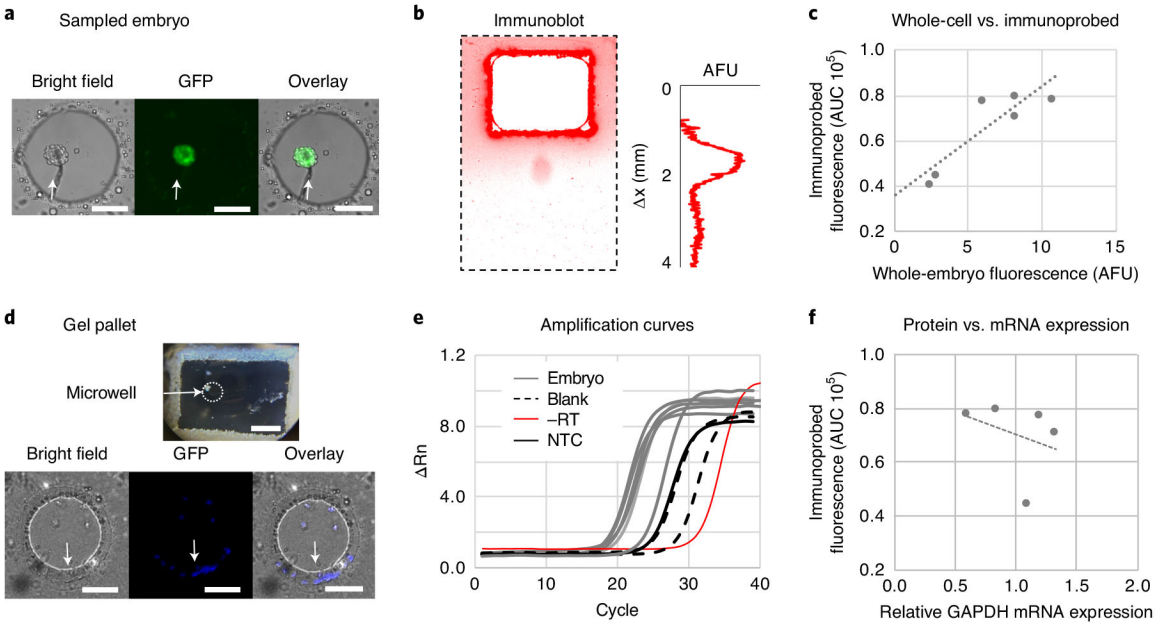

Fig. 8 | Anticipated results.

a, Bright-field and fluorescence micrographs of an enhanced green fluorescent protein (eGFP)-expressing embryo sampled into a snapBlot microwell. Arrowheads point to the embryo. **b**, False-colored fluorescence micrograph of immunoblot, or immunoprobed eGFP protein band, with adjacent fluorescence intensity profile. **c**, Bivariate plot shows a positive correlation among pre-lysis, whole-embryo fluorescence and immunoblot signal after fPAGE (immunoprobed fluorescence) (Pearson correlation $R^2 = 0.788$). **d**, Bright-field and fluorescence micrographs of nuclei-laden gel pallet. Arrowheads point to Hoechst-stained nuclei. **e**, Amplification curves for GAPDH show that mRNA from gel pallets amplify before negative controls (blank gel pallets, −RT and NTC). **f**, Bivariate plot of immunoprobed GFP fluorescence and relative GAPDH mRNA expression (normalized by 18s and blank controls, $n = 5$, Pearson correlation $R^2 = 0.106$). Scale bars, 100 μm. Appropriate institutional regulatory board permission was granted for obtaining and handling of mouse embryos.

Table 1 |

Benchmarking approaches for measuring RNA and protein from the same single cell or embryo

Assay strategy	RNA method	Protein method	Multiplexing (per cell)	Throughput	Protein selectivity
snapBlot	RT–qPCR: detects only nuclear mRNA	Immunoblotting: detects only cytoplasmic and surface proteins	RNA: 10s of targets; proteins: ~11 targets[31]	10s of embryos	Immunoaffinity and protein sizing
Imaging	In situ hybridization[12,14,15] or PLAYR[16]	FACS[12], mass cytometry[13], imaging mass cytometry[14]	~8–17 targets total if using fluorescence[12,40]; ~50 targets total if using metal isotopes[41]	100s (ref.[14]) to 10,000s (refs. [12,13]) of cells	Immunoaffinity
Division of lysate[17,18]	RT–qPCR	PEA[17] or dPLA[18]	RNA: 10s of targets[17]; proteins: 10s of targets[17]	~100 cells	Immunoaffinity (increased specificity due to two antibodies required for PEA/dPLA)
Conversion to common molecular format	RT–qPCR[21]	PEA[21]	RNA: 10s–100s of targets; proteins: 10s of targets	~100 cells	Immunoaffinity (increased specificity due to two antibodies required for PEA)
	Single-cell RNA sequencing[19,20]	Oligonucleotide-labeled antibody labeling[19,20]; detects only surface proteins, unless using reversible fixation[22]	RNA: 10,000s of targets; proteins: 10s–100s of targets	1,000s of cells	Immunoaffinity

Table 2 |

Troubleshooting table

Step	Problem	Possible reason	Solution
Step 1–19	The PA gel adheres to the SU-8 mold	The SU-8 mold was not coated properly with GelSlick	Make sure that GelSlick solution covers entire area of array, including spacers
		The wrong (untreated) side of the GelBond was facing the gel precursor	Check that the more hydrophilic treated side of the GelBond is facing the gel precursor (pipette ~10 μl of water on both sides of the GelBond; the side on which the water spreads out more is treated and should touch the precursor)
	The PA does not polymerize	O_2 inhibits the reaction; the GelBond strip was not pushed down sufficiently onto the SU-8 wafer	Make sure to apply the glass plate (or a weight) immediately after laying the GelBond strip over the drop of gel precursor solution
		O_2 inhibits the reaction; the precursor contained air	Make sure that the precursor was properly de-gassed; take care not to add air bubbles when mixing reagents together
		APS and TEMED are not evenly distributed in the precursor solution	After adding APS and TEMED to the gel precursor, pipette solution up and down multiple times to mix thoroughly so that gel polymerization occurs uniformly
	Bubbles trapped while setting GelBond film over the wafer or large holes seen in PA gel after fabrication	Air bubbles might become trapped at the micro-posts when lowering film onto the SU-8/ silicon wafer	Carefully lift the GelBond film and try lowering over the wafer again. Caution: doing this more than twice might cause the gel precursor to be exposed to O_2, and the gel might not polymerize Carefully use tweezers to push the GelBond film around the SU-8 wafer to let the air bubbles escape through the edges
	Microwells are not circular	The GelBond was moved during/ after polymerization over the SU-8 wafer	Make sure that no component is moved during polymerization, and make sure to lift the GelBond from the SU-8 wafer with an upward movement to prevent sliding across the SU-8 wafer
	Some microwells look dark under a bright-field microscope	Micro-posts were removed from the SU-8 wafer and are now in the PA gel	Make sure to coat the entire patterned area of the SU-8/silicon wafer with GelSlick to ensure that micro-posts are not peeled off of the wafer
	PA gel releasing from GelBond film	The non-treated surface of the GelBond was accidentally used	When marking the corner of the GelBond film, be sure to test the hydrophobicity with a 10–20-μl drop of water. The hydrophilic side where the drop spreads instead of beading up is the treated side, which should be in contact with the PA precursor solution and the SU-8 wafer during fabrication
Steps 20–35	Zona pellucida is not removed	AT solution was not fresh, or the incubation time was too short	Prepare a fresh AT solution droplet and place embryos in the new droplet. Incubate at 37 °C for 5 min and check under the microscope. Repeat if necessary
	Embryo is lysed	Embryo was incubated in AT solution for too long	Discard the embryo and decrease the incubation time. If necessary, incubate at room temperature instead of 37 °C and monitor under the stereoscope
	Embryos do not stay in the microwells	Embryos are not fully settled into the microwell before moving the snapBlot device	After depositing an embryo in a well, wait longer for the embryo to settle to the bottom before moving the pipette tip away from the well and introducing convection
		Microwells are not large or deep enough to hold embryos	Adjust the diameter and/or depth of the microwell to better suit the size of the embryos, by fabricating a new SU-8 wafer (photolithography mask with larger-diameter wells and/or spin coating a thicker layer of SU-8)
Steps 47–62	Microwell is not present in gel pallet or is not intact (partially excised) (Supplementary Fig. 4)	Microwell array was not aligned correctly over cutting grid	Make sure that the microwell is aligned over the cutting grid, as shown in Fig. 5b. If necessary, practice excising microwells from the middle row (empty microwells) before moving on to microwells containing samples
		Laser beam was not aligned correctly over the grid	Make sure that the laser beam is aligned over the top-left corner of the grid rectangle encompassing the microwell, as shown in Fig. 5c. If necessary, practice excising microwells from the middle row (empty) before moving on to microwells containing samples
		Cutting settings (power, gain and speed) are not adjusted correctly	Decrease power, gain and/or speed so that the gel pallet is excised but the microwell is not dehydrated when properly aligned. If necessary,

Step	Problem	Possible reason	Solution
			practice excising microwells from the middle row (empty) before moving on to microwells containing samples
Step 68	Failed PCR amplification	Failed PCR reaction	Test samples for amplification of any of the preamplified targets through regular PCR methodology. Samples might have been stored improperly or contaminated with nucleases Confirm that primers and SSO mastermix are working as expected with positive control samples
Steps 88 and 89	No signal (from either excised wells or embryo loading control wells) (Supplementary Fig. 4)	If signal is missing from only some of the microwells, lysis buffer pouring has displaced embryos from microwells	Avoid tilting the gel between embryo settling and lysis steps. Ensure that pouring of the lysis buffer is toward the corner of the gel (away from the microwells) and slow to minimize convection over the microwells
		Lysis time was too long	Reduce lysis time to prevent excessive diffusional losses during the lysis step
		Primary antibody has low affinity for target	Increase antibody concentration and re-probe
		Primary antibodies were not target specific	Verify the specificity and species reactivity of the antibody and consider using a different antibody
		Secondary antibodies did not recognize primary antibodies	Verify the species reactivity of the secondary antibody
		Protein target is low abundance	Test embryos with higher expression levels of the target protein Search for smaller affinity probes (e.g., antibody fragments) that can reach higher in-gel concentrations due to their higher partition coefficients Probe for the least abundant proteins first, as some protein loss occurs during stripping cycles[42]
	Signal from embryo loading controls but not excised wells	Protein bands excised from device; proteins did not run far enough into the gel	Increase electrophoresis time to ensure that protein bands remain in the device and are not excised with the gel pallet
	Punctate signal	Aggregates from secondary antibody	Make sure to spin down secondary antibodies for at least 5 min at 10,000*g* at room temperature before use
	High background signal	Washing time was insufficient	Wash again in TBST for at least 1 h
		Antibody concentrations were too high	If washing for 1 h in TBST does not remove background, strip for 1 h at 55 °C and re-probe with a lower antibody concentration
	Non-uniform background signal	Antibody solution dried near the edges of the gel or laser-cut gel pallet holes during antibody probe incubation	Ensure that Kapton tape seals the gel pallet holes well, so that the antibody probe solution cannot evaporate from the holes. Ensure that antibody incubation is performed in a humid chamber (made by placing a weighted lid/cover over the antibody incubation and a wet KimWipe)
		Antibody solution was nonuniformly diluted by the fluid layer on the gel	After lowering the gel onto the antibody solution, laterally shift the gel a few times to convectively stir and homogenize the antibody probe solution[43]
	Protein bands are streaky and/or poorly spatially resolved.	Gel density (%T) and/or electrophoresis duration are too low for desired separation resolution	Increase gel density (%T) and/or electrophoresis time to improve separation resolution
		Lysis time might be insufficient, leading to continuous injection	Increase lysis time to allow embryos to fully lyse before electrophoresis
	Protein bands migrated farther than expected (Supplementary Fig. 4)	Lower %T was used to fabricate the gel	Make sure to keep the %T consistent between runs, as a lower %T will cause the pore size to be larger and, thus, proteins to migrate faster for a given electrophoresis time
		PA gel was not fully polymerized during fabrication	Make sure to de-gas the precursor solution, as trapped O_2 inhibits reaction, and polymerization will not reach completion Make sure to work quickly after adding the APS and TEMED to the PA precursor, as having solution in contact with air will cause O_2 to inhibit the reaction and cause lower effective %T
Step 97	High protein signal after stripping	Proteins have not been successfully stripped from the gel	Repeat stripping steps, increasing stripping time up to 3 h if necessary