STEMdiff™ Cerebral Organoid Kit

Culture medium kit for establishment and maturation of human cerebral organoids

Catalog #08570 1 Kit
Catalog #08571 1 Kit

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Product Description

STEMdiff™ Cerebral Organoid Kit is a defined, serum-free cell culture medium that enables the robust generation of unguided cerebral organoids derived from human pluripotent stem cells (hPSCs; e.g. Healthy Control Human iPSC Line, Female, SCTi003-A, Catalog #200-0511), in a simple four-stage protocol. Cerebral organoids are an unguided neural organoid model with a cellular composition and structural organization that is representative of the developing human brain. STEMdiff™ Cerebral Organoid Kit has been optimized to increase efficiency and reproducibility of organoid formation based on the formulation published by Lancaster & Knoblich and Lancaster et al. (2013; 2014; 2017). Cerebral organoid formation is initiated through an intermediate aggregate formation step followed by expansion of neuroepithelia. After a period of maturation, organoids generated using this kit feature cortical-like regions such as the ventricular zone (PAX6+/SOX2+/Ki-67+), outer subventricular zone (Ki-67+/p-Vimentin+), intermediate zone (TBR2+), and cortical plate (CTIP2+/MAP2+/TBR1+), which layer in similar orientations as observed in vivo. For extended periods of organoid culture (> 40 days), the components required for organoid maturation can be purchased as the STEMdiff™ Cerebral Organoid Maturation Kit (Catalog #08571).

Product Information

All components listed below are sold as part of a complete kit (Catalog #08570 or 08571) and are not available for individual sale.

NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Cerebral Organoid Kit (Catalog #08570)				
STEMdiff™ Cerebral Organoid Basal Medium 1	08572	100 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cerebral Organoid Basal Medium 2	08573	250 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cerebral Organoid Supplement A*	08574	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cerebral Organoid Supplement B*	08575	0.5 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cerebral Organoid Supplement C**	08576	0.25 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cerebral Organoid Supplement D*	08577	0.5 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cerebral Organoid Supplement E*	08578	4.5 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cerebral Organoid Maturation Kit (Catalog #08571)				
STEMdiff™ Cerebral Organoid Basal Medium 2	08573	250 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Cerebral Organoid Supplement E*	08578	4.5 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

*This component contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.
**Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.

All media and supplements in STEMdiff™ Cerebral Organoid Kit and STEMdiff™ Cerebral Organoid Maturation Kit are free of antibiotics and based on the formulation published by Lancaster & Knoblich and Lancaster et al. (2013; 2014; 2017).

Materials Required but Not Included

PRODUCT NAME	CATALOG #
100 mm Dish, Non-Treated	38045
6-Well Ultra-Low Adherent Plates for Suspension Culture	100-0083
Axygen™ 200 µL universal pipetter tips; wide bore	e.g. Fisher Scientific 14-222-730
Corning® 96-well round-bottom ultra-low attachment microplate	e.g. Corning 7007
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38017
Costar® 24-well ultra-low attachment plate	e.g. Corning 3473
D-PBS (Without Ca++ and Mg++)	37350
Falcon® Conical Tubes, 50 mL	38010
Falcon® Serological Pipettes, 5 mL or 10 mL	38003 or 38004
Gentle Cell Dissociation Reagent	100-0485
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
Trypan Blue	07050
Y-27632 (Dihydrochloride)	72302

Equipment Required

- Sterile forceps
- Embedding surface (e.g. Organoid Embedding Sheet [Catalog #08579] or Parafilm®)
- Orbital shaker + sticky mat (e.g. Celltron [INFORS HT Catalog #I69222])

Protocol Diagram

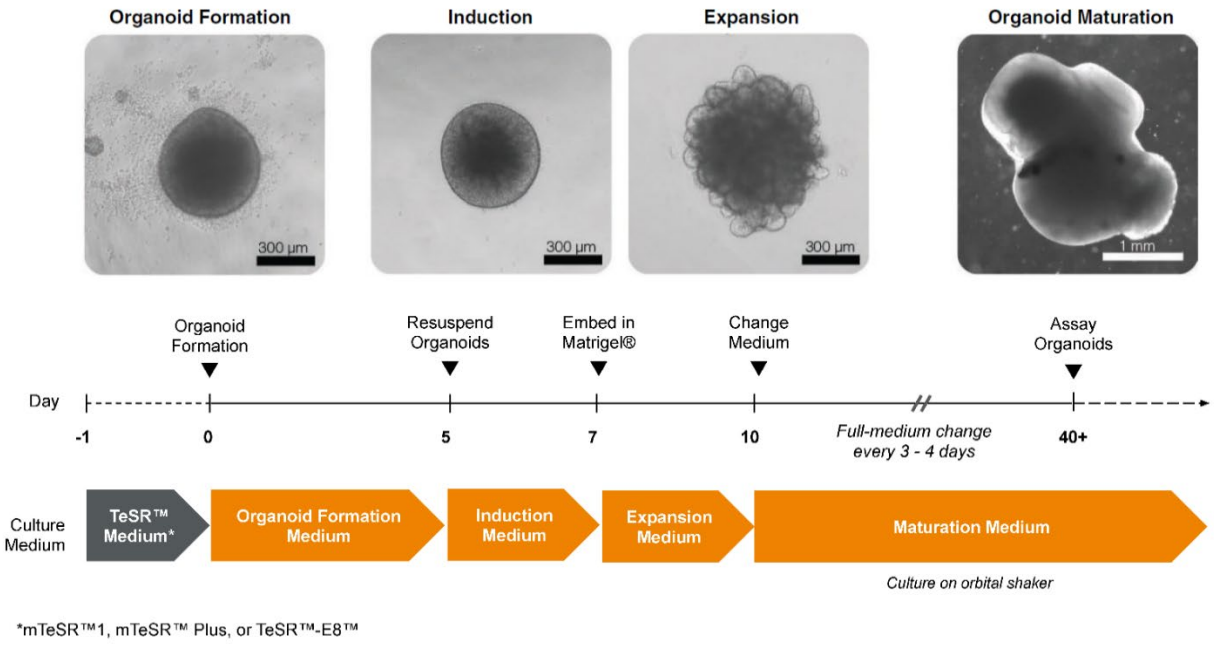

Figure 1. Protocol Diagram for the Generation of Unguided Cerebral Organoids from hPSCs Using STEMdiff™ Cerebral Organoid Kit

Preparation of Media

Use sterile technique to prepare STEMdiff™ cerebral organoid media. Prepare each medium as needed in Directions for Use. Refer to Table 1 for medium components, volumes, and in-use storage and stability.

1. Thaw supplement(s) at room temperature (15 - 25°C). Mix thoroughly.

   NOTE: If not using immediately, aliquot supplement(s) and store at -20°C. Do not exceed the shelf life of the supplement(s). After thawing aliquots, use immediately. Do not re-freeze.

2. Add supplement(s) to Basal Medium as indicated in Table 1. Mix thoroughly. Warm medium to room temperature before use.

   NOTE: If not using immediately, store medium as indicated in Table 1.

Table 1. Preparation of STEMdiff™ Cerebral Organoid Media

MEDIUM	COMPONENT	VOLUME	IN-USE STORAGE AND STABILITY
Organoid Formation Medium (50 mL)	STEMdiff™ Cerebral Organoid Basal Medium 1	40 mL	Store at 2 - 8°C for up to 2 weeks.
	STEMdiff™ Cerebral Organoid Supplement A*	10 mL	
Induction Medium (50 mL)	STEMdiff™ Cerebral Organoid Basal Medium 1	49.5 mL	Store at 2 - 8°C for up to 2 weeks.
	STEMdiff™ Cerebral Organoid Supplement B*	0.5 mL	
Expansion Medium (25 mL)	STEMdiff™ Cerebral Organoid Basal Medium 2	24.25 mL	Store at 2 - 8°C for up to 2 weeks.
	STEMdiff™ Cerebral Organoid Supplement C	0.25 mL	
	STEMdiff™ Cerebral Organoid Supplement D*	0.5 mL	
Maturation Medium (100 mL)	STEMdiff™ Cerebral Organoid Basal Medium 2	98 mL	Store at 2 - 8°C for up to 2 weeks.
	STEMdiff™ Cerebral Organoid Supplement E*	2 mL	

* If thawed supplement appears slightly turbid, it can still be used with no impact on performance.

Directions for Use

Please read the entire protocol before proceeding.

Use sterile technique when performing the following protocols:

A. Organoid Formation (Day 0 - 5)
B. Induction (Day 5 - 7)
C. Expansion (Day 7 - 10)
D. Organoid Maturation (Day 10 - 40+)

A. ORGANOID FORMATION (DAY 0 - 5)

This protocol is for the formation of organoids from hPSCs in a single well of a 6-well plate. For other cultureware, adjust volumes accordingly. Warm cultureware, media, and reagents to room temperature (15 - 25°C) before use.

NOTE: hPSC cultures are ready for passage when the majority of colonies are large, compact, and have dense multi-layered centers. Passage hPSC cultures when they are no more than 70 - 80% confluent and exhibit < 10% differentiation.

Day 0

1. Prepare Organoid Formation Medium (see Preparation of Media) and warm to room temperature.
2. Prepare Seeding Medium as follows: Add 30 µL of 5 mM Y-27632 (Dihydrochloride) to 15 mL of Organoid Formation Medium (final concentration of 10 µM).
3. Use a microscope to visually identify regions of differentiation in the hPSC culture. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
4. Aspirate medium from hPSC culture and wash the well with 1 mL of sterile D-PBS (Without Ca++ and Mg++).
5. Aspirate D-PBS and add 1 mL of Gentle Cell Dissociation Reagent.
6. Incubate at 37°C and 5% CO_2 for 8 - 10 minutes.

   NOTE: Incubation time may vary when using different cell lines or other non-enzymatic cell dissociation reagents.

7. Using a 1 mL pipettor, gently resuspend cells by pipetting up and down slowly 3 - 5 times. Transfer cell suspension to a sterile 50 mL conical tube.
8. Rinse the well with an additional 1 mL of Seeding Medium and add this rinse to the tube containing cells.
9. Centrifuge cells at 300 x g for 5 minutes.
10. Remove and discard supernatant. Add 1 - 2 mL of Seeding Medium to resuspend cells.
11. Count cells using Trypan Blue and a hemocytometer.
12. Dilute an appropriate volume of the cell suspension in additional EB Seeding Medium to obtain a cell concentration of 90,000 cells/mL.
    NOTE: The above cell concentration is recommended for use with hPSCs that have been maintained in mTeSR™1 or TeSR™-E8™. When using hPSCs maintained in mTeSR™ Plus, you may need to reduce initial cell concentration for the formation stage. It is advised to test a range of seeding densities (i.e. 25,000 - 90,000 cells/mL) for any new hPSC line to determine the optimal cell number for ideal organoid growth and morphology.
13. Add 100 µL of cell suspension from step 12 into each well of a 96-well round-bottom ultra-low attachment plate. This will result in 9000 cells per well (or 2500 - 7500 cells per well if reduced seeding densities were used for mTeSR™ Plus-maintained hPSCs).

    NOTE: To improve efficiency and reproducibility of organoid formation, a multi-channel pipettor is recommended for this step.
14. Incubate 96-well plate at 37°C and 5% CO_2. Do not disturb plate for at least 24 hours.
15. Observe plate under a microscope. Small organoids (100 - 200 µm) will be observed with a layer of unincorporated cells around the central organoid.

Day 2 - 5

16. On day 2 and day 4, gently add 100 µL of Organoid Formation Medium per well. A multi-channel pipettor is recommended for this step. Incubate at 37°C and 5% CO_2.
17. On day 5, observe organoids under a microscope. Organoids should reach a diameter of > 300 µm (typically 400 - 600 µm) and exhibit round and smooth edges (see Figure 1).
18. Proceed to section B (Induction).

B. INDUCTION (DAY 5 - 7)

NOTE: Warm cultureware, medium, and reagents to room temperature (15 - 25°C) before use.

NOTE: If ultra-low attachment plates are not available, tissue culture-treated cultureware can be used if it is pre-treated with Anti-Adherence Rinsing Solution (Catalog #07010) to prevent cell attachment.

Day 5

1. Prepare Induction Medium (see Preparation of Media) and warm to room temperature.
2. Add 0.5 mL of Induction Medium to each well of a 24-well ultra-low attachment plate.
3. Add 1 - 2 organoids to each well of the 24-well plate as follows:
   a. Using a wide-bore 200 µL pipette tip, draw up 50 µL from one well of the 96-well plate from section A to obtain organoid(s).
   b. Remove most of the medium by carefully ejecting it back into the well, retaining organoid(s) in the pipette tip.
   c. Dispense organoid(s) into one well of the 24-well plate containing Induction Medium.

   NOTE: Ensure that organoids are evenly distributed in the well by shaking the plate back and forth 3 - 4 times in incubator. Organoids that touch are more likely to merge. If a high number of organoids merge, transfer only a single organoid per well.
4. Incubate plate at 37°C and 5% CO_2 for 48 hours. Organoids will maintain smooth edges and develop optically translucent edges (see the image in Figure 1).
5. Proceed to section C (Expansion).

C. EXPANSION (DAY 7 - 10)

Day 7

1. Thaw Corning® Matrigel® on ice at 2 - 8°C for 1 - 2 hours.

   NOTE: Thaw enough Corning® Matrigel® to have 15 µL/organoid (e.g. 96 wells x 15 µL/well = 1.44 mL Corning® Matrigel®).

   NOTE: Keep Corning® Matrigel® on ice to prevent premature polymerization. All plasticware that comes in contact with Corning® Matrigel® should be chilled at -20°C for at least 30 minutes prior to use.
2. Prepare Expansion Medium (see Preparation of Media) and warm to room temperature (15 - 25°C).
3. Place embedding surface (e.g. Organoid Embedding Sheet or Parafilm®) into an empty, sterile, 100 mm dish.

4. Using a wide-bore 200 µL pipette tip, draw up 25 - 50 µL of medium + organoid from one well of the 24-well plate and transfer to embedding surface. Repeat this step until 12 - 16 organoids are collected on the embedding surface.

   NOTE: Embed no more than 12 - 16 organoids at a time; this will prevent the organoids from drying out and the Corning® Matrigel® from prematurely polymerizing.
5. Remove excess medium from each organoid by carefully drawing up medium with a standard 200 µL pipette tip. Position the opening of the tip so that it is pointing away from the organoid to avoid drawing it up.
6. Using a pipettor with a cold 200 µL standard pipette tip, add 15 µL of Corning® Matrigel® dropwise onto each organoid.
7. Using a new cold 200 µL pipette tip, reposition the organoid to the center of the droplet.
8. Place the plate in an incubator at 37°C for 30 minutes to polymerize Corning® Matrigel®.
9. Use sterile forceps to grasp embedding surface containing Corning® Matrigel® droplets.
10. Position sheet directly above one well of a 6-well ultra-low adherent plate. Using a 1 mL pipettor, draw up Expansion Medium and gently wash Corning® Matrigel® droplets off the sheet and into the well. Use 3 mL of Expansion Medium/well. Repeat until all 12 - 16 Corning® Matrigel® droplets are in the well.
11. Incubate at 37°C and 5% CO_2 for 3 days. Embedded organoids will develop expanded neuroepithelia as evidenced by budding of the organoid surface (see image in Protocol Diagram).
12. Proceed to section D (Organoid Maturation).

D. ORGANOID MATURATION (DAY 10 - 40+)

Day 10

1. Prepare Maturation Medium (see Preparation of Media) and warm to room temperature (15 - 25°C).
2. Using a 5 mL or 10 mL serological pipette at the slowest setting, carefully remove all medium from wells containing organoids. Do not disturb Corning® Matrigel®-embedded organoids.
3. Replace medium with 3 mL/well of Maturation Medium.
4. Place plate of organoids on an orbital shaker in a 37°C incubator. For the INFORS HT Celltron orbital shaker, set the shaker speed (RPM) according to Table 2. For other orbital shaker models, calculate the RPM using the equation in Figure 2.

Table 2. Recommended Shaker Speeds for INFORS HT Celltron Orbital Shaker for Various Cultureware

CULTUREWARE*	VOLUME OF MEDIUM (mL)	RECOMMENDED SHAKER SPEED (RPM)	RELATIVE CENTRIFUGAL FORCE (RCF)† (g)
6-well plate or 60 mm dish	3.0	65	0.11808
12-well plate	1.5	85	0.20194
24-well plate	1.0	100	0.27950

*6-well and 12-well plates are optimal cultureware for organoid culture.
†Calculated using throw (shaking diameter) of 25 mm for the INFORS HT Celltron.

$$RPM = \sqrt{\frac{RCF}{throw \times 1.118}} \times 1000$$

Where:
RPM = shaker speed (revolutions per minute)
RCF = relative centrifugal force (g), provided in Table 2 for various cultureware
throw = shaking diameter (mm), as specified by manufacturer

Figure 2. Conversion of RCF to RPM

5. Perform a full-medium change every 3 - 4 days as follows:
   a. Tilt the cultureware.
   b. Using a 5 mL serological pipette at the slowest setting, slowly remove as much of the medium as possible without disturbing the organoids.
   c. Add 3 mL/well of fresh Maturation Medium.
   d. Return plate to the orbital shaker in a 37°C incubator.

Day 40+

By day 40, organoids will exhibit dense cores with regions of the organoids displaying optically translucent edges, and will typically be ready for analysis (see the image in Figure 1). There is a 50 - 60% success rate for obtaining organoids with dense cores by day 40; this rate will vary with different cell lines. Heterogeneous morphologies will be apparent.

NOTE: As organoids mature, they will exhibit an increased metabolic rate and deplete the medium components more quickly. Gradually decrease the number of organoids per well of the 6-well plate if they are being maintained long-term (e.g. reduce to 8 organoids per well on Day 40 and 4 organoids per well on Day 60+).

6. Assay organoids by cryosectioning/immunolabeling and/or by RT-qPCR. Multiple radial arrangements of PAX6+ progenitor cells should be observed by immunostaining, surrounded by a smaller layer of TBR2+ intermediate progenitors. Closest to the surface of the organoid, a layer of MAP2+ neurons is expected, which should exhibit separation of CTIP2+/TBR1+ deeper layer neurons and SATB2+ upper layer neurons. Refer to Table 3 for the list of antibodies that have been validated for immunostaining of cerebral organoids.

   NOTE: Antibody dilutions are provided as a starting point and further optimization may be required.

Table 3. Recommended primary antibodies for immunostaining cerebral organoids

ANTIBODY / MARKER	CATALOG #	RECOMMENDED ANTIBODY DILUTION
CTIP2	Abcam Catalog #ab18456	1 : 2000
FOXG1	Abcam Catalog #ab18259	1 : 2000
Ki67	Millipore Catalog #MAB4190	1 : 2000
MAP2	Abcam Catalog #ab5392	1 : 5000
PAX6	Biolegend Catalog #901301	1 : 2000
PH3	Millipore Catalog #06-570	1 : 2000
PVIM	MBL Catalog #D076-3	1 : 2000
SATB2	100-1346	2 µg/mL
SOX2	R&D Systems Catalog #MAB2018	1 : 2000
TBR1	100-1341 Abcam Catalog #ab31940	2 µg/mL 1 : 2000
TBR2	Abcam Catalog #ab23345	1 : 200
ßIII-tubulin/TUJ1	60052	1 : 2000

7. Cerebral organoids can continue to be cultured beyond day 40 using STEMdiff™ Cerebral Organoid Maturation Kit (Catalog #08571).

   NOTE: To prepare a full bottle (225 mL) of Maturation Medium, remove 29.5 mL of STEMdiff™ Cerebral Organoid Basal Medium 2 before adding 4.5 mL of STEMdiff™ Cerebral Organoid Supplement E to the bottle.

Troubleshooting

STAGE	PROBLEM	POSSIBLE CAUSE	RECOMMENDED ACTION
Organoid Formation (Day 0 - 5)	Many small organoids do not form into a large central organoid	Starting hPSCs were of poor quality	• Confirm that hPSCs are of high quality by observing morphology and assessing expression of markers of the undifferentiated state (OCT3/4 and TRA-1-60). If OCT3/4 and TRA-1-60 are low (< 90%), restart with high-quality hPSCs. • Start with high-quality hPSCs from an earlier passage number • Passage cells when they are no more than 70 - 80% confluent
		96-well plate was disturbed during culture	• Ensure 96-well plate is not disturbed for at least 24 hours after seeding (section A, step 14)
	Organoid surface has significant outgrowth or does not have brightening along edges	Starting hPSCs were of poor quality	• Confirm that hPSCs are of high quality by observing morphology and assessing expression of markers of the undifferentiated state (OCT3/4 and TRA-1-60). If OCT3/4 and TRA-1-60 are low (< 90%), restart with high-quality hPSCs. • Start with high-quality hPSCs from an earlier passage number • Passage cells when they are no more than 70 - 80% confluent
Induction (Day 5 - 7)	Organoids exhibit large balls of outgrowth	Starting hPSCs were of poor quality	• Do not continue with the experiment; restart with high-quality hPSCs
		Too much Organoid Formation Medium was transferred	Transfer only a small amount of Organoid Formation Medium when transferring organoids (~10 - 20 µL in 500 µL)
	Large amount of cell debris in well	Too much Organoid Formation Medium was transferred	Transfer only a small amount of Organoid Formation Medium when transferring organoids (~10 - 20 µL in 500 µL)
Expansion (Day 7 - 10)	No expansion or increase in surface area, as indicated by bubbling of the organoid surface	Corning® Matrigel® lot was sub-optimal	Use an alternative lot of Corning® Matrigel®
		Improper timing of Corning® Matrigel® embedding	The time required for appearance of the neuroepithelium may vary depending on cell line. Continue Induction phase beyond 7 days (up to day 10 - 11) if sub-optimal expansion continues to occur.
		Starting hPSCs were of poor quality	Do not continue with this experiment; restart with high-quality hPSCs
Maturation (Day 10 - 40+)	Organoid surface is not intact and/or high levels of cell death and debris are visible in the culture	Incorrect orbital shaker speed	Ensure that the calculation in Figure 2 has been performed correctly, using the specific throw (shaking diameter) of the orbital shaker model
		Organoids exhibited high metabolic rate with long-term maturation	• Decrease the number of organoids cultured per well • Increase the volume and/or frequency of medium changes

Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCNCworkflow, or contact us at techsupport@stemcell.com. For available fresh and cryopreserved peripheral blood, cord blood, and bone marrow products, visit www.stemcell.com/primarycells.

References

Lancaster MA et al. (2013) Cerebral organoids model human brain development and microcephaly. Nature 501(7467): 373–9.

Lancaster MA & Knoblich JA. (2014) Generation of cerebral organoids from human pluripotent stem cells. Nat Protoc 9(10): 2329–40.

Lancaster MA et al. (2017) Guided self-organization and cortical plate formation in human brain organoids. Nat Biotechnol 35(7): 659–66.