

Submission ID #: 71970

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21950898>

Title: DNA-FISH Metaphase Spreads to Distinguish Extrachromosomal DNA from Homogeneously Staining Regions in Human Cancer Cell Lines

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**
(Authors: We have modified this response to account for the SCOPE shot. Please specify if this approach is acceptable or if shot 3.4.2 will be visualized on-screen and the videographer can film it directly.) **We attempted to perform this scope shot with the videographer. The scope adapter did not work very well. We still took a video but if it is not good enough we are ok using the image from the paper to be displayed on the screen.**

If **Yes**, can you record movies/images using your own microscope camera? **No**

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

LAXCO LMI3-PH1

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

Scope shot: 3.4.2

Videographer: Please film the above-mentioned shot using a scope kit.

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **Yes, Same Building**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 18

Number of Shots: 54

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Note: The statements have been edited slightly.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Lauren Masters:** Our research focuses on determining whether amplified oncogenes reside on extrachromosomal DNA or within distinct, chromosomally integrated homogeneously staining regions.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Katelin Hagstrom:** Sequencing fails to distinguish ecDNA from HSR amplification, so our metaphase-spread protocol resolves this at single-cell resolution without cytogenetics expertise.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Lauren Masters:** This protocol enables the visualization of locus copy number and physical location at single-cell resolution using fluorescent probes.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Lauren Masters:** After imaging, researchers can deconvolve Z-stacks and analyze them in ImageJ to accurately quantify gene copy numbers and determine ecDNA status.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Lauren Masters:** Broader adoption will help researchers determine amplification state and determine if the cells switch between ecDNA and HSR states.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Metaphase Arrest and Harvest of COLO320DM Cells

Demonstrator: Katelin Hagstrom

- 2.1. To begin, dilute the Colcemid (*KAHL-seh-mid*) stock solution at a 1 in 1 ratio with sterile ultrapure water [1]. For the metaphase arrest, add 100 microliters of diluted Colcemid solution to the COLO320DM (*KOH-loh-three-twenty-D-M*) cells maintained in 10 milliliters of media at logarithmic growth at 70 to 90 percent confluence [2] and place them in a cell culture incubator for 45 minutes to overnight [3].
 - 2.1.1. WIDE: Talent preparing the diluted Colcemid solution with sterile ultrapure water. ***Videographer:** Please obtain CU shot as well for this shot.* **TEXT: Colcemid: N-methyl-N-deacetyl-colchicine**
 - 2.1.2. Talent adding the diluted Colcemid solution to the cell culture. (**Authors:** Thank you for your response. We have incorporated the details in the narration. Please verify if it is appropriate.)
 - 2.1.3. Talent placing the culture into the cell culture incubator.
- 2.2. Prepare a fresh Carnoy's fixative using a 3 to 1 ratio of methanol to acetic acid [1]. Keep the Carnoy's fixative on ice throughout the fixation procedure [2].
 - 2.2.1. Talent preparing the fresh Carnoy's fixative.
 - 2.2.2. Talent placing the Carnoy's fixative on ice.
- 2.3. Prepare a fresh 0.075 molar potassium chloride solution [1] and warm the solution in a 37 degrees Celsius water bath [2].
 - 2.3.1. Talent preparing the potassium chloride solution.
 - 2.3.2. Talent placing the potassium chloride solution into the water bath.
- 2.4. After centrifugation and cell counting, wash the cells with PBS [1]. Gently resuspend in 10 milliliters of the warm 0.075 molar potassium chloride solution [2] and mix the suspension by gentle inversion [3]. Incubate the cells in a 37 degrees Celsius water bath for 30 minutes [4].
 - 2.4.1. Talent washing the cells with phosphate-buffered saline.
 - 2.4.2. Talent resuspending the cells in the warm potassium chloride solution
 - 2.4.3. Talent gently inverting the container to mix.
 - 2.4.4. Talent placing the cell suspension into the water bath.

- 2.5. Slowly add 2 milliliters of the ice-cold Carnoy's fixative while gently mixing the suspension by inversion [1]. After allowing the cells to stand for 5 minutes, centrifuge the cells at 500 *g* for 5 minutes at room temperature [2]. Use a pipette to carefully remove the supernatant [3].
 - 2.5.1. Talent adding the ice-cold Carnoy's fixative while gently inverting the suspension.
 - 2.5.2. Talent placing the sample into the centrifuge.
 - 2.5.3. Talent removing the supernatant with a pipette.
- 2.6. Slowly add 10 milliliters of the ice-cold Carnoy's fixative in 0.5 milliliter increments while gently mixing the suspension after each addition, allowing the cells to stand for 10 minutes [1]. Centrifuge the cells at 650 *g* for 5 minutes at room temperature [2]. During the final wash, leave the cells suspended in Carnoy's fixative [3].
 - 2.6.1. Talent gradually adding the Carnoy's fixative while gently inverting the suspension after each addition.
 - 2.6.2. Talent placing the sample into the centrifuge.
 - 2.6.3. Talent leaving the cells suspended in Carnoy's fixative after the final wash.

NOTE: Treat this as separate section

3. Preparation of Metaphase Spreads

Demonstrator: Lauren Masters

- 3.1. Label glass slides and place them on ice [1]. Use a P1000 (*P-one-thousand*) pipette to resuspend cells to approximately 5 million cells per milliliter of Carnoy's fixative [2]. Transfer approximately 300 microliters of the fixed-cell suspension into a 1.5 milliliter microcentrifuge tube [3].
 - 3.1.1. Talent placing the labeled glass slides on ice.
 - 3.1.2. Talent resuspending the fixed cells with a P1000 pipette.
 - 3.1.3. Talent transferring the fixed-cell suspension into a 1.5 milliliter microcentrifuge tube.
- 3.2. Use a P200 (*P-two-hundred*) pipette to thoroughly resuspend the sample by pipetting up and down several times [1], aspirate 60 microliters of the resuspended cells [2].
 - 3.2.1. Talent pipetting the sample up and down several times with a P200 pipette.
 - 3.2.2. Talent aspirating the resuspended cells with a P200 pipette.
- 3.3. Hold the pipette tip approximately 1 meter above the glass slide on ice and aim for the center of the slide [1]. Slowly dispense 1 drop of the cell suspension onto the slide [2].

Place the slides on a slide incubator at 80 degrees Celsius for 10 minutes [3].

3.3.1. Talent positioning the pipette above the chilled glass slide.

3.3.2. Talent dispensing a single drop of the cell suspension onto the center of the glass slide.

3.3.3. Talent placing the slides into the slide incubator.

3.4. Examine the slides using a phase-contrast microscope with a 10 X (*ten-X*) or 20 X (*twenty-X*) objective [1]. Confirm the presence of multiple well-dispersed metaphase spreads, with minimal overlap [2].

3.4.1. Talent examining the slides with the phase-contrast microscope.

3.4.2. **SCOPE:** Multiple well-dispersed metaphase spreads visible with minimal overlap. (**Authors:** We have instructed the videographer to use scope kit to film this shot. Please specify if this is appropriate. The instructions are provided at the start of the document.)

NOTE: Treat this as a separate section

Slide Pretreatment and DNA-FISH Probe Hybridisation

3.5. For slide pretreatment, place the glass slides in 70 percent acetic acid for 2 minutes [1], wash in 40 milliliters of PBS for 30 seconds [2], followed by a second wash in 45 milliliters of PBS for 5 minutes [3]. Next, dehydrate the glass slides sequentially in 70 percent, 85 percent, and 100 percent ethanol for 1 minute each [4].

3.5.1. Talent immersing the glass slides in 70 percent acetic acid.

3.5.2. Talent transferring the glass slides into 40 milliliters of phosphate-buffered saline.

3.5.3. Talent transferring the glass slides into 45 milliliters of phosphate-buffered saline.

3.5.4. Talent sequentially transferring the glass slides through the ethanol solutions.

~~**In situ Hybridization, Counterstaining, and Imaging**~~

NOTE: Don't put title here, include 4.1 in the above section after 3.5

~~4.~~

4.1. Apply 5 microliters of the probe and hybridization buffer mixture, containing 1 microliter of amplified locus-specific probe and 4 microliters of buffer, to the center of each drop location on the glass slide [1]. Carefully place a coverslip over the sample [2] and seal its edges with rubber cement [3]. Place the glass slides in a humid chamber for *in situ* probe hybridization for 12 to 18 hours as specified in the manufacturer's

instructions [4].

- 4.1.1. Talent applying the probe and hybridization buffer mixture to the center of each drop location on the glass slide.
- 4.1.2. Talent placing a coverslip over the sample.
- 4.1.3. Talent sealing the edges of the coverslip with rubber cement.
- 4.1.4. Talent placing the glass slides into the humid chamber.

NOTE: Treat this as separate section

Post-Hybridization Washing

- 4.2. Remove the glass slides from the humid chamber [1]. Carefully remove the rubber cement [2]. Place the glass slides in 2X (*two-X*) saline-sodium citrate, or SSC (*S-S-C*) solution for 2 minutes at room temperature [3], then carefully remove the coverslips [4].
 - 4.2.1. Talent removing the glass slides from the humid chamber.
 - 4.2.2. Talent removing the rubber cement from the coverslip edges.
 - 4.2.3. Talent placing the glass slides into the 2 times saline-sodium citrate solution.
 - 4.2.4. Talent removing the coverslips.
- 4.3. Next, place the glass slides in the prewarmed 0.4X (*zero-point-four-X*) SSC solution at 72 degrees Celsius for 2 minutes [1], followed by 2X SSC for 1 minute at room temperature [2]. Dehydrate the glass slides sequentially in 70 percent, 85 percent, and 100 percent ethanol for 1 minute each [3]. Air-dry the slides at room temperature in the dark for 10 minutes [4].
 - 4.3.1. Talent placing the glass slides into the prewarmed 0.4 times saline-sodium citrate solution.
 - 4.3.2. Talent transferring the glass slides into the 2 times saline-sodium citrate solution.
 - 4.3.3. Talent sequentially transferring the glass slides through the ethanol solutions.
 - 4.3.4. Talent placing the glass slides on the bench to air-dry in the dark.

NOTE: Treat this as separate section

DAPI Counterstaining and Mounting

- 4.4. Use a pipette to apply 10 microliters of the diluted DAPI (*dah-pee*) to each glass slide at the drop location [1]. Carefully place a coverslip over the sample to ensure the DAPI solution spreads evenly [2]. Incubate the slides in the dark for 5 to 10 minutes [3].
 - 4.4.1. Talent pipetting the diluted DAPI onto the marked drop location of the glass slide. **TXT: DAPI: 4',6-diamidino-2-phenylindole**
 - 4.4.2. Talent placing a coverslip over the sample.
 - 4.4.3. Talent placing the slides in a dark location for incubation.
- 4.5. Place the glass slides in PBS for 30 seconds [1]. Remove the coverslips [2] and place the glass slides in PBS for 2 minutes [3].
 - 4.5.1. Talent immersing the glass slides in phosphate-buffered saline.
 - 4.5.2. Talent removing the coverslips.
 - 4.5.3. Talent placing the glass slides into fresh phosphate-buffered saline.
- 4.6. Dehydrate the glass slides by briefly dipping them in 70 percent, 85 percent, and 100 percent ethanol for approximately 10 seconds each [1]. Air-dry the slides at room temperature in the dark for 10 minutes [2].
 - 4.6.1. Talent sequentially dipping the glass slides into the ethanol solutions.
 - 4.6.2. Talent placing the glass slides on the bench to air-dry in the dark.
- 4.7. Add 15 microliters of the mounting medium to each glass slide [1] and carefully place a coverslip on top [2]. Seal the edges of the coverslip with nail polish, then image and analyze the efficiency of metaphase spread [3].
 - 4.7.1. Talent applying the mounting medium to the glass slide.
 - 4.7.2. Talent placing a coverslip over the mounting medium.
 - 4.7.3. Talent sealing the edges of the coverslip with nail polish.

Results

5. Results

- 5.1. Confirmation of metaphase spreads by phase-contrast microscopy at 10X magnification showed well-dispersed chromosomes, indicating successful metaphase arrest, hypotonic swelling, and nuclear rupture upon contact with the glass slide [1].
 - 5.1.1. LAB MEDIA: Figure 2. *Video Editor: Please emphasize the white boxes in the image.*
- 5.2. Representative DNA-fluorescent *in situ* hybridization metaphase spread images of a COLO320DM cell showed the locus-specific *MYC* (Mick) signal localized to extrachromosomal DNA detected outside the linear chromosomes [1]. A chromosome 8 centromere probe identified the endogenous chromosome 8, which harbors the native *MYC* locus [2].
 - 5.2.1. LAB MEDIA: Figure 3A. *Video Editor: Please emphasize the pink dots away from the blue regions and indicated by white arrows.*
 - 5.2.2. LAB MEDIA: Figure 3A. *Video Editor: Please emphasize the two green spots located within the blue region near the bottom-center of the image.*
- 5.3. In the homogeneously staining region state, the *MYC* probe signal appeared as a dense cluster on a linear chromosome with relatively consistent signal across nuclei within a single sample, reflecting the mitotic stability of chromosomally integrated amplification [1].
 - 5.3.1. LAB MEDIA: Figure 3B. *Video Editor: Please emphasize the pink dots within the blue regions and indicated by white arrows.*

1. Metaphase

Pronunciation link: <https://www.merriam-webster.com/dictionary/metaphase>

IPA: /'mɛtəˌfeɪz/

Phonetic Spelling: met·uh·fayz

2. COLO320DM

Pronunciation link: No confirmed link found

IPA: /'koʊloʊ θri: 'twenti: ,di: 'em/

Phonetic Spelling: koh·loh three twen·tee dee·em

3. Colcemid

Pronunciation link: No confirmed link found

IPA: /'kɑ:lsemɪd/

Phonetic Spelling: kahl·seh·mid

4. N-methyl-N-deacetyl-colchicine

Pronunciation link: No confirmed link found

IPA: /,ɛn 'mɛθəl ,ɛn di:ə'si:təl 'kɑ:ltʃə,sɪ:n/

Phonetic Spelling: en meth·uhl en dee·uh·see·tuhl kol·chuh·seen

5. Logarithmic

Pronunciation link: <https://www.merriam-webster.com/dictionary/logarithmic>

IPA: /,lɒɡə'riðmɪk/

Phonetic Spelling: law·guh·rith·mik

6. Confluence

Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>

IPA: /'kɑ:nfluəns/

Phonetic Spelling: kon·floo·uhns

7. Carnoy's fixative

Pronunciation link: No confirmed link found

IPA: /kɑ:r'nɔɪz 'fɪksətɪv/

Phonetic Spelling: kar·noyz fik·suh·tiv

8. Methanol

Pronunciation link: <https://www.merriam-webster.com/dictionary/methanol>

IPA: /'mɛθəˌnɔːl/

Phonetic Spelling: meth·uh·nawl

9. Acetic acid

Pronunciation link: <https://www.merriam-webster.com/dictionary/acetic%20acid>

IPA: /ə'siːtɪk 'æsɪd/

Phonetic Spelling: uh·see·tik ass·id

10. Potassium chloride

Pronunciation link: <https://www.merriam-webster.com/dictionary/potassium%20chloride>

IPA: /pə'tæsiəm 'klɔːraɪd/

Phonetic Spelling: puh·tas·ee·uhm klor·ide

11. Centrifugation

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>

IPA: /ˌsɛntrɪfjə'geɪʃən/

Phonetic Spelling: sen·trif·yuh·gay·shuhn

12. Phosphate-buffered saline

Pronunciation link: No confirmed link found

IPA: /'fɑːsfert 'bʌfərd 'seɪliːn/

Phonetic Spelling: fos·fayt buf·erd say·leen

13. Resuspend

Pronunciation link: No confirmed link found

IPA: /ˌri:səˈspend/

Phonetic Spelling: ree·suh·spend

14. Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /ˌsu:pərˈneɪtənt/

Phonetic Spelling: soo·per·nay·tuhnt

15. Microcentrifuge

Pronunciation link: No confirmed link found

IPA: /ˌmaɪkroʊˈsentrəˌfjuːdʒ/

Phonetic Spelling: my·kroh·sen·truh·fyooj

16. Aspirate

Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>

IPA: /ˈæspəˌreɪt/

Phonetic Spelling: ass·puh·rayt

17. Phase-contrast microscope

Pronunciation link: <https://www.merriam-webster.com/dictionary/phase-contrast%20microscope>

IPA: /ˈfeɪz ˌkɑːntræst ˈmaɪkrəˌskoʊp/

Phonetic Spelling: fayz kon·trast my·kruh·skohp

18. Objective

Pronunciation link: <https://www.merriam-webster.com/dictionary/objective>

IPA: /əb'dʒektɪv/

Phonetic Spelling: ub·jek·tiv

19. Pretreatment

Pronunciation link: <https://www.merriam-webster.com/dictionary/pretreatment>

IPA: /,pri:'tri:tment/

Phonetic Spelling: pree·treet·muhnt

20. Dehydrate

Pronunciation link: <https://www.merriam-webster.com/dictionary/dehydrate>

IPA: /di:'haɪ,dreɪt/

Phonetic Spelling: dee·hy·drayt

21. Sequentially

Pronunciation link: <https://www.merriam-webster.com/dictionary/sequentially>

IPA: /sɪ'kwɛnʃəli/

Phonetic Spelling: sih·kwen·shuh·lee

22. Ethanol

Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>

IPA: /'ɛθə,nɔ:l/

Phonetic Spelling: eth·uh·nawl

23. DNA-FISH

Pronunciation link: No confirmed link found

IPA: /ˌdiː ɛn ˈeɪ fɪʃ/

Phonetic Spelling: dee-en-ay fish

24. Hybridization

Pronunciation link: <https://www.merriam-webster.com/dictionary/hybridization>

IPA: /ˌhaɪbrəˈdeɪʒən/

Phonetic Spelling: hy-bruh-duh-zay-shuhn

25. Locus-specific

Pronunciation link: <https://www.merriam-webster.com/dictionary/locus>

IPA: /ˈloʊkəs spəˈsɪfɪk/

Phonetic Spelling: loh-kuhs spuh-sif-ik

26. Coverslip

Pronunciation link: <https://www.merriam-webster.com/dictionary/coverslip>

IPA: /ˈkʌvərˌslɪp/

Phonetic Spelling: kuv-er-slip

27. In situ

Pronunciation link: <https://www.merriam-webster.com/dictionary/in%20situ>

IPA: /ɪn ˈsaɪtuː/

Phonetic Spelling: in sy-too

28. Saline-sodium citrate

Pronunciation link: No confirmed link found

IPA: /ˈseɪˌliːn ˈsoʊdiəm ˈsɪˌtreɪt/

Phonetic Spelling: say·leen soh·dee·uhm sit·rayt

29. Counterstaining

Pronunciation link: No confirmed link found

IPA: /'kaʊntərˌsteɪnɪŋ/

Phonetic Spelling: kown·ter·stay·ning

30. DAPI

Pronunciation link: No confirmed link found

IPA: /'dæpi/

Phonetic Spelling: dah·pee

31. 4',6-diamidino-2-phenylindole

Pronunciation link: No confirmed link found

IPA: /fɔːr sɪks daɪˌæmɪ'diːnoʊ tuː ˌfɛnəl'ɪndoʊl/

Phonetic Spelling: four six dye·am·ih·dee·noh two fen·uhl·in·dohl

32. Microscopy

Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>

IPA: /maɪ'krɑːskəpi/

Phonetic Spelling: my·kros·kuh·pee

33. Chromosomes

Pronunciation link: <https://www.merriam-webster.com/dictionary/chromosome>

IPA: /'krɒsəmˌsəʊmz/

Phonetic Spelling: kroh·muh·sohmz

34. Hypotonic

Pronunciation link: <https://www.merriam-webster.com/dictionary/hypotonic>

IPA: /ˌhaɪpəˈtɒːnɪk/

Phonetic Spelling: hy·puh·ton·ik

35. Nuclear rupture

Pronunciation link: <https://www.merriam-webster.com/dictionary/rupture>

IPA: /ˈnuːkliər ˈrʌptʃər/

Phonetic Spelling: noo·klee·er rup·cher

36. Fluorescent

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>

IPA: /flʊˈresənt/

Phonetic Spelling: floo·ress·uhnt

37. Extrachromosomal

Pronunciation link: No confirmed link found

IPA: /ˌɛkstrəˌkroʊməˈsoʊmə/

Phonetic Spelling: eks·truh·kroh·muh·soh·muhl

38. Centromere

Pronunciation link: <https://www.merriam-webster.com/dictionary/centromere>

IPA: /ˈsentrəˌmɪr/

Phonetic Spelling: sen·truh·meer

39. Endogenous

Pronunciation link: <https://www.merriam-webster.com/dictionary/endogenous>

IPA: /ɛnˈdɑːdʒənəs/

Phonetic Spelling: en·dah·juh·nuhs

40. Homogeneously

Pronunciation link: <https://www.merriam-webster.com/dictionary/homogeneous>

IPA: /ˌhoʊməˈdʒiːniəsli/

Phonetic Spelling: hoh·muh·jee·nee·uhs·lee

41. Nuclei

Pronunciation link: <https://www.merriam-webster.com/dictionary/nucleus>

IPA: /ˈnuːkli,aɪ/

Phonetic Spelling: noo·klee·eye

42. Mitotic

Pronunciation link: <https://www.merriam-webster.com/dictionary/mitotic>

IPA: /maɪˈtɑːtɪk/

Phonetic Spelling: my·tot·ik

43. Chromosomally

Pronunciation link: No confirmed link found

IPA: /ˌkroʊməˈsoʊməli/

Phonetic Spelling: kroh·muh·soh·muh·lee

44. Amplification

Pronunciation link: <https://www.merriam-webster.com/dictionary/amplification>

IPA: /,æmpləfə'keɪʃən/

Phonetic Spelling: am·pluh·fuh·kay·shuhn