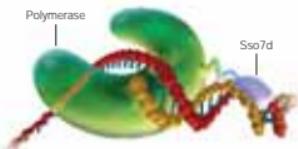




## A Powerful Bond

Bio-Rad's unique *iProof™* high-fidelity DNA polymerase delivers unsurpassed speed and fidelity — with higher yields and fewer reaction failures — for all your PCR needs.



Fusing the double-stranded DNA binding protein Sso7d to iProof gives it a powerful sliding grip on the replicated DNA.

### Tips for Successful Amplification

- Use iProof high-fidelity DNA polymerase at 0.5–1.0 U per 50  $\mu$ l reaction; do not exceed 2 U per 50  $\mu$ l reaction
- Use 15–30 sec/kb for extension; do not exceed 1 min/kb
- Use 200  $\mu$ M dNTPs; do not use dUTP
- Use 98°C for denaturation (high-salt buffer)
- Anneal at  $T_m + 3^\circ\text{C}$  (>20 nt) or use 2-step protocol
- Note: iProof DNA polymerase produces blunt-end products



### Recommended Cycling Parameters

Cycle Step	Temperature	Time	Cycle Repetitions
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	5–10 sec	
Annealing	45–72°C	10–30 sec	25–35
Extension	72°C	15–30 sec/kb	
Final extension	72°C	5–10 min	1

**BIO-RAD**

## Troubleshooting Tips

Observation	Comments and Suggestions
No product or low yield	<ul style="list-style-type: none"><li>Repeat and make sure that there are no pipetting errors</li><li>Use fresh high-quality dNTPs (170-8874); do not use dNTP mix that contains dUTP</li><li>Lower annealing temperature</li><li>Lengthen extension time</li><li>Optimize enzyme concentration</li><li>Denaturation time may be too long or too short; optimize the denaturation time</li><li>Check condition of the primers</li><li>Check primer design</li></ul> <p>Generally applies to G+C-rich or supercoiled templates</p> <ul style="list-style-type: none"><li>Titrate DMSO (2–8% final) in the reaction</li><li>Denaturation temperature may be too low (98°C is optimal for most templates)</li><li>Try using GC buffer if HF buffer has failed</li></ul> <p>Generally applies to templates &gt;5 kb</p> <ul style="list-style-type: none"><li>Sample concentration may be too low; use more template</li><li>Template DNA may be damaged; use carefully purified template</li><li>Increase number of cycles</li></ul>
Nonspecific products —	<ul style="list-style-type: none"><li>Reduce enzyme concentration</li><li>Shorten extension time</li><li>Reduce number of cycles</li><li>Increase annealing temperature or try 2-step protocol</li><li>Vary denaturation temperature</li></ul>
High molecular weight smears	<ul style="list-style-type: none"><li>Raise annealing temperature</li><li>Lower enzyme concentration</li><li>Optimize Mg<sup>2+</sup> concentration</li><li>Titrate template amount</li><li>Lower primer concentration</li><li>Design new primers</li></ul>
Low molecular weight discrete bands	<ul style="list-style-type: none"><li>Optimize extension time</li></ul>
Generally applies to templates >5 kb	

Practice of the patented polymerase chain reaction (PCR) process requires a license.



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