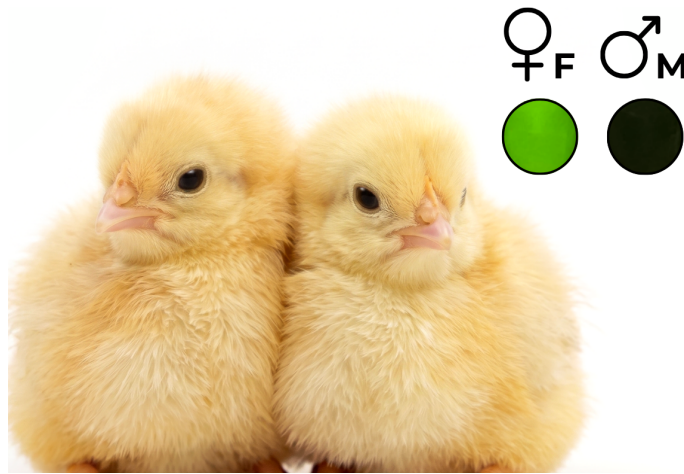


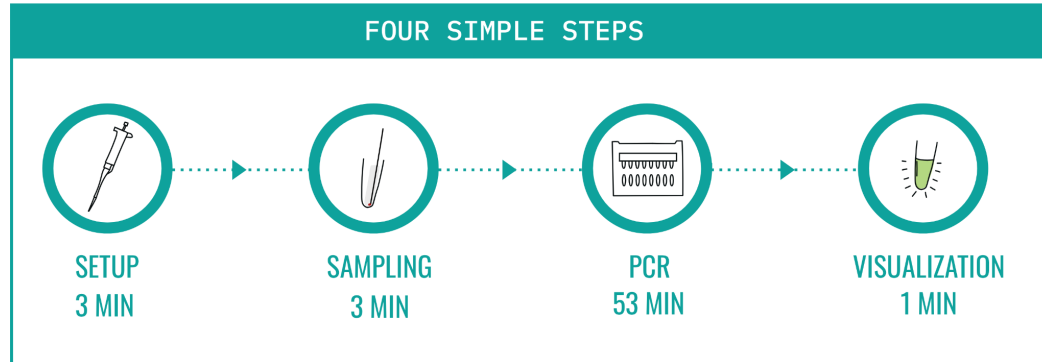


chickView™ DNA Sex Test

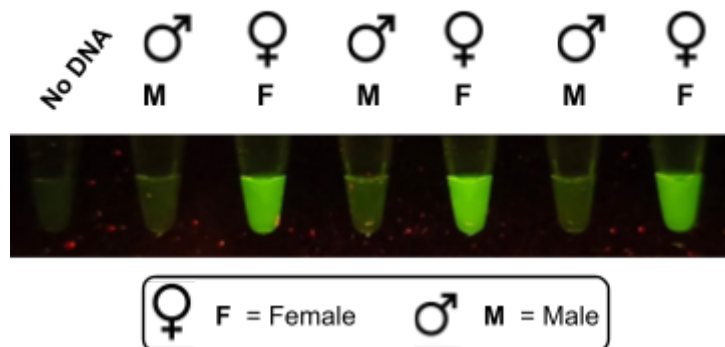
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OVERVIEW

chickView is a quick, user-friendly, PCR-based DNA test to identify chicken pullets from dried blood spot samples. The entire process takes just 1 hour with less than 10 minutes of hands-on work and provides clear visual results.



**Female samples glow green.
Male samples don't glow.**



IMPORTANT CONSIDERATIONS

- chickView™ DNA Sex Test is a rapid screening tool that does not include an internal control. An internal control confirms that a negative test (no glow) is accurate rather than a result of procedural errors.
- Controls.** To ensure accurate results, include the following control samples:
 - Negative Control:** Filter paper without a dried blood spot. This control identifies if female DNA has contaminated the equipment or workspace.
 - Positive Control:** DNA from a known female to confirm that the equipment and reagents are working correctly.
- Inaccurate results.**
 - False positives** may occur if the work area or materials are contaminated with female DNA.

- **False negatives** may occur if the sample is not processed as directed. A positive control helps rule out equipment or reagent failure as the cause of a negative result.
- The chickView™ DNA Sex Test has been validated on samples from domestic chickens (*Gallus gallus domesticus*) using miniPCR equipment. Performance is not guaranteed with other thermal cyclers.

KIT COMPONENTS

The chickView DNA Sex Test (Dx-3543-01) provides material for 48 tests.

SUPPLIED IN KIT

Reagents and Supplies	Quantity	Storage
Reagent A (chickView™ Sex Primers)	1 tube	Freezer
Reagent B (Enzyme)	1 tube	Freezer
PCR tubes (0.2 ml)	1 bag	Room temp.
Collection tubes (1.5 ml)	1 bag	Room temp.
GeneWand™ sample transfer sticks	1 bag	Room temp.
Filter paper	1 bag	Room temp.

SUPPLIED BY USER

Equipment

For starting your lab, we recommend our [chickView Workstations](#). Alternatively, here is the list of all the recommended equipment available at dx.minipcr.com.

Item	Quantity	Recommended product	Cat. Number
Thermal cycler	1	miniPCR® mini8X thermal cycler	QP-1000-08
		miniPCR® mini16X thermal cycler	QP-1016-16
Fluorescence viewer*	1	P51™ Pro Molecular Fluorescence Viewer	QP-1900-10
20-200 µl micropipette	1	20-200 µl H-style	QP-1001-03
Microcentrifuge tube rack	1	Microcentrifuge tube rack, 60-well	CM-1003-10
PCR tube rack	1	miniRack	CM-1003-04
Microcentrifuge (optional)	1	Gyro™ Microcentrifuge, fixed speed	QP-1800-01

*Blue-light transilluminators such as blueBox™, blueGel™, and GELATO™ can be used, but are not recommended because their lower contrast may increase the risk of false negatives.

Consumables

20-200 µl micropipette filter tips ([4AA77](#) or equivalent). You'll need approximately 1 box for every 5 kits.

Other laboratory supplies

- Disposable laboratory gloves
- Protective eyewear
- Lab coat
- Bottle or sprayer with fresh cleaning solution (5% bleach **or** 3% Hydrogen Peroxide)
- Permanent markers
- Containers with lid to dispose of tubes and tips

LABORATORY GUIDELINES

PCR is an extremely sensitive technology that can detect minute amounts of DNA. Always follow the practices outlined below to minimize the risk of contamination.

1. Set up your lab in an area that is removed from possible sources of bird DNA (e.g., away from the husbandry area).
2. Keep each step of the process in separate areas and avoid unnecessary movement of materials between them:
 - Reagents area: for handling and preparing the test solutions.
 - Sampling area: for collecting chicken DNA samples.
 - PCR area: for amplifying DNA and visualizing the results.

	Reagents area	Sampling area	PCR area
Purpose	PCR mix preparation.	Sample collection,	PCR run. Result visualization.
Materials	<u>Reagents</u> Reagent A Reagent B <u>Equipment</u> 20-200 µl micropipette Marker miniRack Trash bin Cleaning solution sprayer Microcentrifuge (optional) <u>Consumables</u> Tissue paper Gloves 200 µl filter tips 0.2 ml tubes 1.5 ml tubes	<u>Equipment</u> GeneWands Filter paper Marker miniRack Trash bin Cleaning solution sprayer <u>Consumables</u> Tissue paper Gloves	<u>Equipment</u> miniPCR P51 Pro Trash bin <u>Consumables</u> Gloves

3. Maintain a clean lab. Spray work surfaces with fresh cleaning solution (5% bleach or 3% hydrogen peroxide) before and after every use.
4. Change gloves between samples, or spray them with the cleaning solution.
5. Keep all tubes closed except for the one that you are actively using.

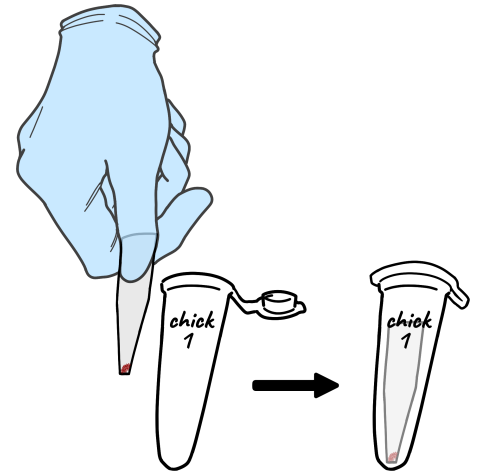
6. Discard tips, GeneWands, tubes, and gloves in containers that can be closed. Empty and clean them often.

Failure to follow good laboratory practices can result in contamination that will invalidate results. A contaminated area is difficult to clean up!

DRIED BLOOD SPOT COLLECTION

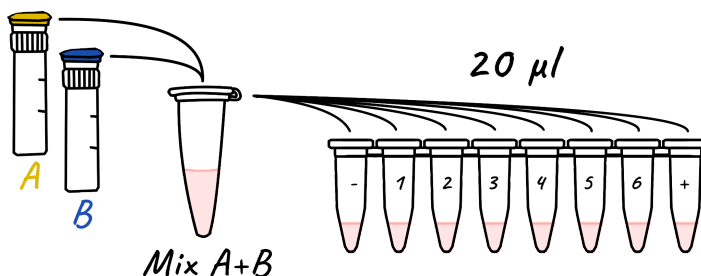
Only properly trained individuals should collect blood for testing.

1. For each chick, remove one collection tube (1.5 ml tube) and one filter paper from the stock bags. Keep the filter paper outside of the tube for now. **Avoid contact with the supply bags while handling the chicks** to help prevent cross-contamination.
2. Tag the chick and write the ID on the tube with a permanent marker.
3. Hold the provided filter paper by the wide end and spot blood onto the short side. **Results are not guaranteed with other filter papers.**
4. Place the filter paper into the labeled collection tube with the blood spot facing inward. Close the tube.
5. For multiple birds, repeat steps 2–4. Sanitize or change gloves between each bird to prevent cross-contamination.
6. Keep dry blood spots at room temperature. Samples are stable for up to one month.



INSTRUCTIONS

STEP 1. PREPARE REAGENTS (3 MIN)



How to use the micropipette

1. Put on gloves and sanitize the work surface with the cleaning solution.
2. You will need one 0.2 ml tube for each sample. Label each tube with a unique sample ID.

- In a 1.5 ml tube, prepare the PCR Mix by combining Reagent A and Reagent B in equal parts. Refer to the table below for the volumes to use based on the number of tests you are running (samples + controls).

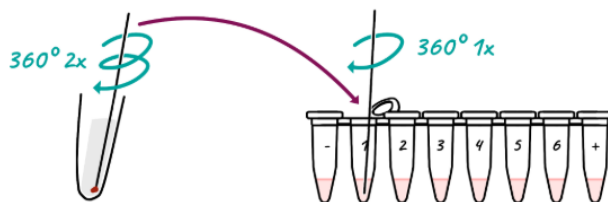
	Number of tests		
	1	8*	16*
Reagent A (μl)	10	88	176
Reagent B (μl)	10	88	176
Total volume (μl)	20	176	352

*These calculations include excess to account for volume loss during pipetting. **The volume provided in the kit accounts for this excess.**

- Mix well by slowly pipetting up and -down 6 times.
- Add **20 μl of the PCR Mix** (A +B) to each 0.2 ml tube.
- Close all 0.2 ml tubes.

Remember to store any remaining Reagent A and Reagent B in the freezer.

STEP 2. ADD SAMPLES (3 MIN)



- Clean the surfaces with the cleaning solution and wear gloves.
- Process the negative control (no DNA):**
 - Insert a clean GeneWand™ stick into the collection tube containing the filter paper without a dried blood spot.
 - Using the wand, push the filter paper to the bottom of the tube.
 - Hold the wand in contact with the filter paper while you rotate the wand for two complete turns.
 - Open only the 0.2 ml tube that corresponds to the negative control sample.
 - Transfer the wand into the liquid PCR Mix and rotate the wand one complete turn.
 - Discard the wand.
 - Close the 0.2 ml tube. This is your negative control.
 - Close the collection tube containing the filter paper.

For each bird sample:

- Insert a clean GeneWand™ stick into the collection tube containing the paper with the dried blood spot.
- Using the wand, push the paper to the bottom of the tube.

- k. Rotate the wand two complete turns, ensuring it stays in contact with the dried blood spot.
- l. Open the PCR Mix tube matching the sample ID.
- m. Transfer the wand into the PCR Mix tube and rotate it one complete turn.
- n. Discard the wand, then close both the collection and 0.2 ml tubes.
3. Process the **positive control** (known female) last.
4. Make sure all tubes are securely closed.

STEP 3. RUN PCR (53 MIN)

1. Load the 0.2 ml tubes into the miniPCR*. Close the lid.
2. Run the **chickView 1** PCR program.

*Other thermal cyclers may be used, but performance is not guaranteed.

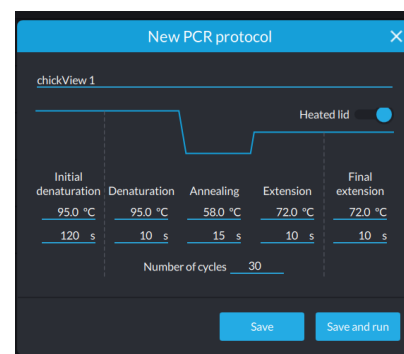
How to program the miniPCR

This step only needs to be done the first time you run the program, as it will be saved in the miniPCR app.

1. Click or tap on the (+) symbol
2. Select "PCR"
3. Enter information:
 - a. Name: "chickView 1" or name of your choice
 - b. Initial denaturation 95 °C, 120 sec
 - c. Denaturation 95 °C, 10 sec
 - d. Annealing 58 °C, 15 sec
 - e. Extension 72 °C, 10 sec
 - f. Number of cycles 30
 - g. Final extension 72 °C, 10 sec
2. Click or tap "Save and run"

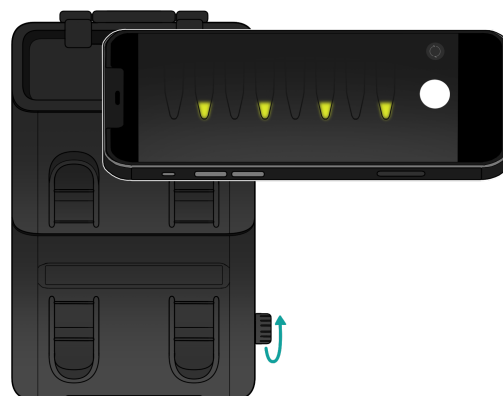


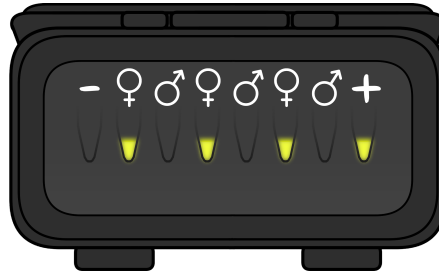
Program the
miniPCR®



STEP 4. RESULTS (1 MIN)

1. Place the PCR strip into the **P51™ Pro Fluorescence Viewer** and turn on the blue light.
2. Close the front flap and align your phone camera with the center hole.
3. Rotate the illumination control knob to adjust brightness until you see maximum contrast between the positive and negative controls.
4. Take a photo to document your results.

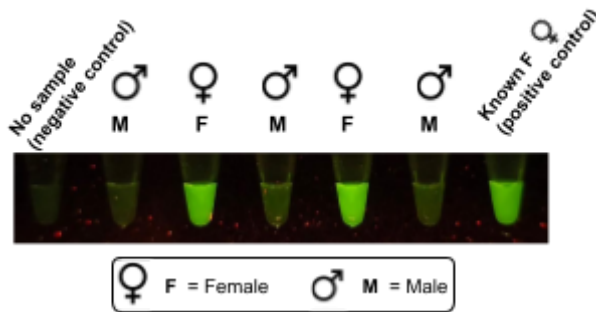




Notes:

- Results can be visualized up to 5 hours after PCR if the samples are kept in the miniPCR, in the P51 Pro Viewer, or in a dark place.
- Blue-light transilluminators such as blueBox, blueGel, and GELATO can be used, but are not recommended because their lower contrast may increase the risk of false negatives.

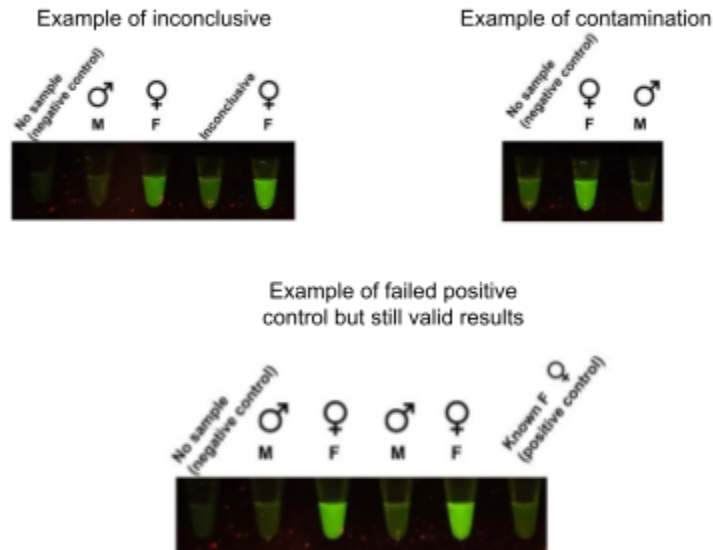
Expected results



**Female samples glow green.
Male samples don't glow.**

Sample	Expected result	Notes
No DNA (negative control)	No glow	Green fluorescence indicates contamination. The entire test run is invalid. Repeat the test.
Male	No glow	The test DOES NOT detect male DNA.
Female	Green glow	The test detects the presence of female DNA and emits green fluorescence.
Known female (positive control)	Green glow	If one or more unknown samples show green fluorescence, they should be recorded as female, even if the positive control does not fluoresce.

Troubleshooting



Issue	What to do
Inconclusive result (pale glow)	Repeat the test.
Glow in the negative control indicates contamination	If contamination is detected repeatedly on multiple runs, we recommend that you: 1. Deep clean the lab by wiping surfaces with fresh cleaning solution 2. Replace or thoroughly clean the trash bins 3. Clean your lab coat 4. Discard open tubes of reagents 5. Discard the remaining filter tips Refer to the Laboratory Guidelines section (page 3) for additional suggestions
The positive control (known female) does not glow	If one or more unknown samples show green fluorescence, they should be recorded as female, even if the positive control does not glow. In this case, the lack of fluorescence in the positive control likely reflects an issue with the control DNA extraction, rather than the test run.

Email us at dx@minipcr.com, we are happy to help!